Associative Learning in a Mouse Model of Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disease defined by severe memory loss and the accumulation of amyloid plaques and neurofibrillary tangles in the brain. The experiments presented in this thesis examined cognitive performance in a well-characterised mouse model of AD, the APPswe/PS1dE9 (TG) mouse, at 4-5 months old prior to extensive amyloid plaque deposition. The experiments employed were associative learning tasks, which are not often used to measure cognitive performance in classical neuroscience research into neurodegenerative disease.

Chapter 1 looked at appetitive Pavlovian cue and context conditioning and extinction, and found some evidence of impaired contextual discrimination during conditioning. Cue conditioning and extinction was found to be intact in the TG mouse model. Chapter 2 looked at appetitive Pavlovian delay and trace conditioning before examining the ability to time the arrival of the unconditioned stimulus (US). No genotype differences were found during delay or trace conditioning; however, TG mice were impaired (lost precision) in their ability to time the arrival of the US during test trials. Chapter 3 examined recognition memory performance, via the spontaneous

novel object recognition (sNOR), relative recency (RR) and context priming (CP) tasks, interpreting the results in terms of Wagner's SOP model of memory. No genotype differences were found in the sNOR or RR tasks, supporting intact stimulus-generated priming; however, evidence from Bonardi et al. (2016) and non-significant trends in the CP task supported impaired retrieval-generated priming. Chapter 4 looked at the levels of neuro-inflammation (microglia) in the cortex, hippocampus and striatum to assess the possible early contribution of inflammation on the development of AD. This chapter reported greater levels of microglia in the hippocampus and striatum of TG mice compared to wild types. Greater microglia clusters were also seen in the cortex and hippocampus of TG mice compared to wild types.

The results from this thesis show evidence of impaired cognitive performance, prior to extensive plaque deposition, in associative learning tasks that are not routinely employed in neuroscience research. Further work is required in these learning tasks to establish whether they could be effectively used to diagnose AD at an early stage.

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Abbreviations

AD	Alzheimer's disease
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
Aβ	Amyloid beta
BLA	Basolateral Amygdala
CFC	Contextual fear conditioning
CR	Conditioned response
CS	Conditioned stimulus
CTA	Conditioned taste aversion
EBC	Eye-blink conditioning
FAD	Familial Alzheimer's disease
MCI	Mild cognitive impairment
MWM	Morris water maze
NFT	Neurofibrillary tangles
OIC	Object in context
PSEN1	Presenilin 1
PSEN2	Presenilin 2
RR	Relative recency
SAD	Sporadic Alzheimer's disease
sNOR	Novel object recognition
SOP	Standard operating procedures
TI	Trace interval
TG	Transgenic
US	Unconditioned stimulus
WT	Wild type

1.0 Chapter 1 – Aims

My aim in this thesis was to examine the early cognitive deficits associated with Alzheimer's disease using a particularly well-characterised mouse model (APPswe/PS1dE9). Alzheimer's Disease (AD) is a progressive and neurodegenerative disease and is the most common type of dementia. Worldwide the prevalence of AD is around 35 million, with 4.5 million new cases each year (ARUK dementia statistics 2012). In the UK there are currently around 800,000 AD sufferers and this is predicted to rise to 2 million by 2051 (Dementia UK 2014). The economic burden of dementia in the UK is around £26.3 billion. (Dementia UK 2014).

Being able to understand the early development of the disease and accurately diagnose the condition early is essential to try and reduce the ever-increasing number of people living with the disease. Early diagnosis is essential as it allows for the accurate differentiation of people with AD and other forms of dementia or clinical conditions, Patients diagnosed with AD would be able to received earlier treatment intervention; from the pharmacological to treat the cognitive impairments to non-pharmacological interventions to treat associated behavioural problems.

A number of issues surround studying AD in the human population. The fundamental limitation is that currently AD can only be fully diagnosed post-mortem, by examining brain tissue for the pathological hallmarks of the disease. This process can be affected by factors such as the preparation of the tissue, the subject's age at death, the stage of AD progression at time of death, the level of treatment received and the presence of co-morbid conditions. Imaging diagnostic techniques are also subject to limitations, such as high cost, and the effectiveness of tracer binding and its indirect nature as a measure of pathology level. As a result, the use of animal models has become increasingly widespread. Animals genetically altered to express specific genetic mutations associated with the disease are guaranteed to develop AD pathology. This allows researchers to accurately measure the full pathological, cognitive and behavioural development of the disease, which is impossible in humans.

Currently a large body of research into the cognitive impairments of AD using mouse models has focused on the later stages, once pathology has extensively developed in the brain. This focus leads to increased chances of finding impaired performance; however, being able to characterize the development of the disease

relies on establishing the earliest impairments in cognitive performance.

The animal behavioural work carried out in this thesis focuses on associative learning as a way of examining early cognitive changes in the development of AD. Most attempts to characterize the development of AD in mouse models have relied on a number of 'standard' experimental tasks measuring spatial, contextual and recognition memory.

The associative learning tasked used in this thesis are often used in psychological experiments examining learning and memory in humans, rodents and other psychopathologies: depression, addiction, schizophrenia and Parkinson's (Diwadkar et al., 2008; Martin-Soelch et al., 2007; Sprengelmeyer et al. (1995). They are however, not commonly seen in neuroscience research examining neurodegenerative disease.

Associative learning tasks are particularly useful as they measure the strength, latency and rate of learning and memory over multiple trials and sessions. This will allow the experiments in this thesis to measure subtle nuances in the development of memories and the strength, latency and rate of learning about contextual cues,

discrete cue, objects and locations between wild type control animals and the APPswe/PS1dE9 mouse model of AD.

1.0.1 Thesis

The forthcoming sections will attempt to broadly outline human Alzheimer's disease focusing on the cognitive, pathological symptoms and genetic factors associated with the disease. The thesis will then go on to outline the pathological and cognitive impairments seen in the specific mouse model (APPswe/PS1dE9) that was used in the forthcoming chapters.

1.1 Alzheimer's disease development

AD goes through a number of different stages before a firm diagnosis can be made. Emerging evidence suggests that there is at least a decade between the start of pathological development and a clinical diagnosis (Bateman et al. 2012). AD is split into three main subcategories: preclinical, mild cognitive impairment (MCI) and Alzheimer's disease. Preclinical AD is seen as the earliest stage of development, in which disease pathology begins to occur but no clear cognitive impairments are observed. The second stage is MCI, although not all patients diagnosed with MCI will go on to develop

AD. MCI is defined by increased abnormal, AD related, biomarkers and memory impairment (Petersen et al., 1999; 2009). Finally, a diagnosis of AD is based on several factors. Biomarker levels are much greater than in either MCI or preclinical dementia and memory impairments are also worse than the previous stages.

1.2 Symptoms of Alzheimer's disease

The following section will classify cognitive symptoms associated with Alzheimer's disease.

1.3 Cognitive symptoms

Cognitive impairments are the hallmark symptom of AD, in particular memory and executive function impairments:

1.3.1 Memory

Memory is the process whereby information is stored and retrieved, and is often divided up into different systems (Henke 2010). A traditional distinction firstly divides memory into short term and long term. Short-term memory is of limited capacity in the duration of seconds but information is readily accessible, whilst long term memory is limitless and retention of information is seen as indefinite, although less accessible (Craik & Lockhart 1972; review see Cowan 2008). Tulving et al. (1972; 1999; 2003) later differentiated long

term memory into two separate types: declarative and non-declarative (Schacter et al., 1987).

1.3.1.1 Non-declarative memory

According to Tulving's theory, non-declarative memory comprises procedural memory, priming and classical conditioning (Ullman et al., 2004; Squire, 2004; Tulving & Schacter 1990). Non-declarative memory is thought not to be open to conscious access, but to occur implicitly. Non-declarative learning and memory remain reasonably unaffected in AD patients until they reach a severe phase of the disease (Kessels et al., 2011).

1.3.1.1.1 Procedural memory

Procedural memory represents unconscious and automatic skills that involve motor learning, e.g. learning to ride a bike, and is unaffected by AD (Libon et al., 1998; Machado et al., 2009).

1.3.1.1.2 Priming

Priming is the process by which performance improves with previous exposure to information. Participants are presented with a series of stimuli (picture or word) before later being presented with some identical and novel stimuli. Priming is observed when responding to the identical stimulus is faster than to the novel. A

number of studies employing this technique have failed to show impaired performance in AD patients (Fleischman et al., 2005; Golby et al., 2005; Lustig et al., 2004), although occasional impairments are seen in semantic priming, faster responding to a semantically related stimulus (Hernandez et al., 2008).

1.3.1.1.3 Classical conditioning

Classical conditioning occurs when a conditioned stimulus, (CS; e.g. a light) is presented with an unconditioned stimulus (US; e.g. food) that results in an unconditioned response (salivation). After repeated pairings of CS and US the CS on its own begins to elicit a conditioned response, CR (salivation). Interestingly patients with AD do show some impairment in simple conditioning tasks. A number of different methods have been employed to measure classical conditioning in humans and clinical populations.

Tasks using aversive USs include eye-blink conditioning, in which an auditory or visual CS is paired with an air puff to the eye (US), and results in a reflexive eye blink CR. Patients with AD show slower acquisition of conditioned responding than controls (Solomon et al., 1995; 1991; Woodruff-Pak et al., 1990; 1996), reflecting slower learning of the CS-US association. A related technique is startle conditioning, in which a CS is paired with a loud noise (US) and the

CR is an increase in sweating, measured by skin conductance. Hamann et al. (2002) paired a green rectangle with the US (CS+), but not a red rectangle (CS-). Healthy volunteers showed a strong CR to the CS+ but not the CS-, whilst patients with mild or moderate AD failed to show greater differential responding to the CS+ than CS-.

Conditioning tasks using appetitive USs have also been employed. Bodi et al. (2009) required participants to learn which cartoon faces A1, A2, B1, B2, were associated with a particular fish, X1 or X2. Participants were presented with a cartoon face (A1) and two possible fishes (X1 and X2), and required to select the correct fish. Depending on the fish selected, appropriate feedback, correct or incorrect, was presented. Participants with AD were able to acquire the CS-US associations, but made a greater number of errors, selecting the wrong fish, than did age-matched healthy participants.

1.3.1.1.4 Summary

Implicit and unconscious non-declarative memory remains markedly unaffected in patients with AD; both procedural and priming tasks show little AD-specific impairment. Interestingly, the few classical conditioning tasks that have been carried out have shown poorer learning in AD patients than age-matched healthy participants.

Slower performance in AD patients is also evident in semantic priming tasks in comparison to controls.

1.3.1.2 Declarative memory

Declarative memory is commonly divided into three sub-categories: semantic, episodic and spatial memory (Tulving, 1972).

1.3.1.2.1 Semantic memory

Semantic memory is for the acquisition and representation of fact-based knowledge. Semantic deficits encompass problems in word finding, knowledge of concept meaning and semantic context. Of the many different tasks that have been employed to measure semantic memory in AD, most involve matching information based on certain relationships. The verbal fluency task (VFT) requires participants to generate words beginning with a certain letter or from a particular category (Henry et al., 2004; Hodges et al., 1990; 1992). The picture-picture matching task (PP) involves matching pictures to a conceptually related picture, and the verbal-picture naming task (VP) naming an item based on a verbal description. Patients with mild AD are impaired on the VFT, PP and VP tasks relative to age-matched healthy volunteers (Henry et al., 2004; Hodges et al., 1990; 1992; Nebes et al., 1989). They were also impaired on a picture-sorting task

(PS), which requires participants to sort pictures into one of three different groups, and a word-picture matching task (WP) which involves matching a word to the appropriate picture (Henry et al., 2004; Hodges et al., 1990; 1992; Nebes et al., 1989).

1.3.1.2.2 Episodic memory

Tulving (1993) defined episodic memory as being for personal experience in subjective space and time. An individual has conscious awareness of a previous event, specifically where it took place, what occurred and when (Tulving., 1993; Ullman et al., 2004; Clayton et al., 2007). It is one of the first types of memory to become disrupted in AD (Nester et al., 2004; Rickert et al., 1998), and has been assessed in a number of different tasks.

Sauzeon et al. (2016) measured episodic memory function in young healthy, old healthy and AD patients in a virtual reality (VR) environment. Participants were led around a virtual apartment (A) that consisted of four rooms, for 50s before having to recall all the items seen in the apartment (free-recall task). Participants then repeated this procedure around apartment B. Then participants underwent a verbal yes/no recognition task, where they were presented with a list of 56 items and they had to say whether or not they had encountered the objects in apartment A. AD patients were

poorer than both control groups in the immediate free-recall tasks and the yes/no recognition task.

Plancher et al. (2012) measured episodic memory performance in cognitively healthy controls, and MCI and mild Alzheimer's disease patients. Participants were required to navigate through two virtual cities in a car, either driving (active) or being driven (passive). They were instructed to memorize all aspects of the virtual scene they were exploring, and were then given a free recall task, which involved identifying as many items from the environment as possible. This was followed by a recognition task where they had to answer yes or no to questions about items and events that occurred in the virtual environment. AD patients were significantly worse than both MCI and control participants in both tasks. Spatial allocentric information, the ability to situate the elements of scenes in relation to each other, was particularly useful at differentiating between MCI and healthy controls. The virtual reality test was also highly correlated with the daily memory complaints of the AD participants.

1.3.1.2.3 Spatial memory

Spatial memory; in particular allocentric memory is the ability to navigate around based on local and global landmarks within your surroundings. Spatial memory has been examined in both patients

with MCI and AD. Kalova et al (2005) presented participants with a schematic of an area that contained landmark cues and the position of a target location (sample phase). Following this participants were taken to a real world replica arena and positioned at the start location, participants were required to navigate target location presented in the sample phase. Particpants could navigate based on landmark cues (allocentric) or based on the start location (egocentric). The AD patients were impaired relative to controls in their ability to use the allocentric landmark cues to navigate towards the target location. Hort et al (2007) replicated the above finding, but also showed that patients with MCI were also impaired using allocentric landmark cues to navigate. Possin et al (2016) established a virtual version of the Morris water maze (WMW) task in which participants had to navigate around a virtual arena to find a hidden platform. Two version of this task were administered: the visible target (VT) and hidden target (HT) versions. In the VT version the position of the target platform was made obvious, and no differences were seen between MCI or control participants. In the HT version where particpants had to navigate to the target location based on the distal cues around the arena (allocentric) the MCI partipants were impaired in their performance compared to controls. Finally Weniger

et al (2010) compared MCI patient's performance with controls on a virtual reality navigation task based on allocentric cues or egocentric memory. MCI patients were impaired compared to controls on both types of navigation task and was linked to a significantly reduced hippocampal volume. Allocentric memory has been consistently linked to normal medial temporal lobe function, in particular the role of the hippocampus (Broadbent et al., 2006; Clark et al., 2005; Maguire et al., 1998; Morris et al., 1982 O'Keefe & Nadel 1978; Parlsow et al., 2004)

1.3.2 Executive function

Another cognitive domain which is particularity affected in AD is executive function - a broad term used to cover top-down control of behaviour, decision-making, self-monitoring, planning, initiating and inhibiting behaviour. Impairments may consist of impaired performance on tasks that require inhibition of a response. Many studies have identified executive function impairments in patients with AD of varying severity (Ashendorf et al., 2009; Baddeley et al., 1991; Rainville et al., 2002).

The Tower of Hanoi (TOH) task, which requires the transfer of disks on pegs from a start position to a finish position, has been used

extensively as a measure of executive function. Two rules govern this task: only one disk can be moved at a time, and all the disks must occupy a peg. TOH requires planning of the sequence of moves, execution of the plan, and monitoring and revision as each move is carried out (see review, Bull et al. 2003), and TOH performance is impaired in mild AD (Rainville et al., 2002).

Sahakian et al. (1990) used a set-shifting paradigm taken from the CANTAB (Cambridge neuropsychological test automated battery) to assess executive function. Here participants are presented with two target stimuli (coloured circles) and required to choose one stimulus based on an undisclosed rule (colour or size). Feedback is given enabling participants to learn the rule. Following acquisition the response contingencies are altered either intra-dimensionally (shift from large circles to small circles) or extra-dimensionally (shift from size to colour). Participants with minimal AD could perform this task, whilst patients with moderate AD were impaired, specifically being unable to learn intra-dimensional shifts (Perry & Hodges 1999).

1.4 General Summary Of Symptoms

AD primarily impairs declarative memory: patients with AD show consistent impairments in both semantic and episodic memory, and

executive function. Episodic memory deficits appear years before a clinical diagnosis of AD (Backman et al., 2001), which could make it an ideal tool for early diagnosis. Impairments in semantic memory and executive function are also found in patients with mild AD. Impaired performance in non-declarative memory also becomes evident as the disease progresses.

1.5 Neurobiology of Alzheimer's disease

A whole host of genetic, pathological and morphological brain changes occur during the age-related decline associated with AD. The next section will discuss these changes, including the underlying genetic mutations, morphological brain atrophy and the brain pathology: amyloid plaques, neurofibrillary tangles and cholinergic function.

1.5.1 Familial vs Sporadic Alzheimer's disease

AD can be divided into two separate pathologies: familial (early-onset; FAD) and sporadic (late-onset; SAD). A strong genetic influence underpins AD development: having a relative (first-order) with SAD doubles the risk of developing SAD in later life (Rao et al., 2013). FAD is linked to genetic mutations on the PSEN1 (presenilin 1), PSEN2 (presenilin 2) and APP (amyloid precursor protein) genes,

and possession of one of these mutations results in FAD. Although FAD only represents 1% of AD cases, it is an essential tool in understanding the more common sporadic form (Bateman et al., 2011). Currently no genetic mutation has been identified to explain the development of SAD; however, similar to FAD, it is clearly a multifaceted disease, caused by complicated interactions between the environment and genes (Table 1.1).

1.5.2 Pathology

The neuropathology associated with SAD includes development and deposition of amyloid plaques and neurofibrillary tangles, and associated cholinergic receptor loss. FAD shows the same neuropathology, but in different amounts and with a different distribution depending on the different familial genetic mutations.

1.5.2.1 Amyloid-plaques - Genetics

Amyloid plaques in both FAD and SAD result from increased intra-cellular aggregation of the Aβ 42 and 40 proteins. Aβ proteins are essential for brain function but their normal role is not fully understood. Aβ is believed to be involved in a number of different physiological processes including controlling synaptic activity and

maintenance of normal neuron function (Pearson & Peers, 2006). The gene that encodes the APP is found on chromosome 21 (Table 1.1). APP is then sliced by either the α- or β-secretase enzymes (Figure 1.1), which reduce its length. Cleavage by α-secretase produces α-APPs and C83 peptide; however cleavage by the β-secretase enzyme produces β-APPs and C99 peptides (Figure 1.1). Then the ϒ-secretase enzyme cleaves the shortened C83 and C99 peptides into non-pathogenic P3, or Aβ 40 and Aβ 42, (Citron et al,. 2004; Liebscher et al,. 2012; Tanzi et al., 2012). The α-secretase pathway produces non-pathogenic proteins, whilst the β-secretase pathway leads to pathogenic Aβ 40/42. Both Aβ 40 and Aβ 42 are neurotoxic, but Aβ 42 is much more prone to aggregate into plaques.

In SAD unknown APP gene mutations result in greater β-secretase cleavage and higher levels of pathological Aβ 40/42, whilst PSEN1 and 2 mutations (Table 1.1) cause greater ϒ-secretase cleavage, also producing increased toxic Aβ 40/42 (Figure 1.1; Vitrivel et al. 2006; Zhang et al., 2013). An excessive amount of Aβ 40/42 is produced relative to healthy controls, resulting in the aggregation and progressive deposition of plaques (Petrella et al., 2003).

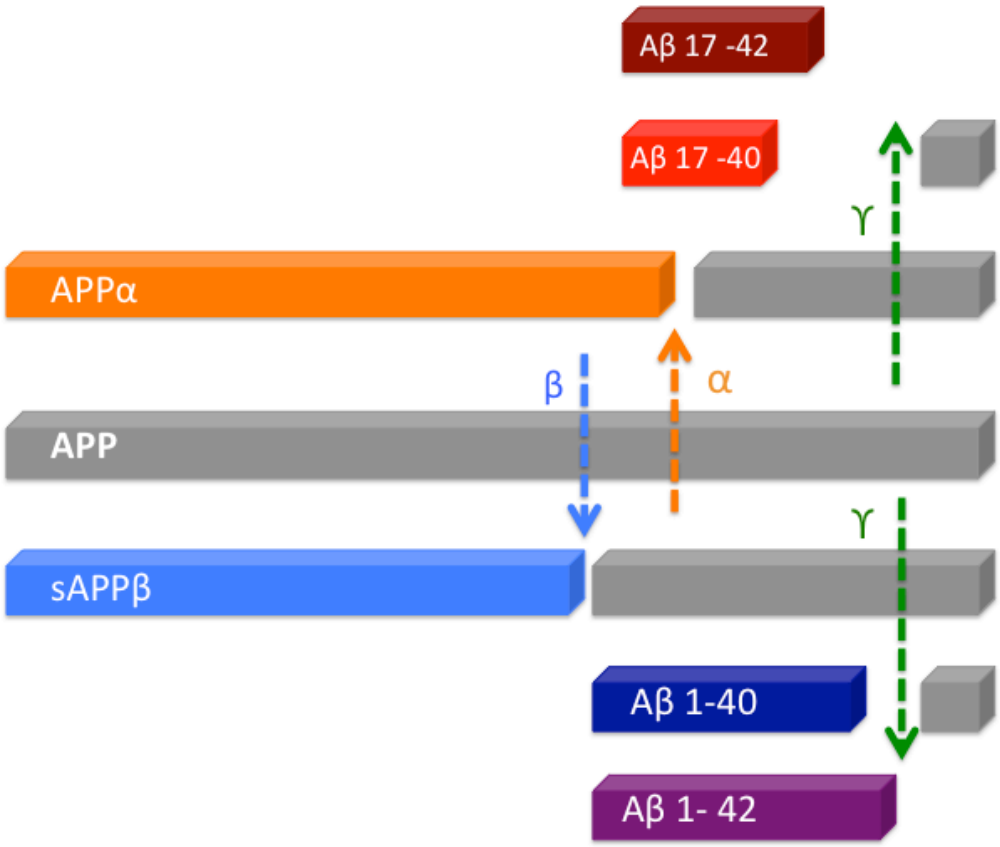


Figure 1.1. A schematic of the cleavage of the amyloid precursor protein.

1.5.2.2 Amyloid-plaques - Pathology

The deposition of amyloid plaques during the development of AD shows considerable inter-individual variability, especially during the early stages of the disease. Braak & Braak (1991) assessed the brains of 83 demented and non-demented individuals for Aβ plaques. Plaque deposition was broken down into three stages. Stage A defines deposition mainly in the basal section of the frontal, temporal and occipital lobes, Stage B in the neo-cortex, with low levels in the

hippocampal formation, and stage C in primary neocortical areas, the cerebellum, striatum, thalamus, hypothalamus, subthalamic nucleus and red nucleus. More recently Thal et al. (2002) developed a more fine-grained version of the staging system for the progression of amyloid pathology. Their Stage 1 is characterised by deposition in the neocortical regions, Stage 2 in the entorhinal cortex, hippocampal formation, amygdala, insular and cingulate cortex, Stage 3 in the striatum, basal forebrain, thalamus, hypothalamus and white matter, Stage 4 in the brainstem and Stage 5 in the pons and cerebellum.

Sophisticated brain imaging techniques have also been used to measure the progression of AD pathology. Positron emission tomography (PET) in conjunction with an Aβ binding agent PIB (*N*-methyl-[^{11}C]2-(4_-methylaminophenyl)-6 hydroxybenzothiazole) can be used to measure the levels of Aβ plaques. PIB binds exclusively to the Aβ plaques, and the degree of binding is used as a measure of plaque levels. Klunk et al. (2004) showed that levels of PIB binding are significantly greater in the frontal and temporal-parietal cortical areas, frontal cortex and neocortical areas of AD patients than controls. Binding in the temporal lobe was also greater, but less so. Beckett et al. (2012) also looked at PIB binding in preclinical, MCI and AD patients, and found increased binding in all

three groups relative to controls. PIB binding was also found to increase over the progression of the disease. Binding was greater in the AD group than the MCI group, and greater in the MCI group than the preclinical group.

1.5.2.3 Neurofibrillary tangles – Genetics

Neurofibrillary tangles are the second major pathological change in AD. They result from mutations in the TAU gene (Table 1.1) located on chromosome 17. This gene codes TAU proteins that function in healthy individuals to assemble microtubules, which form part of the structural architecture of neuronal cells (Iqbal et al., 2005). TAU proteins in a normal state contain 2 - 3 mol of phosphate, but in AD they are hyperphosphorylated, which reduces microtubule binding and assembly. As a result the TAU protein accumulates into intra-neural tangles made up of paired helical filaments (Iqbal et al., 2005). It is currently unclear whether or not increased accumulation of NFT is inherently neurotoxic (SantaCruz et al., 2005; for a review see Cowan & Mudher 2013).

1.5.2.4 Neurofibrillary tangles - Pathology

The formation of NFTs also shows a distinct pattern of development that has been characterized into six stages (Braak & Braak, 1991; Serrano-Pozo et al., 2011.). Stage 1 is defined by NFT formation in the trans-entorhinal and perirhinal areas, Stage 2 in the entire entorhinal cortex and the CA1 region of the hippocampus, Stage 3 the limbic system (subiculum), Stage 4 the amygdala, thalamus and claustrum, Stage 5 the isocortical and associative areas and Stage 6 the primary sensory, motor and visual areas (Braak & Braak, 1991). The topographical distribution of NFTs in AD patients has also been measured in PET scans using an NFT binding agent (FDDNP). FDDNP binding is increased in medial temporal, lateral temporal, parietal, posterior cingulate and frontal cortex (Shin et al. 2008).

1.5.2.5 Cholinergic function

A disruption and loss of cholinergic function also occurs in SAD. The cholinergic system is controlled by the neurotransmitter acetylcholine (ACh) and nicotinic and muscarinic receptors (Scarr et al., 2013), and is made up of three main projections. The first runs from the basal forebrain to the hippocampus and most cortical and subcortical nuclei, the second from the brain stem to the thalamus and midbrain, while the third is a complex network of interneurons

in the striatum and nucleus accumbens (Scarr et al. 2013). There is a significant reduction in choline acetyltransferase, an enzyme involved in ACh synthesis, in AD patients as early as one year from symptom onset (Bowen et al., 1982). Reversing the cholinergic depletion in AD is currently the main route of pharmacological treatment. The three cholinesterase inhibitors currently approved by the USA food and drug administration (FDA) for treating AD are donepezil, rivastigmine and galantamine. All function by inhibiting the breakdown of ACh via blockade of acetylcholinesterase (Birks 2012). This allows ACh to remain in the synaptic cleft of neurons for longer, allowing it more time to bind with other receptors and so increasing its effectiveness (Lleo et al., 2006).

Chromosome	*Gene*	*Results of mutation*
1	*Presenilin 2 (PSEN2)*	*Increased production of amyloid-beta*
14	*Presenilin 1 (PSEN1)*	*Increased production of amyloid-beta*
17	*TAU*	*Increased neurofibrillary tangles*
19	*Apolipoprotein E4*	*Increases density and level of deposition of amyloid-beta*
21	*APP*	*Increased production of amyloid-beta*

Table 1.1. The genes associated with both familial and sporadic AD.

1.5.2.6 Apolipoprotein

A variant of the Apolipoprotein E (ApoE) gene (Table 1.1) has proved the most accurate risk factor for developing AD. ApoE is involved in the transportation of cholesterol to neurons (Liu et al., 2013), by binding with lipids (fats) to form lipoproteins that transport cholesterol. It is not fully understood how the ApoE protein is involved in AD development, but research suggests it may play a role in the production of Aβ 40/42 (Poirier et al., 2000). The ApoE gene has two copies of three allele variations: APOE-e2, APOE-e3 and APOE-e4. The risk of developing SAD based on the ApoE allele variations is 60% in people with two copies of the ApoE4 allele, and 30% in those with one copy (Corder et al., 1993; Huang et al., 2012; Spinney et al., 2014).

1.5.2.7 Summary

The development of FAD is caused by specific genetic mutations of the APP, PSEN1 and PSEN2 genes. SAD involves the abnormal processing of both the amyloid precursor and TAU proteins, which are all mediated by the APP, PSEN1, PSEN2 and TAU genes. The risk of developing AD is increased to between 30% and 60% with possession of one or two ApoE4 variant alleles. The abnormal

processing of the APP, PSEN1, PSEN2 and TAU genes produce a specific pattern of amyloid plaque and NFT deposition in the brain and an associated loss of cholinergic function.

1.5.3 Morphology / Neurodegeneration

There are distinct changes in the brain morphology of AD patients relative to non-demented aged-matched controls. Magnetic resonance imaging (MRI) reveals a significant reduction in cortex, hippocampus and ventricle area volume in AD patients (Islam et al., 2010). Atrophy of the medial temporal lobe (MTL; comprising hippocampus, dentate gyrus, subicular complex, perirhinal, entorhinal and parahippocampal cortex) accurately differentiates demented from non-demented patients, and predicts which MCI subjects will go on to be diagnosed with AD (Clerk et al. 2013). Pennanen et al (2004) has found that hippocampal and entorhinal cortex volume were significantly reduced in AD vs MCI, MCI vs controls and AD vs Controls. The study also showed greater entorhinal volume loss compared to hippocampal in MCI patients and that entorhinal loss preceded hippocampal loss in AD patients. Cortical atrophy is first seen in the temporal lobes, followed by the

frontal and parietal lobes, whilst the occipital and motor cortex remain relatively unaffected (Cvetkovic-Dozic et al., 2001).

1.5.3.1 Inflammation

Brain inflammation also occurs as a result of AD pathology. The inflammatory reaction in the brain is part of a wider immune response to infection or injury, and involves the release of microglia and astrocytes. Microglia become activated on detection of injury, and clear up damaged cells by releasing pro-inflammatory proteins, cytokines, chemokines, prostaglandins, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2). Astrocytes also become reactive in response to central nervous system injury and protect viable neurons by forming scar tissue (McGreer & McGreer 1995; Sofroniew et al., 2005). These reactions occur in response to both Aβ and NFT deposition.

Only a few human studies have measured inflammation levels during early and late stages of the disease. Yasuno et al. (2008) carried out a PET study using the [^{11}CJDAA11006 binding ligand, and showed increased activation of microglia in the prefrontal cortex, temporal, parietal, occipital, anterior cingulate cortex, striatum and cerebellum of mild to moderate AD patients relative to healthy age-

matched controls. Carter et al. (2011) looked at astrocyte levels using PET imaging and the ^{11}C-deuterium-l-deprenyl(^{11}C-DED) agent, in MCI, AD and control patients. Increased astrocyte levels were only observed in the MCI group in the frontal and parietal cortices. Both studies highlight the involvement of neuro-inflammation during the earliest stages of AD, but this research is limited by the ability to measure microglia and astrocyte levels with imaging techniques.

1.5.3.2 Soluble Amyloid

The first major theory to explain the development of AD was the 'amyloid cascade hypothesis' (Hardy et al., 1992). Its main focus was that the development of Aβ plaques drove the development of neuronal atrophy, cholinergic loss and eventually dementia (Hardy et al., 1992). More recently focus has switched to the idea that soluble forms of Aβ drive the development of AD. These are more neurotoxic than their insoluble aggregated counterparts (Shankar et al., 2008), and levels of soluble Aβ correlate much better with cognitive deficits associated with AD than plaque levels (Kuo et al., 1996; Lue et al., 1999; Naslund et al., 2000). Also cognitive impairments in several AD mouse models have been observed before plaques (Holcomb et al., 1999; Hsia et al., 1999; Jankowsky et al., 2005; Muckle et al., 2000;

Pistell et al., 2008; Westerman et al., 2002). This all suggests a strong link between soluble forms of Aβ and the early development of AD.

1.5.4 Summary

AD pathology appears to develop in a specific manner. Amyloid plaques and neurofibrillary tangles develop and deposit in specific brain regions as the disease progresses, eventually resulting in widespread pathology. The loss of cholinergic function and brain atrophy also shows a specific pattern as the disease progresses.

1.6 Animal models of Alzheimer's Disease

This section gives a brief overview of the history behind genetic manipulation of mouse models of disease, before discussing different manipulations that have been employed in AD research.

The first reproducible method for creating lines of genetically altered mice was developed in the early 1980s (Brinster et al., 1986; Gordon et al., 1980; Palmiter et al, 1986), via the direct microinjection of DNA into the pronucleus of a mouse egg. This paved the way for increasingly complex transgenic mouse models (see Hanahan et al., 2007 for a review). The ability to genetically manipulate the mouse genome became of particular interest to

scientists studying the link between genes and disease, particularly hereditary illness. In the field of AD, the identification of the causative APP, PSEN1, PSEN2 gene mutations associated with the familial form of the disease allowed for development of genetic mouse models of FAD. Although so far no single mouse model successfully recapitulates all the different aspects of SAD, models that express different aspects of the disease have been successfully created (Laferla & Green, 2012). Several genetic manipulations have been employed. The most common is the introduction of a foreign gene into the genome, creating mice that express the phenotypic characteristics of the mutant gene. Researchers can also eliminate expression of a protein coded from a gene (knock out, KO) or introduce a new mutation, resulting in altered protein expression (knock in, KI; Mineur et al., 2005).

A large number of transgenic, KI and KO mouse models have been developed to characterise the pathology of AD. The most common transgenic mice possess specific human mutations, taken from families with FAD, in the APP, PSEN1 and PSEN2 genes (Table 1.2).

The following section will discuss the variety of single and double transgenic mouse models of AD, before specifically focusing

on the double transgenic APPswe/PS1dE9 mouse model used in the experiments presented in the later chapters.

1.6.1 APP transgenic mice

The APP transgenic mouse model is the most common genetic manipulation employed in the production of AD transgenic mice (Table 1.2). It is created via the introduction of a mutant form of the human APP gene (hAPP), using the pronuclear injection method (Ittner & Gotz, 2007). This results in overexpression of APP, causing the development of both diffuse and dense amyloid plaques. Plaque deposition is consistently seen in the cerebral cortex and hippocampus (Games et al., 1995; Hsiao et al., 1996; Moechars et al., 1999; Sturchler-Pierrat et al., 1997). These mice also show deficits in cognition, as assessed by a variety of behavioural procedures (see Raslam & Kee, 2013, for a review).

1.6.2 Presenilin transgenic mice

PSEN1 and PSEN2 transgenic mouse models have also been created, based on the causal role of PSEN1 and PSEN2 in FAD. Overexpression of PSEN1 results in minor behavioural problems but no plaque development, while overexpression of PSEN2 results in poor spatial

navigation (Mineur et al., 2005). PSEN1 mice have been created using a conditional knock out (cKO), which allows researchers to turn genes on and off at will. PSEN1/cKO mice show impaired spatial reference memory and a reduction in Aβ production (Dineley et al. 2002, Feng et al. 2001, Mineur et al. 2005, Yu et al. 2001). Knocking out the PSEN2 gene results in no behavioural or pathological abnormalities.

Mouse	*Mutation*	*Plaque Deposition*	*Study*
PDAPP	*APP Indiana*	*Cortex, Hippocampus, Corpus callosum*	*Games et al. (1995)*
Tg2576	*APP Swedish*	*Cortex, hippocampus*	*Hsiao et al. (1996)*
APP23	*APP Swedish*	*Neocortex, hippocampus, thalamus, olfactory nucleus, caudate putamen*	*Sturchler-Pierrat et al. (1997)*
APP/Ld	*APP London*	*Cortex, hippocampus*	*Moechars et al. (1999)*

Table 1.2. The most common APP transgenic mouse models of AD

1.6.3 APP & PS transgenic mice

Separate APP and either PSEN1 or PSEN2 transgenic mice have also been crossed to more accurately reproduce AD pathology. These double transgenic mice show the same pathology and behavioural deficits as the single APP transgenic mouse, but the rate at which these develop is much greater (Borchelt et al., 1997). Borchelt et al. (1997) found that the double transgenic APP (APPswe) + PSEN1 (PS1A246E) mouse showed plaque pathology from around 9 months, compared to 18 months in the single APP (APPswe) mouse, while the double transgenic APP (tg2576) + PSEN1 (PS1A246E) mouse showed plaque deposition at 2 months of age, compared to 8 months for the single APP (tg2576) mouse (see Howlett et al., 2009 for a review).

APP/PS1 mice also show evidence of pre-plaque alterations in hippocampal function. Busche et al (2013) showed that 1-2 month APPswe/PS1G384A expressed elevated rates of spontaneous calcium ion (Ca^{2+}) activity in the CA1 region of the hippocampus. The level of hyperactive neurons was significantly greater than wild types. This early hyperactivity has also been linked to soluble forms of Aβ, as treatment with a ϒ-secretase inhibitor, which reduces soluble Aβ

levels, restored hippocampal activity to normal (also see Davis et al. 2014).

Earlier development of AD pathology in these double transgenic mice allows for faster characterization of the pathological and cognitive development, without being affected by the natural aging of the mouse.

1.6.4 APPswe/PS1dE9 mouse

The mouse model chosen for the experimental chapters within the thesis is the double transgenic (APP/PS) APP Swedish/PSEN1 deleted exon 9 (APPswe/PS1dE9) first created in 2001 by Jankowsky. This model is based on the human APP695 Swedish transgene, which includes the FAD-related mutation K595N/M596L plus deletion of exon 9 on the PSEN1 gene (Jankowsky et al. 2001). There are a number of distinct reasons as to why this particular model was chosen which will be discussed bellow.

The model recapitulates most of the neurological symptoms seen in SAD (Table 1.2.1), including the development and deposition of amyloid plaques, neurodegeneration of specific brain areas and a reactive inflammatory response. The mouse is not a perfect model of SAD, as it does not show development of TAU pathology (NFT).

Extensive research has been carried out into the onset and time course of these symptoms results in a strong understanding of the pathological development of AD symptoms in the model.

Age (months)	Alzheimer's Pathology	Article
2 - 3	Cholinergic swelling in cortex and hippocampus	Volianskis et al. 2010
2 - 4	Hypo-excitability frontal cortex neuron	Kellner et al., 2014
3	Soluble Aβ42/40 in cortex and hippocampus	Volianskis et al., 2010; Garcia-Alloza et al., 2006
3	Reduced hippocampal LTP	Volianskis et al. 2010
3	Reduced hippocampal LTP	Vegh et al 2014
4	Small number of Aβ plaques – cortex and hippocampus	Garcia-Alloza et al., 2006; Jankowsky et al., 2004
4	Increased microglia in cortex and hippocampus	Ruan et al. 2009
5 - 6	Cholinergic swelling in striatum	Machova et al. 2010
6	Impaired CA3 LTP	Viana Da Silva (2016)
6	Larger numbers of Aβ plaques – cortex and hippocampus	Garcia-Alloza et al., 2006; Jankowsky et al., 2004
6	Increased astrocytes in cortex and hippocampus	Zhang et al., 2012
7	Muscarinic transmission impaired	Machova et al. 2008
9-18	Hyper-excitability frontal cortex neuron	Kellner et al., 2014
12	Cortex and hippocampus neuro-degeneration	Liu et al. 2008
13	Increased hippocampal NMDA binding	Metaxas et al. (2012)

Table 1.2.1. Shows the development of Alzheimer's pathology in the APPswe/PS1dE9 mouse by age.

1.6.4.1 Plaques

The APPswe/PS1dE9 model shows greater early production and deposition of Aβ than other single APP and APP/PS1 models. Increased levels of soluble Aβ42/40 can be detected in these mice by 3 months of age and these levels rise with age (Volianskis et al., 2010; Garcia-Alloza et al., 2006). Aβ plaques then begin to appear in the cortex and hippocampus between 4 - 6 months (Garcia-Alloza et al., 2006; Jankowsky et al., 2004). A small number of plaques are detectable at 4 months; however by 6 months plaques are easily observed. APPswe/PS1dE9 mice show a higher ratio of Aβ42 to Aβ40 (.75/1) compared to other AD mouse models, which also helps to increase the plaque burden.

1.6.4.2 Morphology

Neurodegeneration also occurs in this mouse model. At 4 months of age APPswe/PS1dE9 mice show normal levels of forebrain monoaminergic axon density, but from 12 months onwards these axons show increased degeneration, which correlates with Aβ plaque

load. All the monoaminergic neurons that project to both cortex and hippocampus appear to undergo some level of neurodegeneration by 12 months, and neuronal death appears from 18 months onwards (Liu et al. 2008). Cholinergic neurons appear to stay intact throughout disease progression, but transmission is affected (Perez et al. 2007). Machova et al. (2010) found increased Ach release in cortex, but a reduction in both hippocampus and striatum, whilst Ach synthesis was reduced in cortex and hippocampus but remained unchanged in the striatum.

Neurodegeneration is also evident in the hippocampus as impaired synaptic transmission, plasticity and neurogenesis. Volianskis et al. (2010) found a reduction in amplitudes of transient long-term potentiation (t-LTP) in the hippocampus of 3-month-old APPswe/PS1dE9 mice. LTP reflects an increase in synaptic strength as a result of persistent stimulation and plays a role in learning and memory (Volianskis et al., 2010). Vegh et al (2014) has also shown evidence of impaired hippocampal LTP in 3-month-old APPswe/PS1dE9 mice compared to wild types. By 6 months of age Viana Da Silva (2016) and colleague have shown that

APPswe/PS1dE9 mice show impaired synaptic plasticity in the CA3 region of the hippocampus, via a disruption in LTP.

In addition intracellular recordings of neurons in the frontal cortex in young mice (2-4 months) indicated a reduction in excitability, thought to reflect the increase in damaged neurons. Older (9-18 months) mice showed hyper-excitability in response to the electric stimulation, thought to represent an attempt to compensate for damaged synapses (Kellner et al., 2014).

1.6.4.3 Inflammation

Ruan et al. (2009) reported an increased inflammatory response in the APPswe/PS1dE9 mouse, including higher levels of microglia in hippocampus and cortex from 4 months, and positive astrocytes in the same areas from 6 months onwards (Zhang et al., 2012). Microglial and astrocyte clusters appeared around amyloid plaque deposits, highlighting their involvement in clearing the dispositions . They also observed activated inflammatory proteins from 8-10 months, and a significant increase in reactive astrocytes in 6 and 12-month mice. Only a few microglial cells were detected in 3.5-month-old mice; however, the levels increased at 6 and 12-months in the transgenic mice but remained unchanged in the controls. In addition

significantly higher levels of the inflammatory proteins IL-1B, IL-6, TNFa and PGE2, were observed in 3.5-month-old mice and increased with age (Zhang et al. 2012).

1.6.5 General summary

There are a number of practical reasons for using the APPswe/PS1dE9 mouse model. The animals used in the experimental chapters are an inbred strain of the APPswe/PS1dE9 mouse, initially purchased from the Jackson laboratories, before being maintained by the University of Nottingham's transgenic unit. The inbred nature of the strain increases the level of genetic uniformity. Secondly, transgenic mice are maintained on the C57BL/6 genetic background, which is a very reliable background strain for behavioural experiments, which allows for much more complex learning and memory tasks to be carried out.

Due to the early development of AD pathology in the model, the wild type controls will be reasonably young and not affected by cognitive and physical impairments caused by nature aging, affecting comparisons between genotypes. Finally, the fast pathological development also reduced the financial cost of running transgenic

animal experiments, via reducing breeding and husbandary time between experimental groups.

Many different transgenic models of AD have been developed, ranging from single to multiple mutations. The APPswe/PS1dE9 model used in this thesis was chosen due to its ability to model most of the pathological hallmarks of AD seen in humans.

1.7 Behavioural and Cognitive Impairments

This section will summarise the wide variety of tasks used to examine cognition and behaviour in mouse models of AD, before reviewing the literature on those tasks in the APPswe/PS1dE9 model. It is worth noting that some of the tasks used in animal research differ vastly from the techniques employed to measure behavioural and cognitive impairments in humans with AD, which usually involve language comprehension (e.g. the tasks used in measures of semantic and episodic memory described above). Animal tasks typically assess the ability of transgenic animals to navigate around an environment or their preferences for a newly encountered object as measures of memory performance.

This section will be divided into cognitive and innate measures of behaviour. Innate tasks measure spontaneous animal behaviours, such as anxiety, hyperactivity or perception, whilst cognitive tasks

are those that measure learning, memory, executive function and attention. The cognitive tasks will be subdivided into those that measure spatial information and those that do not.

Spatial navigation tasks have been extensively used to examine the cognitive decline observed in mouse models of AD, and are often the only type of task employed. Spatial navigation procedures involved the use of specific apparatus, including the Morris water maze (MWM), radial arm water maze (RAWM) and the Barnes maze. This emphasis on spatial tasks is in part due to the strong relationship between hippocampal lesion and impaired spatial navigation (Broadbent et al., 2006; Morris et al., 1982; for review see Clark et al., 2005), and the progressive damage to the hippocampus during the development of AD (Parslow et al., 2004; Risacher et al., 2010; for a review see Vicek & Laczo, 2014). Non-spatial tasks are not dependent on navigation, and include novel object recognition and Pavlovian conditioning tasks.

1.7.1 Innate behaviours

1.7.1.1Spontaneous Alternation

Spontaneous alternation tasks in a T- or Y-maze exploit rodents' innate tendency to alternate their arm entries on successive trials. They have been used as a measure of spatial working memory as

remembering which arm has just been entered is necessary to successfully alternate the next choice (Hughes, 2004). Performance can also be explained in terms of novelty preference, as rodents' innate preference for novelty will allow them to successfully alternate between the maze's arms. The basic continuous spontaneous alternation (CSA) task allows the rodent free access to the maze for a period of time and the sequence of arms entered is recorded. In the two-trial spontaneous alternation (2TSA) task the rodent is placed in the start arm and only allowed access to one other arm during the learning phase. It is then removed for a period of time before being replaced in the start arm and given access to the remaining arms (test phase); the first arm entered is used as a measure of performance; rodents should show a preference for entering the previously unexplored arm.

1.7.1.1.1 APPswe/PS1dE9 mouse

No differences have been observed in spontaneous alternation performance in the APPswe/PS1dE9 mouse over a wide range of ages in comparison to controls (Table 1.3) (Arendash et al., 2001; Bonardi et al., 2011; Frye et al., 2008; Lalonde et al., 2005; Maroof et al. unpublished; Park et al., 2010; Pietropaolo et al., 2012; Reiserer et al., 2007).

Spontaneous alternation in the APPswe/PS1dE9 mouse			
Age (months)	*Maze*	*Test*	*Study*
4	*T*	*I*	*Bonardi et al., (2011)*
4	*T*	*I*	*Maroof et al. (2014)*
5-7	*Y*	*I*	*Arendash et al. (2001)*
6		*I*	*Pietropaolo et al. (2012)*
6	*T*	*I*	*Maroof et al. (2014)*
7	*Y*	*I*	*Reiserer et al. (2007)*
8	*Y*	*I*	*Park et al. (2010)*
8	*T*	*X*	*Maroof et al. (2014)*
9	*T*	*I*	*Frye et al. (2008)*
12	*T*	*I*	*Lalonde et al. (2005)*
15-17	*Y*	*I*	*Arendash et al. (2001)*

Table 1.3. Spontaneous alternation results in APPswe/PS1dE9 mice. A blank cell = not measured, X = impairment, I = intact

1.7.1.2 Exploration (Open-Field (O-F))

The open-field task has been used as a measure of activity, exploration and anxiety (Walsh & Cummins, 1976) and is used to categorize the effects of lesions, drug treatments and genetic manipulations. A rodent is placed in an open-field and allowed to

explore the apparatus. Locomotor activity is seen as a measure of activity and exploration. The amount of time spent in the middle versus the edges of the open-field is used as a measure of anxiety, with greater time in the edges reflecting higher anxiety levels.

1.7.1.2.1 APPswe/PS1dE9 mouse

APPswe/PS1dE9 mice generally show greater locomotor activity (Table 1.4) than controls in O-F studies. Armstrong et al. (unpublished) and Maroof et al (2014), using the same apparatus, showed that 4, 6 and 8-month APPswe/PS1dE9 mice are hyperactive. Maroof et al. (2014) also showed no difference in exploration of the centre or periphery of the apparatus. Interestingly both Lalonde et al. (2004) and Arendash et al. (2001) found no difference in locomotor activity between 5 and 7 months of age in the APPswe/PS1dE9 mice, but did show hyperactivity at 12 and 15-17 months respectively, whilst Park et al. (2010) and Lim et al. (2011) also showed no difference in activity at 8 or 10 months of age.

Open – Field			
Age (months	*Time (mins)*	*O-F Activity*	*Study*
4	*10*	*Increase*	*Armstrong et al. (unpub)*
4	*30*	*Increase*	*Maroof et al. (2014)*
5-7	*5*	*I*	*Arendash et al. (2001)*
6	*30*	*Increase*	*Maroof et al. (2014)*
7	*5*	*I*	*Lalonde et al. (2004)*
8	*5*	*I*	*Park et al. (2010)*
8	*30*	*Increase*	*Maroof et al. (2014)*
10	*5*	*I*	*Lim et al. (2011)*
12	*5*	*Increase*	*Lalonde et al. (2005)*
15-17	*5*	*Increase*	*Arendash et al. (2001)*

Table 1.4 Results of Open-field activity in the APPswe/PS1dE9 mouse model. A blank cell = not measured, I = intact, Increased = increased activity in the APPswe/PS1dE9 mouse.

1.7.1.3 Ataxia

Ataxia is the loss of bodily control or movement, and is measured in rodents in tasks including the stationary beam (SB) task, the wire-hang (W-H) task and the accelerated rotarod (ARR) task. These assess a number of normal abilities such as balance, grip strength and gait. The SB task measures a mouse's ability to walk across a thin stationary beam placed above the ground. Measures include distance travelled, latency before first fall and number of falls. The W-H task

involves placing a mouse on a thin wire; the amount of time spent hanging measures global muscle function and coordination. Finally the ARR involves placing a mouse on a rotating plastic beam, which is raised above the ground. The mouse is required to maintain balance and position by walking forward along the beam. The rotarod rotates increasingly quickly and the time taken to fall off is recorded. Performance on these tasks is used in combination to assess whether or not mice show any ataxia.

1.7.1.3.1 APPswe/PS1dE9 mouse

Both Lalonde et al. (2004) and Lim et al. (2012) assessed the activity/movement of 7 and 10-month APPswe/PS1dE9 mice in these tasks. Lalonde et al. (2004) found no movement impairments in the SB, ARR and W-H tasks. Lim et al. (2012) found that APPswe/PS1dE9 mice showed shorter walking distances than controls on the stationary beam but no other deficits in SB measures. No impairments were seen in the ARR or W-H tasks in 10-month APPswe/PS1dE9 mice. The results show that APPswe/PS1dE9 mice show no real movement impairments and have intact motor function and coordination.

1.7.1.4 Elevated Plus Maze (EPM)

The elevated plus maze (EPM) is a simple apparatus that is used to measure anxiety in rodents. It is made up of four arms arranged in a plus shape and elevated above the ground. Two of the arms are enclosed with sidewalls whilst the other two arms have none. The task relies on rodents' innate anxiety of both height and open spaces (Walf & Frye, 2007). Rodents are placed onto an open arm and allowed to explore the apparatus. The number of entries, time spent freezing, time spent in open and enclosed arms, defecation are recorded and analyzed. The best measure of anxiety is a ratio of time spent in the open versus closed arms, this measure is the most effective at eliminating locomotor confounds. An appropriate anxiety response would produce a significant preference for the enclosed arms, as well as freezing and defecation in the open arms.

1.7.1.4.1 APPswe/PS1dE9 mouse

Arendash et al. (2001) carried out the EPM task using 5-7 and 15-17-month-old APPswe/PS1dE9 mice (Table 1.5). They found no genotype difference at any age, using the number of open and closed arm entries and the time spent in the open arm as measures of anxiety. Lim et al (2012) also found no genotype effect of the EPM tasks, but did use the ratio measure of anxiety. However, Lalonde et

al. (2004, 2005) found that both 7 and 12-month transgenic mice spent significantly more time in the open arms, and made more open arm entries than controls, indicating decreased anxiety; see also Reiserer et al., 2007, who found reduced anxiety in 7 month mice using the ratio measure of anxiety. In contrast Chen et al. (2013) found greater levels of anxiety in 7-month APPswe/PS1dE9 mice, who spent less time in the open arms and made fewer open arm entries than controls. These inconsistencies suggest more research is required to fully understand the performance of APPswe/PS1dE9 mice on the EPM task, and is most likely due to methodological differences in carrying out the procedure.

Age (months)	*Anxiety*	*Study*
5-7	*I*	*Arendash et al. (2001)*
7	*Decreased*	*Lalonde et al. (2004)*
7	*Decreased*	*Reiserer et al. (2007)*
7	*Increased*	*Chen et al. (2013)*
10	*I*	*Lim et al. (2012)*
12	*Decreased*	*Lalonde et al. (2005)*
15-17	*I*	*Arendash et al. (2001)*

Table 1.5. Results of elevated-plus maze in the APPswe/PS1dE9 mouse.

I = no genotype difference in anxiety, Decreased = decreased anxiety in the APPswe/PS1E9 mouse, Increased = increased anxiety in the APPswe/PS1dE9 mouse.

1.7.1.5 Summary

APPswe/PS1dE9 show normal performance on spontaneous alternation and ataxia measures, whilst there is evidence of hyperactivity relative to wild type mice. Normal body function, movement and coordination are a prerequisite for more advanced measures of cognitive performance, so this provides a solid base for assessing performance on more complex cognitive tasks.

1.7.2 Cognitive - Spatial tasks

1.7.2.1 Morris water maze (MWM)

The Morris water maze (MWM; Morris et al., 1981) consists of a circular pool half filled with water. The inside of the maze is designed to be featureless, and a small platform (hidden beneath the surface of the water) is placed in the pool. The animals must navigate a path to the hidden platform using distal cues around the pool (training). The water is usually made opaque to camouflage the platform (Morris et al., 1981; 1982; Vorhees & Williams, 2006). The MWM task exploits

the fact that rodents readily try to escape onto the platform. After training a probe trial is often added in which the platform is removed (D'Hooge & De Deyn, 2001; Morris et al., 1982, Vorhees & Williams, 2006). The standard procedure usually consists of 5 training days each comprising 4 trials, followed by a probe trial on the last day. The hidden platform is usually moved to a new location on each training day. The procedure can, however, vary in the number of trials per day, training days, trials per platform location and probe trials. A visible platform test should also be carried out alongside the standard task, to assess the visual acuity of the animals and their swimming ability. This involves placing a visual marker on the hidden platform, which the mice can use to navigate. During acquisition of both tasks performance is based on the time it takes the animal to reach the platform (escape latency). Better performance is associated with faster escape latencies. For the probe trial in the standard procedure, the pool is divided into four quadrants and the amount of time the rodents spent swimming in each is recorded. Greater time in the target quadrant, where the hidden platform was during training, compared to the remaining quadrants reflects good performance.

1.7.2.1.1 APPswe/PS1dE9 mouse

The standard version of the MWM has been used extensively in the APPswe/PS1dE9 mouse (Table 1.6). Unless otherwise stated all experiments discussed were performed using the standard procedure defined above. Zhang et al. (2012) used 3.5-month-old APPswe/PS1dE9 mice, and observed no genotype differences during training; however, transgenic mice showed reduce time in the target quadrant compared to wild types mice during the probe trial. Edwards et al. (2014) carried out a modified version of the task with 10 training days, and probe trials, after training, only on days 4, 7 and 10. They found greater latencies to find the hidden platform in APPswe/PS1dE9 mice aged 9.3 and 14.8 months, but not 3.6 months. They also found reduced time in the target quadrant, compared to wild types, during the probe trials at 9.3 and 14.8 months, but not 3.6 months. All mice regardless of age showed normal performance on the visual acuity task. Lalonde et al. (2005) and Savonenko et al. (2004) used 12, 6 and 18-month-old APPswe/PS1dE9 mice and found significantly slower escape latencies during the learning trials and impaired performance on the probe trials in 12 and 18-month APPswe/PS1dE9 mice. They found no genotype differences on the visual platform task, suggesting this difference between genotypes was the result of a cognitive impairment. In Savonenko et al. (2004)'s

version of the task 10 training trials were administered per day; they paralleled results in 6-month-old transgenic mice, showing no genotypes differences in performance. Jankowsky et al. (2005) and Frye et al. (2005) used 8-month and 12-month-old APPswe/PS1dE9 mice, and both showed significantly longer escape latencies than controls during the training phase, whilst Jankowsky et al. (2005) also found more errors during the probe trial. Stover & Brown (2012) found that 20-26-month-old APPswe/PS1dE9 mice were impaired during the classic MWM procedure, showing longer escape latencies than wild types. However, they also found impaired performance on the visual platform version of the task in these animals, suggesting impaired visual acuity or swimming performance in the older transgenic mice could be responsible for the apparent spatial deficit. The results suggests that the spatial learning deficit is more likely to be due to either their impaired vision or swimming performance than their memory for the platform location.

In summary, performance on the MWM task appears to be consistently impaired in the APPswe/PS1dE9 mouse from around 6 months of age.

Morris Water Maze			
Age (months)	*Impairment*		*Study*
APPswe/PS1dE9	*Training*	*Probe*	
2.5	*I*	*I*	*Zhang et al. (2012)*
3.5	*I*	*X*	*Zhang et al. (2012)*
3.6	*I*	*I*	*Edwards et al. (2014)*
5-7	*I*	*I*	*Arendash et al. (2001)*
6	*I*	*I*	*Savonenko et al. (2004)*
7.5	*X*		*Jeon et al. (2011)*
8	*X*	*X*	*Jankowsky et al. (2005)*
8	*I*	*I*	*Park et al. (2010)*
9	*X*		*Frye et al. (2008)*
9.3	*X*	*X*	*Edwards et al. (2014)*
12	*X*	*X*	*Lalonde et al. (2005)*
14.8	*X*	*X*	*Edwards et al. (2014)*
15-17	*X*	*I*	*Arendash et al. (2001)*
18	*X*	*X*	*Savonenko et al. (2004)*
20-25	*X*	*I*	*Stover et al. (2012)*

Table 1.6. The table shows experiments involving the APPswe/PS1dE9 mouse and the spatial navigation MWM. A blank cell = not measured, X = impairment, I = intact.

1.7.2.2 Radial arm water maze (RAWM)

The radial arm water maze (RAWM) is a modified version of the radial arm maze (RAM). It comprises a radial arm maze, constructed from a central platform with eight horizontal arms radiating around

the center (Olton and Samuelson 1976), into a circular pool, similar to the MWM. The rodent is placed into one of the arms (start arm); four of the remaining arms contain a submerged platform at the end, and the rodent has to navigate to each of the arms containing a platform. After it reaches the platform the trial is terminated, and both rodent and platform are removed from the maze. The task usually consists of four trials per day, for 11 consecutive days. After each day the position of the platforms between the arms changes. The rodents must avoid making reference memory errors (entering an arm which has never contained a platform) and also working memory errors (re-entering an arm which previously contained a platform) (Hyde et al. 1998). The RAWM may also employ a probe trial, in which the rodent must navigate to the platform positions from the last test day; however, all platforms have been removed. The time spent exploring the arm in which a platform was previously presented and the latency to reach the platform is used as a measure of memory. A visible platform version can also be carried out to exclude any nonspecific difficulties in performing the task. The standard task can be modified, changing the number of arms, platforms, trials, training days and altering the inter-trial interval

(Clements et al., 2007; Gresack & Frick 2003; Hyde et al., 1998; Penley et al., 2013; Shukitt-Hale et al., 2004).

1.7.2.2.1 APPswe/PS1dE9 mouse

A few studies have examined the effect of age in the APPswe/PS1dE9 mouse on the RAWM task. Unless otherwise stated all experiments discussed were performed using the standard procedure defined above.

Savonenko et al. (2005), using a variation of the classic RAWM, found that 18-month-old APPswe/PS1dE9 mice made more reference memory errors than controls over 4 consecutive days. On days 2-4 the number of working memory errors, relating to the previous days target arms, on the first trial were used as a measure of long-term memory. Wild types demonstrated working memory errors implying a long-term memory for the position of the platforms on the previous day. Transgenic mice performed at chance, suggesting impaired long-term memory. Xiong et al. (2011) carried out a similar variation of the RAWM to Savonenko et al. (2005) and found that both 6-month and 12-month-old APPswe/PS1dE9 mice took longer to find the platform than controls. 6 and 12-month transgenic mice also made more reference memory errors than controls. Arendash et al. (2001) only found longer escape latencies

for 15-17-month-old transgenic mice than controls during a test trial, carried out 30 minutes after the last acquisition trial.

1.7.2.3 The Barnes Maze

The Barnes maze (Barnes 1979) takes advantage of the fact that rodents tend to avoid brightly lit, open spaces. It is a large circular platform with 18 holes around the edge; an enclosed dark box is attached to the bottom of one of the holes, and the platform is lit with bright lights to induce a need to escape. Extra-maze cues are placed around the maze, which can be rotated to change the relationship between the escape box and extra-maze cues. During testing a rodent is placed in the middle of the maze and given time to successfully escape into the dark box. Latency to escape is used as a measure of performance, whilst escape errors (nose/head/neck entrance into an incorrect hole) are also recorded. A probe trial can also be administered in a similar fashion to the MWM, during which the rodents are required to escape the apparatus, just as in acquisition; however, the escape holes have been removed. The standard protocol typically comprises two trials per day for a period of six consecutive days (Barnes 1979). The task can be altered in a number of ways, for example, number of trials per day and number of days.

1.7.2.3.1 APPswe/PS1dE9 mouse

All experiments presented employed the generic procedure above unless stated. O'Leary & Brown (2009) conducted the standard procedure but increased the number of test days to fifteen consecutive in 16-month-old APPswe/PS1dE9 mice and controls. The transgenic mice showed longer escape latencies and made more hole entry errors than controls in acquisition, suggesting an impairment in spatial learning. The APPswe/PS1dE9 mice also spent less time in the correct escape area than controls during the single probe trial, suggesting possible memory impairment.

Reiserer (2007) conducted two versions of the Barnes maze, the conventional task and a cued version, in 7-month-old transgenic mice. The cued version differed from the conventional task by having an intra-maze cue next to the target hole. When the conventional task was carried out first, no genotype differences in escape latency or hole entry errors were found in either version; but if the cued version was performed first, transgenic mice made more hole entry errors than controls in the conventional, but not the cued, task. They argued that this could be because the cued-version is easier to perform, and APPswe/PS1dE9 mice failed to adjust their search strategy to the harder conventional version.

1.7.2.4 Summary

APPswe/PS1dE9 mice show reliable impairments in spatial navigation between the ages of 6-18 months, specifically in the MWM and RAWM, which is consistent with the literature showing impaired spatial navigation performance on these tasks following hippocampal lesion (Crusio et al., 2005; Fox et al., 1998; Morris et al., 1982; Sutherland et al., 1983).

The mostly likely source of the spatial impairments is the considerable deposition of amyloid plaques within the hippocampus starting at 6 months of age, and the associated impaired function of the hippocampus (Garcia-Alloza et al., 2006; Hu et al., 2010; Jankowsky et al., 2004; Shemer et al., 2006 Volianskis et al., 2010).

Performance in the probe trial during the MWM also suggests some tentative support for impaired performance at 3.5 months of age (Zhang et al. 2012) in the APPswe/PS1dE9 mouse, prior to amyloid plaque development.

1.7.3 Cognitive - Non-Spatial tasks

1.7.3.1 Spontaneous Novel object recognition (sNOR)

The spontaneous novel object recognition (sNOR) task is a relatively simple task that has been used extensively to measure recognition

memory. It takes advantage of the innate preference of rodents to explore novel rather than familiar stimuli. The standard experiment involves a pre-exposure phase in which animals are exposed to two identical objects inside the apparatus. After a retention interval, the animals are placed back in the apparatus and presented with one familiar object from the pre-exposure phase and a novel object. Animals should show a greater amount of exploration of the novel object (Ennaceur & Delacour, 1988). The duration of the retention interval can be varied to increase task difficulty. A novel object preference has been successfully demonstrated in rodents with a retention interval of 8 weeks (Broadbent et al., 2009).

1.7.3.1.1 APPswe/PS1dE9 mouse

sNOR has been assessed in the APPswe/PS1dE9 mouse (Table 1.7) across its lifespan. Impaired performance has been consistently observed from 10 months onwards (Donkin et al., 2010; Jardankhazi-Kurutz et al., 2010; Li et al., 2014; Yan et al., 2009 but see Barbero-Camps et al., 2014; Frye & Walf, 2008), whilst impaired performance has only occasionally been observed between 6 – 8 months (Cheng et al., 2014; Pedros et al., 2014). No impairments have been found in mice younger than 6 months (Bonardi et al., 2011; Lin et al., 2015; Pedros et al., 2014).

Impairments in sNOR are dependent on the perirhinal cortex and to some lesser extent the hippocampus (for a review see Warburton & Brown 2015); both of which are effected during the development of the disease. Laakso et al (2000) has shown a correlation between the level of hippocampal volume loss and performance on a delay recall tasks in patients with a diagnosis of probable AD.

Further research is currently required to assess any histological differences in perirhinal function during the development of AD in the APPswe/PS1dE9 mouse.

Novel Object Recognition			
Age (months)	*Interval*	*Test*	*Study*
2	*2 hours & 24 hours*	*I*	*Lin et al., 2015*
3	*2 hours*	*I*	*Pedros et al. (2014)*
3	*2 hours & 24 hours*	*I*	*Lin et al., 2015*
4	*2 hours & 24 hours*	*I*	*Lin et al., 2015*
4	*1 hour*	*I*	*Bonardi et al. (2011)*
4	*1 hour*	*I*	*Jardanhazi-Kurutz et al. (2010)*
6	*2 hours*	*X*	*Pedros et al. (2014)*
6	*1 hour*	*I*	*Jardanhazi-Kurutz et al. (2010)*
7	*1 hour*	*I*	*Barbero-Camps et al. (2013)*
8	*1 hour*	*X*	*Cheng et al. (2014)*
9	*-*	*I*	*Frye et al. (2009)*
10	*4 hours*	*X*	*Donkin et al. (2010)*
12	*-*	*I*	*Frye et al. (2009)*
12	*1 hour*	*X*	*Jardanhazi-Kurutz et al. (2010)*
12	*-*	*X*	*Yan et al. (2013)*
23	*10 minutes / 24 hours*	*X*	*Li et al. (2014)*

Table 1.7. Novel object recognition summary results in the APPswe/PS1dE9 mouse.. A blank cell = not measured, X = impairment, I =intact.

1.7.3.2 Pavlovian conditioning

Pavlovian conditioning has been used to investigate learning and memory in a variety of species. Conditioning tasks can vary in a

number of different respects, including CS type, duration and US type and duration. The following review will be split based on US type: aversive or appetitive.

1.7.3.2.1 Aversive - Conditioned taste aversion (CTA)

During a conventional CTA task (Garcia et al. 1955), rodents are placed onto a water deprivation schedule before being given free access to water from a graduated bottle for a fixed time each day for several days to establish baseline levels of water consumption. During acquisition, rodents have access to a saccharin solution for a limited period, before receiving an intraperitoneal (i.p) injection of LiCl, a nauseating agent, some time later. On the following test days the animals are given access to the saccharin. The amount of saccharin consumed, or preference of saccharin over water in a two-bottle test, is used as a measure of learning (Nunez-Jaramillo et al., 2012).

1.7.3.2.1.1 APPswe/PS1dE9 mouse

Pistell et al. (2008) gave mice free access to water (habituation) for six days in two bottles for 7 hours a day, during which consumption was measured during the first 30 minutes. On day seven the mice were given access to 0.5% saccharin solution for 30 minutes in one of

the bottles, and one hour later received an i.p injection of 0.14 ml of LiCl. On day 8 all animals received a habituation session, and on day 9 a two-bottle choice test with free access to both water and the saccharin solution. At test neither 2-5 month nor 10-17 month APPswe/PS1dE9 mice showed a difference in consumption of water and saccharin. The wild type controls in both age groups, showed a significant avoidance of the saccharin solution.

In a study by Ramírez-Lugo et al. (2009) APPswe/PS1dE9 and wild type mice received restricted access to water, 60 minutes at noon and 30 minutes five hours later, for 10 consecutive days. On the conditioning day they were given 0.3% saccharin solution during the 60-minute noon drinking period, followed by an i.p. injection of LiCl (0.3 milliliters), twenty minutes later. Two further habituation days were followed by six consecutive test days with free access to saccharin during the noon drinking period. 3-4 month APPswe/PS1dE9 mice showed no difference in the level of CTA during test sessions. 7-8 month APPswe/PS1dE9 mice showed greater saccharin consumption than controls during test sessions 3 and 4. 15-16 month APPswe/PS1dE9 mice also showed significantly greater saccharin consumption during test sessions 1 – 4.

Stover & Brown (2012) placed 5-8 and 20-26 month APPswe/PS1dE9 mice on a water restriction schedule for four days, in which they had access to two water bottles for 8 hours. During acquisition the animals were given free access to 0.5% saccharin solution for 30 minutes before immediately receiving an i.p. injection of 0.14 ml LiCl . Two days later the animals received a 30-min test of free access to both water and saccharin. They found normal CTA during test regardless of genotype.

Although both Pistell et al. (2008) and Ramirez-Lugo et al. (2009) found genotype differences in the CTA task, Stover & Brown (2012) did not. This discrepancy could be due to differences in procedure. Both Ramirez-Lugo et al. (2009) and Pistell et al. (2008) used a trace conditioning design, as there was an interval between the saccharin consumption (CS) and the injection (US). This is a less effective conditioning procedure than Stover & Brown's (2012) delay conditioning design in which the LiCi (US) immediately followed saccharin exposure. It could be argued that delay CTA is intact, whilst trace CTA is impaired in the APPswe/PS1dE9 mouse. The discrepancy between the intact CTA in 3-4 month (Ramirez-Lugo et al. 2009) and impaired CTA in 2-5 month (Pistell et al. 2008) APPswe/PS1dE9 mice could be due to the longer trace interval (1

hour) used by Pistell et al. (2008), which would have increased the task difficulty. Deficits in conditioning may be easier to detect using trace procedures in the APPswe/PS1dE9 mouse.

1.7.3.2.2 Aversive - Contextual fear conditioning

Contextual fear conditioning (CFC) is also used to assess Pavlovian conditioning. CFC involves placing a rodent into a conditioning chamber (CS), which is then paired with a foot shock (US). The animal then receives 1 or more test trials on consecutive days, in which it is returned to the test chamber without presentation of shock. The rodent quickly shows a freezing CR during the CS. Freezing during the conditioning and test trials may be used to measure the strength of the CS-US association, and the rate of extinction of the CR can also be assessed from freezing during the test trials. The CFC task can be varied in a number of ways, including the magnitude and number of foot shocks, and the number of acquisition or test sessions.

1.7.3.2.2.1 APPswe/PS1dE9 mouse

APPswe/PS1dE9 mice, regardless of age, show normal acquisition (Table 1.8) in the CFC task (Bonardi et al., 2011; Chen et al., 2013;

Jacobsen et al., 2006; Kilgore et al., 2010; Lin et al., 2015; Maroof et al., 2014; Park et al., 2010; Vegh et al., 2014).

Using similar procedures only four studies have found a deficit in conditioned freezing, in the transgenic mice, during the first test session compared to wild types. Vegh et al., (2014) placed 3-4 month APPswe/PS1dE9 mice into a conditioning chamber for 180s before administering a single 2s foot shock (0.7 mA). The following day the mice were returned to the chamber for a 180-s test session; APPswe/PS1dE9 mice showed less freezing during this session than control mice. Lin et al. (2015) placed 2, 3 and 4-month APPswe/PS1dE9 mice into a conditioning chamber for 2 minutes, before a 10-s 72-dB white noise was presented, co-terminating with a foot shock (0.4mA). The noise-shock pairing was repeated 90s later. Animals were tested in the chamber 30 minutes (test session 1) and 24 hours (test session 2) after conditioning. 4 month APPswe/PS1dE9 mice showed lower levels of freezing than wild types, but only during the first test session. Several other studies have, however, failed to show any differences in CFC in mice between 2 and 4 months (Jacobsen et al., 2006; Kilgore et al., 2010; Lin et al., 2015; Maroof et al., 2014).

In older mice both Jacobsen et al., 2006 and Kilgore et al., 2010 found deficits (but see, Chen et al., 2013; Maroof et al., 2014; Park et al., 2010). Jacobsen et al., (2006) used 5-month APPswe/PS1dE9 mice, a 5-minute conditioning session and two 1.5 mA foot shocks, while Kilgore et al. used 6-month old mice, a 2.5-minute conditioning session and a 2-s, 0.75 mA shock. Both studies carried out the test session 24 hours later and found less freezing in the transgenic mice than wild types.

The different CFC results in the APPswe/PS1dE9 mice could be explain in terms of learning during the CFC task its self. There is evidence that there is differential involvement of the hippocampus in rapid (one-trial) learning versus incremental conditioning. Rodents with pre-training hippocampal lesions show impaired contextual fear following a single CS-US pairing (one-trial learning), but show normal contextual fear compared to shams when they received multiple (three) CS-US pairings (incremental) (Wiltgen et al., 2006; see also Lehmann et al., 2009).

The four studies that found reduced contextual fear during test carried out either single CS-US pairings (Kilgore et al. 2010; Vegh et al., 2014) or two pairings (Jacobsen et al., 2006; Lin et al., 2015) more likely reflecting rapid one-trial learning, dependent upon the

hippocampus. Whilst studies that carried out multiple (3-10) CS-US paring found no reduced freezing in the APPswe/PS1dE9 mice (Bonardi et al., 2011; Maroof et al., 2014; Park et al. 2010).

Between the ages of 2-4 months this rapid versus incremental argument is not completely correct as multiple studies using single or double CS-US pairings found no genotype differences (Jacobsen et al., 2006; Kilgore et al., 2010; Lin et al., 2014), but this could be confounded by the low levels of AD pathology. Between the ages of 6 – 10 months the argument seems more plausible as only one study using two CS-US pairings (Chen et al., 2013) failed to show reduced freezing in the APPswe/PS1dE9 mouse compared to wild types.

Interestingly two separate studies carried out in the same laboratory found an opposite pattern of results. Bonardi et al. (2011) placed 4-month-old APPswe/PS1dE9 mice into a conditioning chamber for 60s, before administering six (1s, 0.4mA) foot shocks each separated by 60s. The animals were tested 24 (test 1) and 48 (test 2) hours later. Bonardi et al. (2011) found a reduction in freezing, in the wild types, compared to the APPswe/PS1dE9 mice, during the second test session. Maroof et al. (2014) carried out an almost identical replication, with 10 shocks in total, in 4, 6 and 8-

month APPswe/PS1dE9 mice, but found no differences in freezing between genotypes at 4-months, unlike Bonardi et al. (2011), but did replicate their findings at 8 months.

Maroof et al (2014) gave more CS-US pairings during training (10) compared to Bonardi et al (2011) (6 pairings). The increased number of pairings may well have caused a very strong association that could have hidden the more subtle, possible rapid learning, impairment in the young APPswe/PS1dE9 mice.

Further experiments in the same age cohorts need to be carried out to establish any difference in contextual fear conditioning using both a rapid one-trial and incremental multiple trial CFC procedures.

Contextual fear conditioning and extinction				
Age (months)	*mA-Shock (N)*	*Test 1*	*Test 2*	*Study*
2	*1.5 (2)*	*I*		*Jacobsen et al. (2006)*
2	*0.4 (2)*	*I*	*I*	*Lin et al. (2015)*
3	*0.4 (2)*	*I*	*I*	*Lin et al. (2015)*
3-4	*0.7 (1)*	*Reduced*		*Vegh et al. (2014)*
4	*0.4 (2)*	*Reduced*	*I*	*Lin et al. (2015)*
4	*0.4 (6)*	*I*	*Increased*	*Bonardi et al. (2011)*
4	*1.5 (2)*	*I*		*Jacobsen et al. (2006)*
4	*0.75 (1)*	*I*		*Kilgore et al. (2010)*
4	*0.4 (10)*	*I*	*I*	*Maroof et al. (2014)*
5	*1.5 (2)*	*Reduced*		*Jacobsen et al. (2006)*
6	*0.75 (1)*	*Reduced*		*Kilgore et al. (2010)*
6	*0.4 (10)*	*I*	*I*	*Maroof et al. (2014)*
7	*0.4 (2)*	*I*		*Chen et al. (2013)*
8	*0.5 (3)*	*I*		*Park et al. (2010)*
8	*0.4 (10)*	*I*	*Increased*	*Maroof et al. (2014)*
10	*0.5 (3)*	*I*		*Park et al. (2010)*

Table 1.8. The table shows contextual fear conditioning results in the APPswe/PS1dE9 mouse. A blank cell = not measured, I = no genotype difference, reduced = reduced freezing in the APPswe/PS1dE9 mouse, increased = increased freezing in the APPswe/PS1dE9 mouse.

1.7.3.2.3 Appetitive conditioning

Significantly less research in the APPSwe/PS1dE9 mouse has employed appetitive Pavlovian conditioning procedures, a useful tool in understanding the processes that govern reward learning and memory. In these experiments, goal directed exploration is usually used as a measure of conditioning.

1.7.3.2.3.1 APPswe/PS1dE9 mouse

Bonardi et al. (2011) carried out a number of different appetitive conditioning experiments with 4-month-old APPswe/PS1dE9 mice. The first experiments evaluated classical conditioning. After a 40-minute food magazine training session the mice underwent six acquisition sessions, which each comprised 15 presentations of an auditory cue paired with a sucrose pellet (CS+) and 15 presentations of an alternate auditory cue paired with no reward (CS-). Then the mice underwent 2 extinction sessions during the CS+ and CS- were presented without reward. The results showed no difference between the wild type and transgenic mice in acquisition or extinction of appetitive associative learning.

Stover & Brown (2012) carried out an appetitive conditioned odour preference test in 5-8 and 20-16 month-old APPswe/PS1dE9 mice. The mice were given four 10-minute conditioning sessions; in

two they were exposed to a bowl containing a particular odour and sugar (CS+), and in the remainder to a bowl containing only a different odour (CS-). 24 hours after conditioning the mice were exposed to a three-chamber apparatus; one chamber contained the CS+ odour, the other the CS- odour (the bowls were not reinforced) and the final chamber separated the CS+ and CS- chambers. The relative amount of time spent exploring the CS+ and CS- odours was used as a measure of conditioning. No genotype difference in either age-group was found. The results of both Bonardi et al. (2011) and Stover & Brown (2012) suggest intact appetitive Pavlovian cue conditioning in young and old APPswe/PS1dE9 mice.

1.7.3.2.4 Summary – Pavlovian Conditioning

APPswe/PS1dE9 mice show impaired performance on aversive conditioning procedures. Results from both the CFC and CTA literature show evidence for faster extinction of conditioned responding to aversive USs (Jacobsen et al. 2006; Kilgore et al., 2010; Ramirez-Lugo et al. 2009; Vegh et al. 2014). This disruption in memory has been observed in the APPswe/PS1dE9 mouse from as early as 2-4 months of age.

1.8 Hippocampus as a starting point

The hippocampus plays an essential role in learning and memory, being implicated in episodic memory and processing of spatial (O'Keefe, 1976) and contextual information (for reviews see Myers & Gluck, 1994).

In relation to Alzheimer's disease, the hippocampus also plays a central role. As discussed above, the hippocampus and the MTL are one of the first areas to experience considerable deposition of both amyloid plaques and neurofibrillary tangles (Braak & Braak, 1991). Hippocampal atrophy is also seen during the development of AD with consistent evidence of a loss of hippocampal volume, and its ability to differentiate between patients with AD and MCI (Clerk et al., 2013; Cvetkovic-Dozic et al., 2001; Pennanen et al., 2004). Patients with AD also show a loss of functional contectivity between the hippocampus and the cerebral cortex, limbic system, subcortical regions and the cerebellum compared to healthy controls (Allen et al., 2007).

Mouse models of AD have also found further evidence of the role of the hippocampus in the early development of Alzheimer's. Animal models show the early development of both plaques and NFT in the hippocampus (for a review see Elder et al., 2010). Models also seem to consistently show evidence of impaired hippocampal synaptic function; in particular an inhibition of long-term

potentiation and plasticity (for a review see Rowan et al., 2003), often prior to the deposition of amyloid plaques. Some more recent studies have also started to show evidence of increase hippocampal excitability via recordings of hippocampal neuron activity. This excitability has also been linked to soluble forms of amyloid, prior to plaque deposition (Busche et al., 2013; Davis et al., 2013).

Mouse models of AD also show evidence of impaired cognitive performance on tasks know to be dependent upon hippocampal function. Spatial navigation tasks: MWM, RAWM, barnes maze T maze alternation, and tasks that measure contextual learning: contextual fear conditioning (for a review see Webster et al., 2014).

The APPswe/PS1dE9 mouse model used in this thesis also shows evidence of early hippocampal dysfunction. Aβ plaque deposition in the hippocampus appears as early as 4 months of age (Garcia-Alloza et al., 2006; Jankowsky et al., 2004), whilst hippocampal atrophy occurs from 3 months (Volianskis et al., 2010). APPswe/PS1dE9 mice also show elevated levels of soluble Aβ in the hippocampus by 3 months (Hu et al., 2010; Shemer et al., 2006), at least 3 months before plaque deposition is reliably observed. Evidence of impaired synaptic transmission and LTP has also been

found in the hippocampus of these mice at the same age (Savonenko et al., 2005; Shemer et al., 2006). There is already evidence that the APPswe/PS1dE9 mouse shows impaired performance on tasks known to be dependent on the hippocampus. Young APPswe/PS1dE9 mice are impaired in spatial navigation in the MWM and in processing contextual information in the CFC task as early as 3 months of age.

We will therefore focus on deficits produced by damage to the hippocampus as an initial guide on how to look for early cognitive impairments in the APPswe/PS1dE9 mouse model of AD.

1.9 Experimental Chapters

Chapter 2 will examine learning and memory of both discrete and contextual cues. Previous research has shown impaired contextual fear conditioning in young 3-4 month APPswe/PS1dE9 mice; however, contextual conditioning has not yet been examined using rewarding USs; if processing of contextual cues is impaired in the APPswe/PS1dE9 mouse then this should be true for both aversive and appetitive tasks.

Chapter 3 examines differences in learning delay and trace conditioning tasks. Procedural differences between CTA tasks has suggested the possibility of impaired trace conditioning in young

APPswe/PS1dE9 mice; however, no attempt has yet been made to specifically assess the differences using appetitive USs.

Chapter 4 examines recognition memory. Previous research looking at sNOR performance has found evidence of impaired novelty preference from 6 months onwards in these mice. There are, however, several variants of the classic sNOR task that have not been examined at all in the APPswe/PS1dE9 mouse model, which reflect different aspects of recognition memory.

Chapter 5 examines the underlying pathological changes in the brains of 4/5 month APPswe/PS1dE9 mice which may be involved in cognitive impairments. Neuroinflammation during the early stages of AD has recently become a topic of interest, given that inflammation occursr around amyloid plaques in the brains of both patients and transgenic animals. The chapter will look at the levels of inflammatory indicators around the hippocampus and striatum, two areas affected by the disease.

2.0 Chapter 2. Conditioning and Extinction

The hippocampal-dependent nature of contextual conditioning makes it a useful tool to examine memory performance in an AD mouse model for a number of reasons. The hippocampus is one of the earliest areas of the brain to be affected by AD pathology, and plays a central role in early episodic memory deficits seen in human patients.

In the APPswe/PS1dE9 mouse increased levels of soluble Aβ42/40 have been detected in the hippocampus at around 3 months of age (Volianskis et al., 2010; Garcia-Alloza et al., 2006), whilst plaques begin to develop in the hippocampus between 4 and 6 months (Garcia-Alloza et al., 2006; Jankowsky et al., 2004). APPswe/PS1dE9 mice also show signs of early alterations in hippocampal function, including reduced long-term potentiation (Volianskis et al. 2010; Vegh et al., 2014), reduction in dendritic neuron complexities in the hippocampal CA3 region and increased inflammation (microglia) in the hippocampus (Ruan et al., 2009) between 3 and 4 months.

The early changes in hippocampal function in the APPswe/PS1dE9 mouse mean that tasks that are dependent on the hippocampus may be impaired.

2.1 Contextual fear conditioning (CFC)

CFC has been used to assess the function of the hippocampus in learning and memory. Some have found lesions of the hippocampus (ventral, dorsal or complete) reduce levels of conditioned freezing to the context (Zhang et al., Kim & Fanselow 1992, 1997; Good & Honey 1997; Maren et al., 1997; Phillips & LeDoux 1992, 1994 Wiltgen et al. 2006), although others have failed to observe a difference (Maren et al., 1997; Frankland et al., 1998; Chen et al., 1996; Cho et al., 1999; Richmond et al., 1999). This lack of consistency could be influenced by procedural differences, such as number of trials: Wiltgen et al. (2006) found that animals with dorsal hippocampus lesions showed reduced freezing during test compared to shams when they received one context-US pairing; but no differences in freezing were seen between lesioned and sham animals when three context-US pairings were administered. Maren et al. (2007) has shown that pre-training neurotoxic dorsal hippocampal lesions have no effect on contextual freezing during test, but post-training lesions do disrupt freezing compared to shams. Whilst pre-training electrolytic dorsal hippocampal lesions do disrupt contextual freezing compared to shams. Procedural differences in trial number, lesion type and when the lesions are carried all affect the lack of consistency in results.

2.2 Cued fear conditioning

The hippocampus does play a role in fear conditioning to a discrete CS. Specifically lesions of the dorsal portion of the hippocampus (*D*HP) have no effect of conditioned freezing (Bangasser et al., 2006;; Fox et al., 1998;; Gerlai et al., 1998; Holland et al., 2003; Kim & Fanselow, 1992; Maren & Holt 2004; McEchron et al., 1998; Phillips & LeDoux, 1992; Saxe et al., 2008; Solomon et al., 1986; and Weiss et al., 1986) However, Maren et al. (1997) showed that neurotoxic lesions of the dorsal hippocampus, before (7 days) or after (1, 28, 100 days) conditioning attenuated conditioned freezing to a tone. Procedural differences during test may explain these discrepancies. Maren et al (1997) presented the CS for 10-s during training but 512-s during test, whilst Phillips & LeDoux (1992) presented the CS for 20-s during acquisition and test, and found no hippocampal lesion effect. Hippocampal lesions have no effect in experiments were the duration of presented CS during training and test are equated, where as the presentation of an extended CS during test does produce freezing after hippocampal lesion (for review see Sanders et al., 2003). Lesions of the ventral portion of the hippocampus (*V*HP) in contrast does seem to impair conditioned freezing to a cue (Bast et al., 2001; Chen et al., 2016; Maren et al.,

1999; Maren & Holt; 2004; Richmond et al., 1999; Zhang et al., 2001; 2014)

2.3 Cued and contextual extinction

Extinction is the decline in conditioned responding seen following non-reinforced exposure to the CS. In fear conditioning this is a reduction in freezing to either a discrete CS or context when they are presented without reinforcement, during a test session. The first test session can be seen as a measure of learning or extinction, whilst further test sessions are used to assess the rate of extinction. Frohardt et al. (2000) found that hippocampal lesions had no effect on extinction of cued fear, either when the cue was presented in the same context as conditioning or a novel context. Hippocampal lesions did abolish the renewal effect, which is the context-specific recovery of conditioned freezing to the CS, when the CS is presented in the conditioning context after extinction. Corcoran & Maren (2001) conditioned rats on a CS-shock association in context A, before extinction in either context A or B and a test in context A. Control rats froze less to the CS during test if they were extinguished in context A. Infusion of muscimol into the dorsal hippocampus before test resulted in low levels of freezing regardless of the extinction context.

The results show how hippocampal lesions have no effect on conditioning or extinction to discrete cues; however, do disrupt extinction of context specific discrete CS-US associations.

2.4 CFC in APPswe/PS1dE9 mice

The CFC task has been used to measure hippocampal-dependent memory in many different mouse models of AD. APPswe/PS1dE9 mice between 3 and 6 months of age show significantly less freezing to the context at test than controls, despite intact fear conditioning to the same context (Jacobsen et al., 2006; Kilgore et al., 2010; Lin et al., 2015; Vegh et al., 2014). This pattern of responding is the same as that seen in post-training hippocampal-lesioned animals (Maren et al., 2007; Wiltgen et al. 2006).

Bonardi et al. (2011) has shown a contradictory finding in 4-month APPswe/PS1dE9 mice, showing greater freezing to the context during a second test session, suggesting slower extinction of the context-fear association (Bonardi et al., 2011); no genotype differences were seen in the first test session. The majority of studies only carried out a single test (extinction) session, presentation of the context without US. As a whole the evidence suggests impaired

expression of conditioning in the APPswe/PS1dE9 mice at an age prior to substantial plaque development.

2.5 Cued fear in APPswe/PS1dE9 mice

Cued fear conditioning in the APPswe/PS1dE9 mouse model has not been examined to the same degree as contextual fear, the only study being by Lin et al. (2015). In their study mice received four presentations of a 10-s auditory CS co-terminating with a 0.4mA foot shock. The animals were given two identical test sessions, consisting of a 3-min CS presentation without a shock in a novel context, 30 minutes and 24 hours after training.

They found 2-, 3- and 4-month old (pre-plaque) APPswe/PS1dE9 mice exhibited reduced freezing to the auditory CS relative to wild types, during both test sessions. It would be interested to see if the same effect was observed using as conditioning procedure that only involved a single CS-US association, not multiple trials. It would also be interested to see if presenting the test CS for the same duration as training (10-s) could produce the same effect.

2.6 Appetitive contextual conditioning

Contextual processing and conditioning also occur with appetitive USs (Bouton & Peck 1989). Whilst the effect of hippocampal lesions has not been investigated to the same degree in appetitive as in aversive tasks, appetitive tasks have, to some degree, implicated the hippocampus in contextual processing. Ferbinteanu & McDonald (2001) tested rats with either complete, dorsal or ventral hippocampal lesions on an appetitive conditioned place preference (CPP) task in which one arm of an eight-armed maze was reinforced with food, whilst the opposite arm was not; the remaining arms were blocked. Following two days of training the rats were returned to the apparatus without reinforcement and the time spent exploring both arms was recorded. Rats with total and dorsal hippocampal lesions showed no preference for the reinforced arm, whilst animals with ventral lesions did. Meyers et al. (2006) also examined the effect of dorsal hippocampal lesions on cocaine-conditioned place preference in rats. In four sessions one context was paired with cocaine and the other with saline. At test the rats were allowed free access to both contexts. Microinjections of muscimol into the dorsal hippocampus either before training or test disrupted CPP during test. This shows

that the hippocampus is essential for the expression of contextual place preference.

2.7 Appetitive cue conditioning

Appetitive conditioning to discrete cues also appears to be relatively unaffected by hippocampal lesion (Benoit et al., 1999; Fox et al., 1998; Good et al., 1998; Han et al., 1995; Holland et al., 2003). However, this is not always the case. For example, Ito et al. (2005) gave excitotoxic hippocampal lesions to rats before training on an appetitive discrimination in which a 10-s presentation of a rectangle on the left of a liquid crystal display (LCD) screen was reinforced with a sucrose pellet (CS+); presentation of the rectangle on the right was non-reinforced (CS-). Hippocampal rats achieved a significant discrimination between CS+ and CS- more quickly than sham rats, and showed significantly faster approach latencies during the CS+. Another example of the hippocampus disrupting delay conditioning is a study by Tam & Bonardi (2012), who gave rats ibotenic acid lesions of the dorsal hippocampus before appetitive conditioning. Hippocampal rats showed lower levels of conditioned responding to a relatively long (40-sec) auditory CS than sham animals.

2.8 Appetitive conditioning in the APPswe/PS1dE9 mouse

Few appetitive conditioning studies have been carried out in mouse models of AD. As mentioned in Chapter 1, Bonardi et al. (2011) found normal discrimination between a CS+ and CS- in both genotypes and the same rates of conditioned responding during acquisition and extinction session, with discrete auditory cues in 4.5-month APPswe/PS1dE9 mice. This experiment did highlight possible genotype difference in contextual conditioning; they gave the animals a 40-minute food magazine training session in the Skinner box before the start of acquisition. Background responding (in the absence of the CS) was higher in the APPswe/PS1dE9 mice than in the wild types during acquisition. During conditioning both the discrete cue and context predict US delivery, but as the animals learn that the discrete cue best predicts the US, responding to the context decreases. The APPswe/PS1dE9 mice also extinguished this background (context) responding more slowly than controls. This increased background responding could either be a result of context conditioning to the chamber occurring during the magazine training session or the APPswe/PS1dE9 mice spent more time eating food in the food cup from previous US deliveries than the wild types. The possible slower

rate of extinction to the background context could be similar to the slower extinction in the CFC task (Bonardi et al. 2011).

2.8.1 Summary

Previous research in APPswe/PS1dE9 mice has shown impaired fear conditioning to both contextual and discrete cues (Jacobsen et al., 2006; Kilgore et al., 2010; Lin et al., 2015; Vegh et al., 2014), and some evidence for a disruption in extinction of both contextual (Bonardi et al. 2011) and discrete (Lin et al, 2015) cues.

2.8.2 Chapter overview

Based on the early increase in soluble Aβ42/40 in the hippocampus of 3 month APPswe/PS1dE9 mice and the changes in hippocampal function (Volianskis et al., 2010; Garcia-Alloza et al., 2006; the Experiments in this chapter will attempt to investigate Pavlovian conditioning to contextual (Zhang et al., Kim & Fanselow 1992, 1997; Good & Honey 1997; Maren et al., 1997; Phillips & LeDoux 1992, 1994 Wiltgen et al. 2006) and discrete cues Bast et al., 2001; Chen et al., 2016; Maren et al., 1999; Maren & Holt; 2004; Richmond et al., 1999; Zhang et al., 2001; 2014) that are dependent upon an intact hippocampus under certain conditions, using novel appetitive

reinforcement procedures in the APPswe/PS1dE9 mouse. The Experiments in this chapter will attempt to investigate the tentative evidence presented above by Bonardi et al. (2011) that young pre-plaque APPswe/PS1dE9 mice show impaired contextual conditioning. Appetitive conditioning, unlike aversive, is a useful tool as conditioning is acquired much more slowly than aversive tasks and as a result can observe the development of conditioned responding over time, and specifically genotype differences during conditioning and extinction.

2.9 Experiment 1

The aim of Experiment 1 was to simultaneously assess appetitive conditioning and extinction to both contextual and discrete cues in a young (4 month old) APPswe/PS1dE9 mouse model of AD.

Experiment 1 employed a similar, appetitive, version of the Phillips & LeDoux (1992) task, which simultaneously assesses cue and context conditioning and extinction (Table 2.2). In this appetitive version, APPswe/PS1dE9 mice were presented with a short auditory cue X and Y in context A and context B correspondingly. Then both cues and contexts were tested. In the context test the mice were placed in both contexts A and B, while the cue test comprised presentations of X and Y in a novel context (C). Both tests were

conducted without reinforcement. This allowed us to assess acquisition and extinction of conditioned responding to contextual and discrete cues individually.

	4-month APPswe/PS1dE9 mice		
		Reinforcement	
	Modality	*Aversive*	*Appetitive*
Acquisition	*Contextual*	*Intact* *Bonardi et al (2011),* *Lin et al (2015)* *Maroof et al (2014)*	#
	Discrete	*Intact* *Lin et al (2015)*	*Intact* *Bonardi et al (2011)* Stover & Brown (2012)
Extinction	*Contextual*	*Impaired* *Bonardi et al (2011),* *Lin et al (2015) Maroof et al (2014)*	#
	Discrete	*Impaired* *Lin et al (2015)*	*Intact* *Bonardi et al (2011)* Stover & Brown (2012)

Table 2.1. Summary of contextual and cue conditioning experiments in the APPswe/PS1dE9 mouse. # = not examined.

Based on the fear conditioning literature reviewed above (Table 2.1), we predicted that both wild types and APPswe/PS1dE9 mice would show the same level of conditioned responding during acquisition to both contextual and discrete cues. We also predicted that at test, APPswe/PS1dE9 mice would extinguish normally to the discrete cue, relative to wild types (cf. Bonardi et al, 2011) but show a slower rate of extinction to the contextual cues. This prediction is

based on the slower rate of extinction to the background context observed during the discrete CS conditioning procedure carried out by Bonardi et al., 2011.

	Acquisition	*Test*	
		Context	*Cue*
Experiment 1	*AX+*	*A*	*CX*
	BY-	*B*	*CY*

Table 2.2. Design of Experiment 1. Note: A, B, C = contexts: X, Y = auditory cues: +, - = reinforced & non-reinforced.

2.10 Methods

2.10.1 Animals

24 naïve 16-week-old male mice were used. The experiment was carried out in two identical replications: replication one used 6 APPswe/PS1dE9 (mean ad lib. weight 27.2g, range 24.2g – 29.6g) and 6 wild type mice (mean ad lib. weight 30.7g, range 28.6g – 33.9g), and replication two 6 APPswe/PS1dE9 (mean ad lib. weight 31.5g, range 29.3g – 34.9g) and 6 wild type mice (mean ad lib. weight 32.5g, range 30.6g - 35).

2.10.2 Apparatus

The equipment consisted of six identical sound-attenuating conditioning chambers equipped with ventilation fans (Med Associates). Each chamber had a floor made from 24 stainless steel bars with a 7.9-mm gap between each, above a stainless steel waste pan. Two of the chamber sides were made from stainless steel panels measuring 15.9cm x 14.0cm x 12.7cm, whilst a polycarbonate transparent back wall, ceiling and front-loading door made up the remaining sides. The food cup was mounted in the bottom centre of the right wall 1cm above the floor, with an opening measuring 2.5cm x 2.5 cm x 1.9cm. The well was connected to a pellet motor which delivered 12-mg sucrose pellets (Formula P). Head entry to the food well was measured via recording breaks of an infra-red photo-beam across the opening of the well. The house light, a 28-V 12-W bulb partially hooded to project light towards the ceiling, was situated at the top back left wall of the chamber. The house light was kept on throughout the experiment. An audio generator that delivered a 74-dB white noise and a 2-Hz 74-dB click. These were presented through loudspeakers mounted in the right-hand wall of the chamber (top left and top right). Med-PC for Windows was used to control the experiment (Tatham and Zurn, 1989).

To differentiate between the two contexts, the chambers were equipped with two salient cues. First, two different types of removable flooring were used to cover the metal bar floor; one consisted of a clear grey Perspex sheet and the other of a textured rubber mat (Homebase, UK), which contained 2.2cm diameter holes that were spaced 1.8cm apart; 8 raised pimples arranged in a triangular pattern surrounded each hole. Second, the transparent walls, ceiling and door of the chamber were covered in either black and white vertical stripes (11cm in width) or black spots (6mm diameter) on a white background.

For all animals context A and cue X were paired with sucrose, whilst context B and cue Y were non-reinforced. For half the mice in each genotype (Table 2.2) A was the grey flooring and striped wall inserts and the white noise was X, whilst B was the rubber flooring and spotted wall inserts and the clicker was Y. The remaining mice received the reverse configuration. Context C was the original chamber without any inserts or sounds.

2.11 Procedure

2.11.1 Food Restriction:

A week before the start of the experiment the mice were gradually reduced to 85% of their ad lib. weight. Each mouse received a

controlled food ration in an individual feeding cage, and another portion on being returned to the home cage.

2.11.2 Habituation:

On day 1 all mice received two 40-minute sessions in context C without CS presentation.

2.11.3 Acquisition:

On days 2 and 3 all mice received four training sessions, each comprising one placement in context A and one in context B. The order of the context placements was counterbalanced in an alternating pattern (A, B, B, A, A, B, B, A etc). After each placement the chambers were cleaned with alcohol. During each placement 15 trials were presented, each comprising a 20-s presentation of X or Y proceeded by a 20-s preCS period; on X trials a sucrose pellet was delivered at CS offset. Each trial was separated by a fixed inter-trial interval (ITI) of 60s plus a variable period (drawn from an exponential distribution with a mean of 60 s). Head-entry responding was measured during the pre-CS and CS presentations. Each acquisition session began with an ITI before the first trial commenced, and lasted 40 minutes.

2.11.4 Test

On day 4 there were two context test sessions, which were identical to acquisition sessions except that no auditory CSs were presented. On day 5 there were two cue test sessions that were identical to acquisition sessions except that they took place in context C. No sucrose pellets were delivered during any of the test sessions.

2.12 Data Treatment

Each break in the photobeam was defined as a single response. For each session the responses in the pre-CS and CS periods were pooled into three 5-trial bins and converted into responses per minute (RPM). Pre-CS responding was used as a measure of conditioning to the contextual cues, and difference scores (CS responding – pre-CS responding) as a measure of conditioning to the CS. The results were analysed using repeated measures ANOVA, and simple main effects analysis (SME) with the pooled error term to analyze significant interactions. Where the assumption of sphericity was found to be violated the Greenhouse-Geisser correction was used.

2.13 Results

2.13.1 Habituation

APPswe/PS1dE9 (TG) mice showed higher levels of responding during habituation than the wild type (WT) mice in context C (Figure

2.1). ANOVA with genotype (APPswe/PS1dE9 & wild type) as a factor confirmed the greater level of responding by the TG mice $F(1, 22) = 16.30$, $MSE = .459$, $p = .001$, $\eta_p^2 = .42$. The results suggest that the TG mice showed greater activity than the WT, possibly a result of hyperactivity in this transgenic strain (Arendash et ak., 2001; Llonde et al., 2005; Maroof et al., 2014; but see Arendash et al., 2001; Lalonde et al., 2004; Park et al., 2010; Lim et al., 2001 for no difference in hyperactivity).

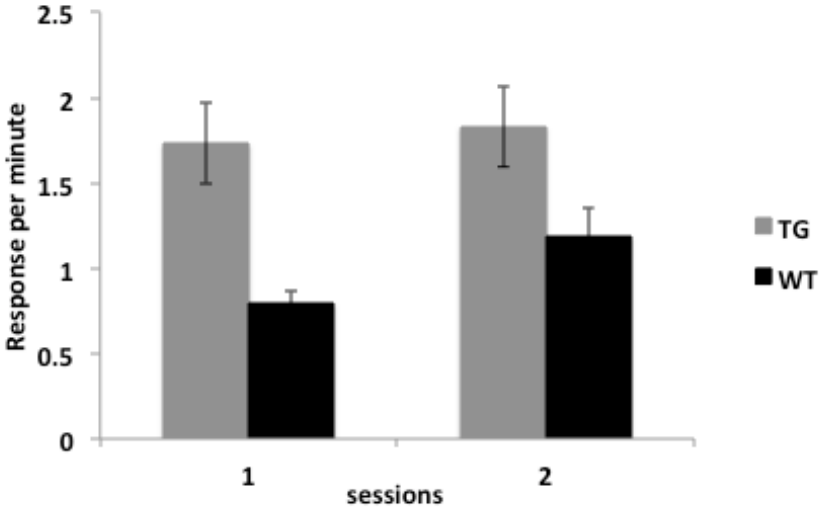


Figure (2.1). Group mean number of head entries in context C) during the habituation sessions. The error bars show standard error of the mean.

2.13.2 Acquisition – Context

Both genotypes showed greater conditioned responding to context A, which was paired with sucrose, than to context B. Figure 2.2 (upper

panel) shows the group mean rates of preCS responding in 5-trial bins for all four sessions. ANOVA with context (A or B), bins (1-12) and genotype as factors revealed a main effect of context $F(1, 22) = 75.77$, $MSE = 137.5$, $p < .001$, $\eta_p^2 = .775$ and of bins $F(11, 242) = 11.46$, $MSE = 54.85$, $p < .001$, $\eta_p^2 = .342$ (Greenhouse-Geisser corrected), and an interaction between these two factors $F(11, 242) = 13.72$, $SME = 42.7$ $p < .001$, $\eta_p^2 = .384$ (Greenhouse-Geisser corrected); there was greater responding to context A than to B from session three, smallest $F(1, 22) = 7.52$, $MSE = 28.12$, $p = .006$, onwards. There was also an increase in responding across bins in context A. $F(11, 242) = 24.01$, $MSE = 18.35$, $p < .001$, but not context B $F(11, 242) = 1.13$, $MSE = 18.35$, $p = .328$. Nothing else was significant (smallest $p = .336$); in particular there was no effect of genotype, or interaction between genotype and context or bins ($Fs < 1$). These data suggest successful Pavlovian discrimination between context A and B in both genotypes.

2.13.3 Acquisition – CS

Both groups responded more to X than to Y (Figure 2.2, lower panel), whilst TG mice showed slightly higher levels of responding overall. ANOVA with CS (X or Y), bin (1-12) and genotype as factors revealed significant main effects of CS $F(1, 22) = 11.29$, $MSE = 23.80$, $p = .003$,

η_p^2 = .339, and bins $F(11, 242) = 5.49$, $MSE = 16.49$, $p < .001$, η_p^2 = .200 and a significant interaction between these factors, $F(11, 242) = 5.23$, MSE = 14.83, $p < .001$, $\eta_p^2 = .192$; there was greater responding to X than to Y on bin 1, $F(1, 22) = 4.71$, $MSE = 15.58$, $p = .03$, and from bin 9 onwards (for bin 9 $F(1, 22) = 5.20$, $MSE = 15.58$, $p = .02$). There was also a change in responding across bins for X, $F(11, 242) = 10.11$, $MSE = 15.66$, $p < .001$, but not Y, $F(11, 242) = .62$, $MSE = 15.66$, $p = .80$. Nothing else was significant (smallest $p = .317$); there was no effect of genotype, ($F < 1$), or interaction between genotype and CS ($F < 1$) or bins $F(11, 242) = 1.158$, $MSE = 16.49$, $p > .05$, $\eta_p^2 = .05$.

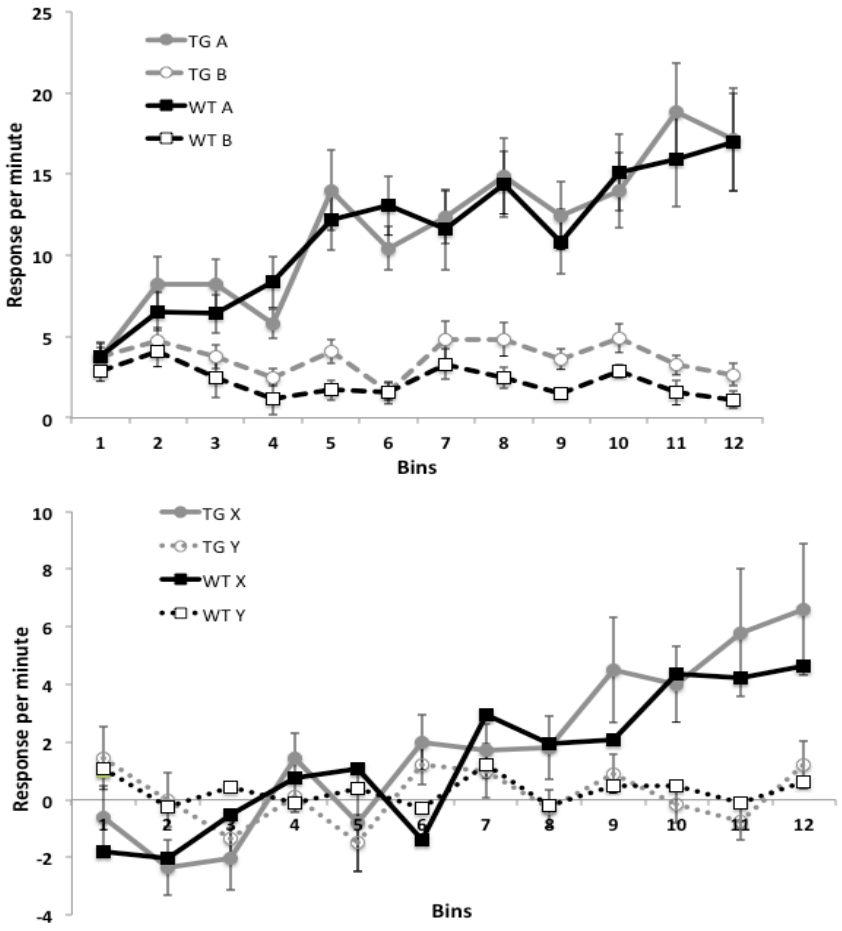


Figure (2.2). Group mean preCS response rates (context; top panel) and difference scores (CS; bottom panel) in each 5-trial bin of the four acquisition sessions of Experiment 1. Error bars show standard error of the mean.

2.13.4 Test – Context

Both genotypes showed a similar pattern of responding. Responding to context A decreased over the two test sessions, whilst responding to context B remained constant (Figure 2.3, upper panel). ANOVA with context, bins and genotype as factors revealed a main effect of context $F(1, 21) = 18.43$, $MSE = 9.93$, $p = .000$, $\eta_p^2 = .492$, and bins $F(5, 105) = 9.573$, $MSE = 10.06$, $p = .001$, $\eta_p^2 = .320$ (Greenhouse-Geisser corrected), and a Context x Bin interaction $F(5, 105) = 6.62$, $MSE = 9.30$, $p = .001$, $\eta_p^2 = .252$ (Greenhouse-Geisser corrected); there was a difference in responding between context A and B during bins 1, $F(1, 21) = 54.22$, $MSE = 6.04$, $p < .001$, and 2, $F(1, 21) = 6.79$, $MSE = 6.04$, $p = .01$. There was also a reduction in conditioned responding over bins for context A, $F(5, 105) = 17.41$, $MSE = 7.65$, $p < .001$, but not context B, $F(5, 105) = 1.26$, $MSE = 7.65$, $p = .28$. Nothing else was significant (smallest $p = .200$); in particular there was no effect of genotype $F(1, 22) = 1.13$, $MSE = 12.88$, $p > .05$ or interaction between genotype and context or bins ($Fs < 1$).

2.13.5 Test – CS

For both genotypes responding to X decreased at the same rate over the two test sessions, while responding to Y remained at the same level (Figure 2.3, lower panel). ANOVA with genotype, CS, session and bins as factors revealed a main effect of CS, $F(1, 22) = 19.55$, $MSE = 23.31$, $p < .001$, $\eta_p^2 = .472$, bins, $F(5, 110) = 4.85$, $MSE = 20.16$, $p < .001$, $\eta_p^2 = .181$ and an interaction between CS and bins, $F(5, 110) = 7.03$, $MSE = 8.84$, $p < .001$, $\eta_p^2 = .242$: there was more responding to X than to Y during bins 1, 2 and 3, smallest $F(1, 22) = 10.92$, $MSE = 11.25$, $p = .001$ for bin 3. The analysis also showed a reduction in conditioned responding to X over bins, $F(5, 110) = 10.95$, $MSE = 10.20$, $p < .001$ but not for Y, $F(5, 110) = .63$, $MSE = 10.20$, $p = .67$. Nothing else was significant (smallest $p = .153$); regarding genotype the analysis showed no main effect ($F < 1$) or interaction between genotype and either CS, $F(1, 22) = 2.18$, $MSE = 23.31$, $p > .05$ or bins ($F < 1$).

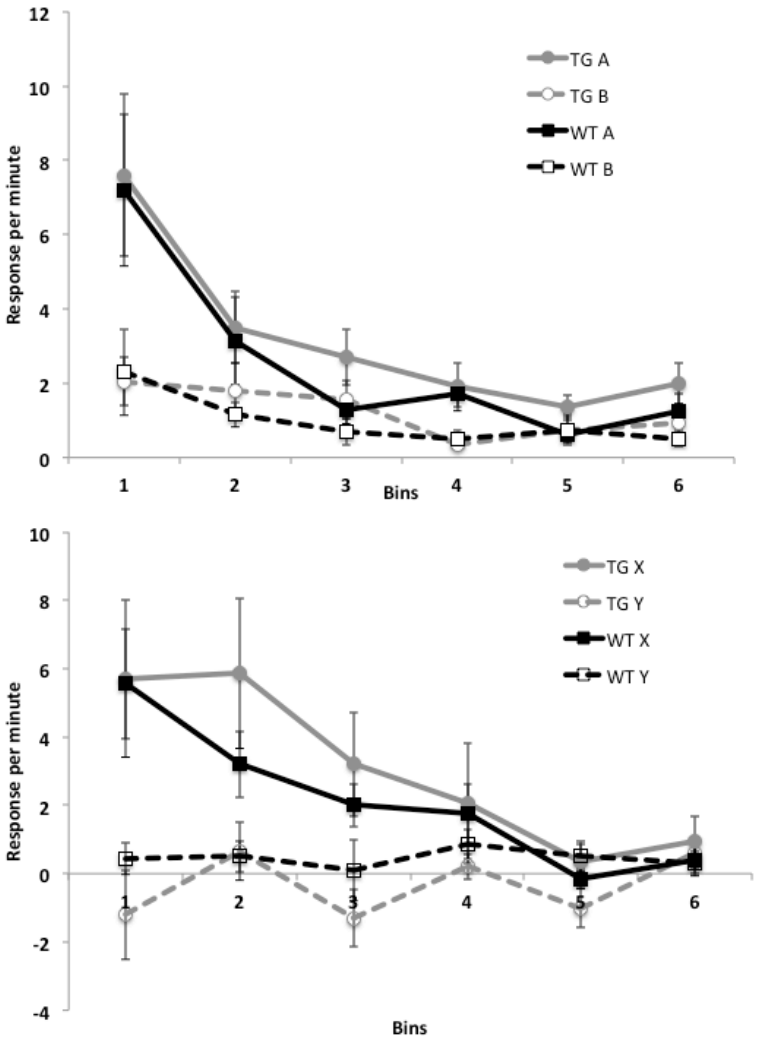


Figure (2.3). Group mean preCS responses (context; top panel) and difference scores (CS; bottom panel) during each each 5-trial bin of the two extinction sessions of Experiment 1. Error bars show standard error of the mean.

2.14 Discussion

In the habituation sessions there was greater responding in the APPswe/PS1dE9 mice than the wild types. This can interpreted as general hyperactivity in APPswe/PS1dE9 mice, or alternatively a

reduction in anxiety in the transgenic animals (Lalonde et al., 2004, Reiserer et al., 2007). Both could result in higher levels of exploration of novel environments in transgenic than in wild types. No genotype differences were observed in the rate of conditioned responding to either stimulus X or context A over the six acquisition sessions. Both genotypes were also able to discriminate between the reinforced (stimulus X and context A) and non-reinforced (stimulus Y and context B) events. These results agree with previous research showing normal Pavlovian conditioning and discrimination to discrete auditory cues in 4-month APPswe/PS1de9 mice (Bonardi et al., 2011). Experiment 1 also produced a novel result, of normal appetitive Pavlovian conditioning to a context in the young (4-month-old) APPswe/PS1dE9 mice.

During extinction both genotypes showed the same reduction in conditioned responding to both the discrete (X) and contextual (A) stimuli. This supports normal extinction of appetitive Pavlovian conditioning to a discrete cue, which has previously been shown (Bonardi et al., 2011), and to contextual cues, which has not previously been assessed.

We predicted that both genotypes would show the same level of conditioning and extinction to a discrete auditory CS. Previous

research had already shown that APPswe/PS1dE9 mice between 4 – 16 months are not impaired on that type of task using appetitive rewards (Bonardi et al., 2011; Stover & Brown., 2012). Lin et al (2015) found reduced freezing during test to discrete auditory cues using aversive conditioning procedures, between the ages of 2 – 4 months in the APPswe/PS1dE9, reflecting a discrepancy in the findings.

The second prediction was that APPswe/PS1dE9 mice would show a specific impairment in extinction of conditioned responding, but only to the contextual cues. This prediction was based on previous contextual appetitive and fear conditioning experiments, which showed disruption in the rate of extinction of contextual information. Specifically, Bonardi et al. (2011) demonstrated slower extinction of contextual cues using a fear conditioning procedure, whilst slower extinction to background cues was observed in an appetitive conditioning procedure. When specifically measuring appetitive contextual conditioning and extinction, Experiment 1 failed to show any genotype differences during extinction to the contextual cues, which is inconsistent with the original prediction.

The failure to observe an impairment in contextual extinction could be explained in a number of ways. The first is whether or not

the US is aversive or appetitive; the APPswe/PS1dE9 mice may only be impaired in aversive preparations, suggesting that whatever processes aversive stimuli in the brain is impaired. Namburi et al. (2015) argued for a circuit mechanism, which differentiates between aversive and appetitive stimuli. The aversive circuit consists of projections from the basolateral amygdala (BLA) to the centromedial amygdala (CeM), whilst the appetitive circuit runs from the BLA to the nucleus accumbens (NAc). Namburi et al. (2015) measured synaptic plasticity, via electrophysiology, in the BLA, CeM and NAc during Pavlovian aversive fear conditioning and appetitive sucrose conditioning. Fear conditioning resulted in increased LTP, a measure of synaptic plasticity, in the BLA to CeM pathway, whilst a decrease was observed in the BLA to NAc pathway. The opposite pattern of results was observed during appetitive conditioning (Namburi et al., 2015). Evidence from lesion studies also supports this idea. Lesions of the CeM impair fear conditioning (Jimenez & Maren., 2009; Kalin et al., 2004; Zimmerman et al., 2007), whilst lesions of the NAc disrupt appetitive conditioning (Di Ciano et al., 2001; Parkinson et al., 1999; 2002). The APPswe/PS1dE9 mouse appears to show neurodegeneration of the amygdala as early as 2 months of age (Lin et al., 2015), whilst levels of soluble Aβ40 and 42 were significantly

greater in the amygdala than in the hippocampus at 3 and 4 months of age (Lin et al., 2015). These early alterations in amygdala function and soluble Aβ levels may have had a specific effect on the processing of aversive stimuli, as seen by impaired cued and contextual fear conditioning between 3 – 6 months of age (Bonardi et al., 2011; Jacobsen et al. 2006; Kilgore et al. 2010; Lin et al., 2015; Vegh et al. 2014), whilst appetitive cue (Bonardi et al., 2011; Experiment 1) and context (Experiment 1) conditioning, which is more dependent on NAc function, remains intact at 4 months of age.

A second possible explanation for the failure to observe an impairment in contextual appetitive extinction is the fact that the discrete cue and the context were conditioned simultaneously, making them both accurate predictors of the US. During acquisition both context and CS could have become associated with the sucrose and possibly each other as well. As a result, during extinction the context could have activated a memory of the CS, which in turn could have increased responding to the context, and vice versa. As a result this procedure does not allow for a separate measure of conditioning and extinction to the context and discrete cue.

To address this concern Experiments 2 and 3 separated the two components of Experiment 1. Experiment 2 examined

conditioning to just the discrete CS, and Experiment 3 conditioning to just the context.

2.15 Experiment 2

Experiment 2 examined appetitive conditioning and extinction of a discrete cue in young (4-month-old) APPswe/PS1dE9 mice. Bonardi et al. (2011) have already shown intact discrimination between a reinforced and a non-reinforced CS using an appetitive US. They observed no genotype differences in extinction of conditioned responding to the reinforced CS.

A variation of this classic cue discrimination task is reversal learning, during which stimulus X is reinforced and stimulus Y is not (X+, Y-). After this discrimination has been acquired the reward contingencies are reversed, so the previously reinforced stimulus is now non-reinforced and the previously non-reinforced stimulus is now reinforced (Y+, X-; Table 2.3). During the reversal phase responding to X extinguishes and conditioned responding starts to be elicited by Y (Dube et al., 1995; Weyant, 1966).

Reversal learning can be explained in a number of different ways. First, during acquisition reinforced presentations of X result in an association between X and the US and conditioned responding, whilst the presentation of Y without the US results in no association

between the two. During reversal learning presenting X without the US results in extinction and a reduction of conditioned responding, whilst presenting Y, now with the US, results in an association being formed and an increase in conditioned responding. Y-US association formation does, however, take longer than formation of a novel association due to latent inhibition (Lubow & Moore., 1959)- the phenomenon whereby a previously exposed CS takes longer to acquire conditioned responding than a novel CS.

Reversal learning can also be explained through the perspective of impulsive control and cognitive flexibility, both of which are elements of executive function in humans (Stopford et al., 2012). Executive function is consistently impaired in patients with early and late AD (Baudic et al., 2006; Stopford et al., 2012; Yuspeh et al., 2002). According to this interpretation animals learn a set of associations between X and the US (X+) and Y and US absence (Y-) during acquisition, and another set of associations (X- and Y+) during reversal learning. Cognitive flexibility is required to learn a new set of associations during the reversal phase, and impulse control to inhibit the associations learnt during acquisition.

2.15.1 Neural substrates

The main neural structure implicated in reversal learning is the orbitofrontal cortex (OFC), with lesions impairing reversal learning to visual, auditory, spatial olfactory and tactile cues (Bissonette et al., 2008; Bohn et al., 2003; Burke et al., 2009; Ghods-Sharifi et al., 2008). The OFC also has projections to the striatum, in particular the NAc and dorsomedial striatum (DMS). Some studies have shown that lesions of the DMS impair visual reversal learning, whilst lesions of the NAc disrupt spatial versions (Burk & Mair 2001; Clarke et al., 2008). The OFC is part of the prefrontal cortex which is a specific area targeted by AD pathology during the development of the disease (Braak & Braak 1991; Levine et al., 2015; Sepulcre et al., 2013; Whitwell et al., 2008)

2.15.2 APPswe/PS1dE9

The prefrontal cortex of young (2-4 month) pre-plaque APPswe/PS1dE9 mice has been examined by Kellner et al (2014) using in vivo-cellular recordings of electrical activity of the pyramidal neurons. During the earl stages of the disease when only soluble amyloid was present within the prefrontal cortex, there was a significant decrease in excitability in response to stimulation,

possibly reflecting a loss of information transfer. These early stage changes in prefrontal activity may well effect tasks dependent on prefrontal cortex function, such as reversal learning.

In the APPswe/PS1dE9 mouse model only two studies have looked at reversal learning. Montgomery et al. (2009) trained mice on several novel two-odour discriminations (odour1+/odour2-); after they reached a performance criterion on each pair, the odour-reward pairings were reversed (odour1-/odour2+). They found slower learning during the reversal phase in 3-month old transgenic mice; however, intact reversal learning was found in 12-month old transgenic mice.

It is interesting that Montgomery et al. (2009) found impaired reversal learning at 3 months but not at 12 months. The 12-month replication was carried out in an identical fashion to the 3-month procedure; however, all new stimuli were used. One possible explanation is that it was due to a practice effect. It is possible that learning the reversal rule was impaired at 3 months but that by the end of reversal the mice had understood the reversal process, and with further practice at 12 months, showed intact performance on subsequent reversal problems. To reduce any possible practice effect different cohorts at different ages should have been tested once each,

to test the effect of age. There is evidence that increasing training or prior experience of reversal learning improved their performance during the reversal phases (Clarke et al., 2011; Costa et al., 2015; Riceberg & Shapiro 2012; Rygula et al., 2010). Prior experience of carrying out reversals also attenuated lesion effects (Clarke et al., 2011; Riceberg & Shapiro 2012; Rygula et al., 2010).

Filali & Lalonde (2009) also looked at reversal learning in APPswe/PS1dE9 mice at 3-12-months of age. Mice were trained in a T-maze filled with water, and required to navigate to a submerged platform in either the left or right arm of the maze. After they had acquired this location discrimination, the platform was switched to the opposite arm and the mice were required to learn the new location. Impaired reversal learning in 6-month-old transgenic mice was observed. However, APPswe/PS1dE9 mice are severely impaired in spatial navigation tasks between 3.5 and 18 months (Arendash et al., 2001; Jankowsky et al., 2005; Lalonde et al., 2001; Savonenko et al., 2004; Zhang et al., 2012). Thus the impaired performance could be due to the spatial component of the task and not the reversal of outcome contingencies.

2.15.3 Aims

Experiment 2 sets to examine auditory cue discrimination in 4-month APPswe/PS1dE9 mice, before examining extinction using a Pavlovin reversal learning procedure following conditioning. Based on previous experiments carried out in the same laboratory (Bonardi et al., 2011), Experiment 2 predicted normal acquisition of the auditory cue discrimination; however, we predicted that 4-month APPswe/PS1dE9 mice would show slower reversal learning to the auditory cues than wild types, based on the small amount of evidence previously showing impaired non-spatial reversal learning in young APPswe/PS1dE mice (Montgomery et al., 2009).

2.16 Methods

All methods and procedures were identical to those of Experiment 1 unless stated.

2.16.1 Animals

Experiment 2 used 18 naïve mice in total, in two replications. The first replication consisted of 6 16-week old mice, 3 APPswe/PS1dE9 transgenic mice (mean ad lib. weight 30.6g, range 28g – 34g) and 3 wild type littermates (mean ad lib. weight 32.3g, range 31g – 34g), whilst the second replication used 12 16-week old mice, 6

APPswe/PS1dE9 transgenic mice (mean ad lib. weight 28.2g, range 19.1g – 33.5g) and 6 wild type littermates (mean ad lib. weight 30.9g, range 29.3g – 34.2g).

2.16.2 Apparatus

The apparatus was identical to that of Experiment 1; all training and testing was carried out in context C.

2.17 Procedure

During Experiment 2 all animals received one session per day.

2.17.1 Acquisition

Acquisition consisted of four sessions. Each was of 2.5 hours duration and involved 20 trials with each of the auditory stimuli (X & Y) presented in a semi-random order. For half of each genotype X was the white noise and Y the clicker, and for the remaining animals the reverse. Each trial consisted of a 15s pre-CS period followed by a 15-s presentation of the auditory cue. Offset of X was immediately followed by the delivery of a sucrose pellet; no sucrose was delivered after presentations of Y. Trials were separated by an ITI of a fixed 60s plus a variable interval with a mean time of 150s.

2.17.2 Reversal

The reversal phase consisted of six sessions, which were identical to the acquisition sessions, except that X presentations were no longer reinforced, whilst Y presentations were now reinforced with a sucrose pellet.

2.18 Data treatment

Mean pre-CS and difference scores (CS-preCS) were computed separately for X and Y for each acquisition and reversal session in rpm. The first reversal session was also analysed separately, by computing difference scores in four, 5-trial bins for both the CS and pre-CS periods. The results were analysed in the same way as in Experiment 1.

2.19 Results

2.19.1 Acquisition – Difference scores

During acquisition both genotypes showed greater responding to X than to Y (Figure 2.4). ANOVA with genotype (APPswe/PS1dE9 & wild type), session (1-4) and CS (X & Y) as factors revealed a significant main effect of session $F(3, 48) = 46.30$, $MSE = 33.68$, $p < .001$, $\eta_p^2 = .743$, (Greenhouse-Geisser corrected) and CS $F(1, 16) = 164.16$, $MSE = 27.90$, $p < .001$, $\eta_p^2 = .911$, and a significant interaction between these factors $F(3, 48) = 49.05$, $MSE = 24.79$, $p < .001$, $\eta_p^2 =$

.754, (Greenhouse-Geisser corrected); there was greater responding to X than to Y during sessions 2, $F(1, 16) = 16.10$, $MSE = 19.74$, $p < .001$, 3 $F(1, 64) = 116.38$, $MSE = 19.74$, $p < .001$ and 4, $F(1, 16) = 226.02$, $MSE = 19.74$, $p < .001$; there was also an increase in responding over sessions for X $F(3, 48) = 94.12$, $MSE = 18.76$, $p < .001$ but not Y $F < 1$. Nothing else was significant (smallest $p = .44$); there was no main effect of genotype ($F < 1$) or interaction between genotype and either session or CS type (Fs < 1).

2.19.2 Acquisition – background responding

Both genotypes showed a similar level of pre-CS responding, pooled over both CSs, which declined over sessions for both genotypes (Table 2.4). ANOVA with genotype and session revealed only a main effect of session $F(3, 48) = 8.96$, $MSE = 7.86$, $p < .001$, $\eta_p^2 = .359$ (Greenhouse=Geisser corrected). Nothing else was significant (smallest $p = .195$).

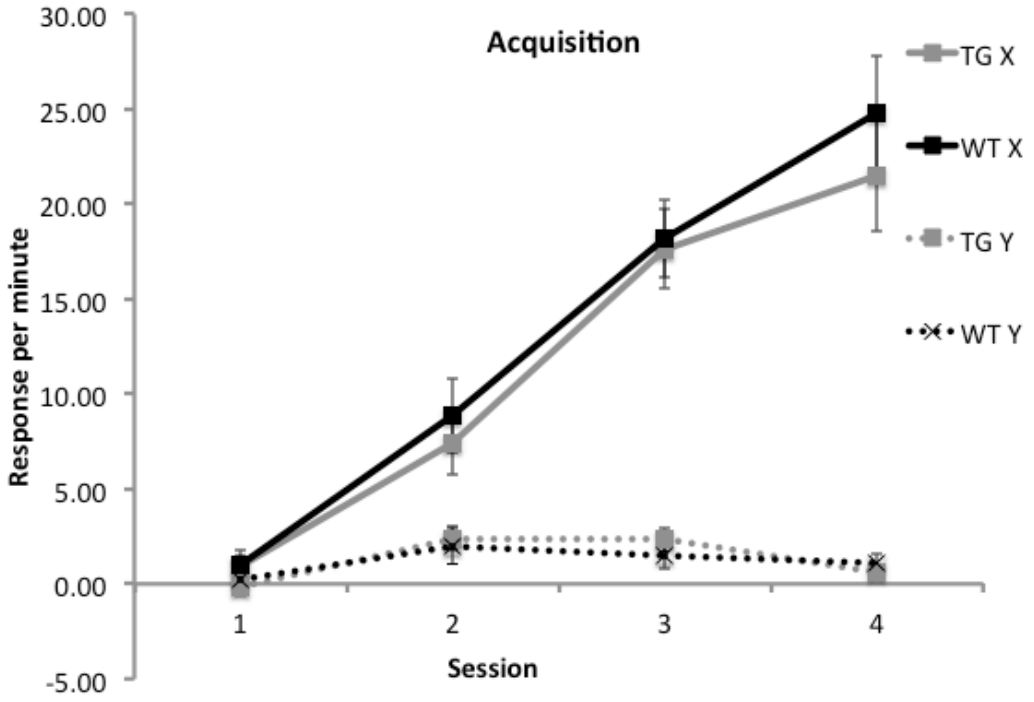


Figure 2.4. Group mean difference scores for X and Y during the four acquisition sessions of Experiment 2. Error bars show standard error of the mean.

Session	*1*	*2*	*3*	*4*
APPswe/PS1d E9	*5.71 (.97)*	*4.78 (.68)*	*3.71 (.93)*	*1.52 (.31)*
Wild type	*4.32 (.65)*	*3.66 (.68)*	*2.41 (.80)*	*1.95 (.67)*

Table 2.4. Group mean rates of pre-CS responding (rpm) during the four acquisition sessions of Experiment 2. The brackets show standard error of the mean.

2.19.3 Reversal – Difference scores

The APPswe/PS1dE9 mice appeared to show slightly faster extinction of conditioned responding to X, but normal acquisition of conditioned responding to Y (Figure 2.5). ANOVA with genotype, CS and session revealed a main effect of sessions $F(5, 80) = 7.34$, $MSE = 38.40$, $p < .001$, $\eta_p^2 = .315$ (Greenhouse-Geisser corrected), and also an interaction between session and CS $F(5, 80) = 44.46$, $MSE = 26.89$, $p < .001$, $\eta_p^2 = .735$ (Greenhouse-Geisser corrected); there was a significant difference in responding to X and Y during all sessions apart from session three (smallest $F(1, 96) = 6.08$, $p = .015$, from session two). The analysis also showed a reduction in responding to X over sessions, $F(5, 80) = 6.50$, $MSE = 15.84$, $p < .001$ and an increase in responding to Y, $F(5, 80) = 40.06$, $MSE = 15.84$, $p < .001$. Nothing else was significant (smallest $p = .416$). In particular, the analysis failed to show a significant effect of genotype, $F < 1$, or interactions between genotype and either session $F(5, 80) = 1.01$, $MSE = 18.08$, $p > .05$, $\eta_p^2 = .06$ or CS, $F < 1$, reflecting no difference in conditioned responding to X or Y between genotypes.

2.19.4 Reversal – background responding

Both genotypes showed a similar level of pre-CS responding, which declined over the six sessions for both genotypes (Table 2.5). An

ANOVA with genotype and session revealed only a main effect of session $F(5, 80) = 13.79$, $MSE = 1.47$, $p < .001$, $\eta_p^2 = .463$ (Greenhouse-Geisser corrected). No other main effects or interactions were significant (smallest $p = .210$).

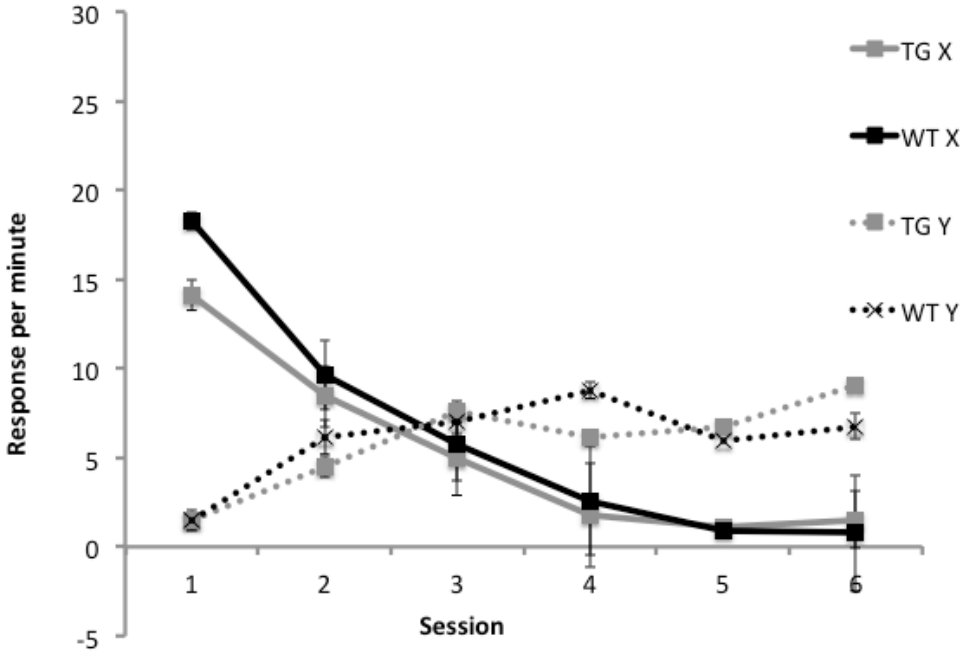


Figure 2.5. Group mean difference scores (rpm) for X and Y in the six reversal sessions of Experiment 2. Error bars show standard error of the mean

Session	*1*	*2*	*3*	*4*	*5*	*6*
APPswe/PS1dE9	*3.13 (.43)*	*3.12 (.61)*	*2.08 (.49)*	*1.48 (.24)*	*1.31 (.26)*	*0.77 (.11)*
Wild type	*4.32 (.65)*	*3.66 (.61)*	*1.47 (.22)*	*1.37 (.20)*	*1.43 (.25)*	*0.6 (.11)*

Table 2.5. Group mean pre-CS responding (rpm) during reversal sessions. Brackets show standard error of the mean.

2.19.5 Reversal session 1 – Conditioned responding

The most striking difference in conditioned responding during reversal, the numerically lower response rates to X in the transgenic mice, appeared in the first session. To establish these differences were significant during the first session before disappearing, the data from this session were analysed independently (Figure 2.6). An ANOVA with genotype, CS and bin (1-4) showed a main effect of CS $F(1, 16) = 95.68$, $MSE = 81.79$, $p < .001$, $\eta_p^2 = .857$, and bins $F(3, 480) = 5.25$, $MSE = 16.99$, $p = .003$, $\eta_p^2 = .247$, and an interaction between these factors $F(3, 48) = 10.46$, $MSE = 18.27$, $p < .001$, $\eta_p^2 = .395$; there was a difference between X and Y on all four sessions (smallest $F(1, 16) = 23.61$, $MSE = 34.15$, $p < .001$, for session 4). The analysis also revealed an increase in responding over bins for Y, $F(3, 480) = 15.28$, $MSE = 17.63$, $p < .001$ but no change over sessions for X, $F < 1$. No thing else was significant (smallest $p = .102$). Thus despite the APPswe/PS1dE9 mice showing numerically lower response rates to the extinguished X, no significant effect of genotype was observed or interactions between genotype and either CS or bins, $Fs < 1$.

2.19.6 Reversal session 1 – Background responding

The genotypes did not differ in pre-CS responding in the first reversal session; the group mean rates were 3.13 rpm (.43) for the

APPswe/PS1dE9 mice and 4.32 rpm (.65) for the wild types, ANOVA confirmed this was not significant (p = .179).

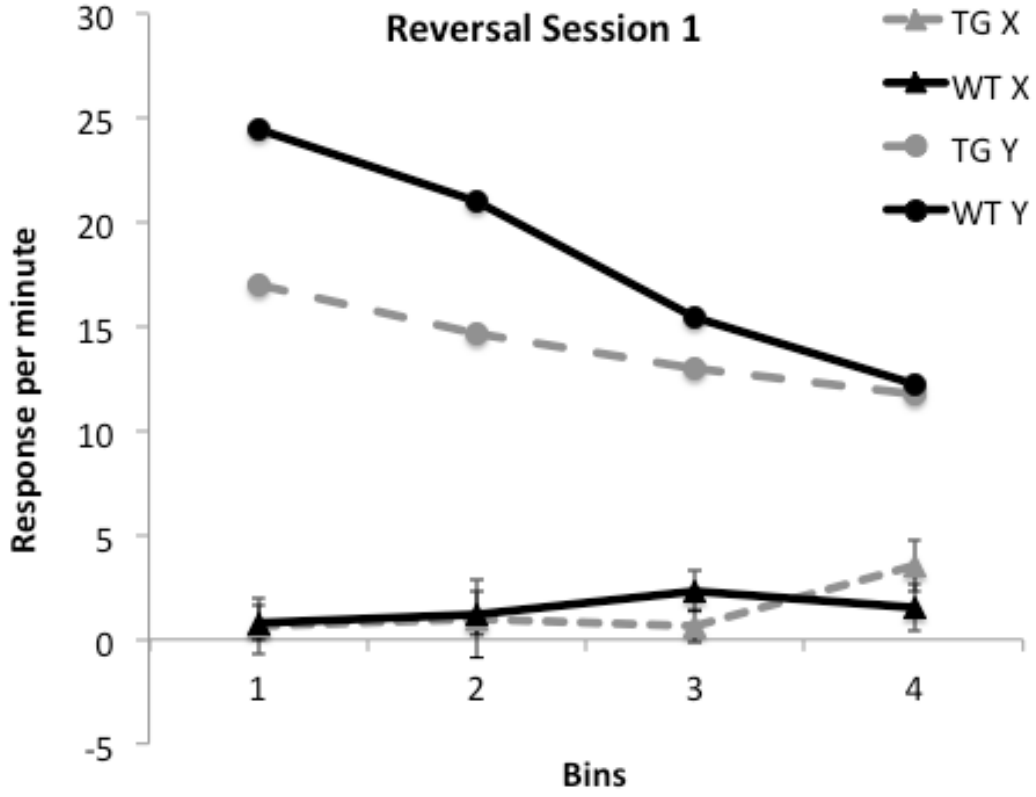


Figure 2.6. Group mean difference scores (rpm) during the first reversal session to X and Y. The error bars show standard error of the mean

2.20 Discussion

Both genotypes showed the same level of background (pre-CS) responding during both the acquisition and reversal sessions, and a reduction in pre-CS response rates over the ten sessions. This reduction in responding reflects extinction of the conditioning chamber (context). Unlike Experiment 1 that showed greater

responding by APPswe/PS1dE9 mice during habituation, Experiment 2 showed no genotype differences in activity. .

During acquisition no genotype differences were observed in discrimination between X and Y. Both genotypes also showed a similar rate of conditioned responding to X over the four sessions; in the last acquisition session APPswe/PS1dE9 mice showed numerically lower responding to X than wild types.

During reversal learning both genotypes showed a reduction in responding over sessions to X, reflecting extinction of conditioned responding, whilst an increase in responding was observed for Y, reflecting acquisition of conditioned responding. In the first reversal session response rates to X in the APPswe/PS1dE9 mice were lower than in the wild types, although no significant difference was observed in the rate of learning about Y during the reversal sessions. However, separate analysis of the first reversal session revealed (non-significant) lower levels of responding to X in the APPswe/PS1dE9 mice, but no difference in responding to Y.

We initially predicted that 4-month-old APPswe/PS1dE9 mice would show slower learning during the reversal phase, based on Montgomery et al., (2009)'s finding of slower reversal learning on an odour discrimination and reversal learning task in the same type of

mouse of the same age. Experiment 2 failed to reveal any significant genotype differences in reversal learning to an auditory cue.

The numerically lower levels of responding in the APPswe/PS1dE9 mice in the first reversal session could be reflecting faster extinction of the CS+, similar to the lower levels of conditioned freezing to an auditory cue during test sessions 1 and 2 in 2, 3, and 4-month APPswe/PS1dE9 mice (Lin et al., 2015). But an alternative, more plausible explanation is that it was a carryover from the numerically lower level of responding to X in the APPswe/PS1dE9 mice during the last acquisition session.

No genotype differences were observed in the rate of learning about Y during the reversal phase, suggesting the APPswe/PS1dE9 mice acquired the contingency reversal at the same rate as wild types; unlike previous reversal-learning tasks that showed evidence of a slower rate of CS+ acquisition during the reversal phase (Filali & Lalonde 2009; Montgomery et al., 2009).

2.21 Experiment 3

Most evidence from the literature and the results presented in Experiments 1 and 2 point towards normal appetitive conditioning and extinction of discrete cues in the APPswe/PS1dE9 mouse.

However, appetitive conditioning to contextual cues in mouse models of AD, and in particular the APPswe/PS1dE9 mouse, has been ignored in the literature. Although there was no evidence in Experiment 1 of impaired conditioning or extinction of the contextual cues in the APPswe/PS1dE9 mice, Bonardi et al. (2011) found some evidence of impaired contextual extinction in a discrete CS appetitive conditioning procedure. As mentioned in the chapter introduction (2.8) prior to conditioning, Bonardi et al. (2011) gave APPswe/PS1dE9 and wild type mice magazine training, comprising presentations of food pellets, in the conditioning context before a discrete CS conditioned discrimination procedure. As mentioned, APPswe/PS1dE9 mice showed slower extinction of responding to the background compared to wild types, which could be interpreted as a slower rate of contextual extinction. Further evidence comes from a second study carried out by Bonardi et al. (2011). They carried out an evaluation of latent inhibition in 4.2-month-old APPswe/PS1dE9 mice. Latent inhibition is the phenomenon in which conditioning to a stimulus (CS) is retarded by pre-exposure to the same stimulus (Lubow & Moore 1959). Bonardi et al. (2011) pre-exposed 4-month-old APPswe/PS1dE9 mice to either a click or white noise for seven, 40-trial sessions. Then the mice underwent the same acquisition and

extinction sessions as in the previous task. The results indicate intact latent inhibition in the transgenic mice. Interestingly no magazine training was given in the latent inhibition task, and no genotype differences in background responding were seen during conditioning. This supports the idea that the slower extinction of background responding in the cue-conditioning task described above may have been due to a deficit in contextual extinction.

Experiment 3 aimed to directly measure appetitive conditioning and extinction to contextual cues. APPswe/PS1dE9 mice and wild types were reinforced with sucrose pellets in context A and received no reinforcement in context B (Table 2.6). Once the discrimination between contexts A and B had been acquired, the animals underwent extinction by being placed into both contexts without any sucrose pellets. Based on the role of the hippocampus in contextual processing, the early pathological changes associated with AD in the hippocampus, and the evidence highlighting impaired aversive contextual extinction in the APPswe/PS1dE9, we predicted a higher rate of extinction to the contextual cues in the APPswe/PS1dE9 mice than in the wild types.

2.22 Methods

2.22.1 Animals

23 naïve 16-week-old male mice were used for the experiment, which was carried out in two replications; the first consisted of 6 APPswe/PS1dE9 transgenic mice (mean ad lib. weight 23.6g, range 21.4g – 29.7g) and 6 wild type littermates (mean ad lib. weight 28.8g, range 27.1g – 32.9g), and the second 6 APPswe/PS1dE9 transgenic mice (mean ad lib. weight 31.6g, range 28.3g – 34.2g) and 6 wild type littermates (mean ad lib. weight 33.6g, range 29.7g – 39.2g). Two APPswe/PS1dE9 and 1 wild type mouse died during the experiment, and their data are excluded from the analysis.

2.22.2 Apparatus

Identical to those of Experiment 1, except that a continuous auditory stimulus was also present throughout all context presentations. This was the clicker for context A, and the noise for context B. The contextual cues were counterbalanced in the same way as in Experiment 1.

2.23 Procedure

All the animals in Experiment 3 had context A paired with a sucrose reward and context B paired with no reward. The order of context exposures was counterbalanced in the same way as in Experiment 1.

2.23.1 Acquisition

There were four acquisition sessions, each consisting of one placement in context A and one in B. Each context placement lasted 60 minutes and consisted of six pseudo-trials, each comprising a 5-minute recording period (pre-CS); trials were separated by a fixed ITI of 190 seconds. In context A each pseudo-trial was followed by delivery of three sucrose pellets; no sucrose was delivered in context B.

2.23.2 Extinction

There followed two extinction sessions; these were identical to acquisition sessions except that no sucrose pellets were delivered.

2.24 Data treatment

The first recording period (pre-CS) from the first pseudo-trial of each session was used to analyze the acquisition data. This was done first to observe responding that was not affected by animals eating the pellets. The level of conditioned responding during the first pseudo-trial will be a measure of the learning that occurred during the previous acquisition session, reflecting a pure measure of conditioning.

Following the first analysis, the acquisition data was subsequently analysed using a discrimination ratio, this was carried

out post-hoc to assess the level of conditioned responding throughout each session. The discrimination ratio using the response rates *a* and *b*, for contexts A and B respectively, were calculated in three blocks of two pseudo-trials per session. A discrimination ratio (a-b)/(a+b) was computed for each block. Positive values reflect greater responding in context A than B, and negative values the opposite. Extinction sessions were analysed in exactly the same way as acquisition sessions.

2.25 Results

2.25.1 Acquisition – Responding in context

During acquisition responding to context A increased over sessions, whilst responding to B decreased. The only exception was a sudden increase in responding to B during session three for the APPswe/PS1dE9 mice (Figure 2.7, left panel). An ANOVA with context (A & B), session (1 – 4) and genotype (APPswe/PS1dE9 & wild type mice) as factors revealed main effects of context $F(1, 19) = 7.84$, $MSE = 4.92$, $p = .01$, $\eta_p^2 = .292$, session $F(3, 57) = 13.77$, $MSE = 5.06$, $p < .001$, $\eta_p^2 = .420$, an interaction between session and context $F(3, 57) = 14.39$, $MSE = 3.67$, $p < .001$, $\eta_p^2 = .431$ and a three way interaction between context, session and genotype $F(3, 57) = 4.12$, $MSE = 3.67$, $p = .01$, $\eta_p^2 = .178$. To explore this three-way interaction,

separate ANOVAs with genotype and block as factors were carried out on the data from contexts A and B. The context A analysis revealed a main effect of session $F(3, 57) = 18.21$, $MSE = 10.36$, $p < .001$, $\eta_p^2 = .490$ (Greenhouse=Geisser corrected), reflecting an increase in responding over blocks (smallest $p = .429$). The context B analysis revealed a main effect of session, $F(3, 57) = 7.40$, $MSE = 3.37$, $p < .001$, $\eta_p^2 = .280$, and an interaction between session and genotype, $F(3, 47) = 4.36$, $MSE = 3.37$, $p = .008$, $\eta_p^2 = .187$; there was significantly greater responding by APPswe/PS1dE9 mice than wild types during session 3 $F(1, 76) = 11.82$, $MSE = 4.32$, $p = .001$. An increase in responding over sessions was observed in the APPswe/PS1dE9 mice, $F(3, 57) = 9.08$, $MSE = 3.37$, $p < .001$, but not wild types, $F(3, 57) = 2.36$, $MSE = 3.37$, $p = .08$. From the main ANOVA no other main effects or interactions were significant (smallest $p = .298$).

2.25.2 Acquisition – Discrimination ratio

Wild type mice showed greater discrimination ratios than APPswe/PS1dE9 mice during bins 7 – 12 (Figure 2.8 right panel). ANOVA with genotype and bins (1-12) revealed a significant main effect of bins, $F(11, 209) = 26.50$, $MSE = .083$, $p < .001$, $\eta_p^2 = .582$, and a Bins x Genotype interaction , $F(11, 209) = 2.64$, $MSE = .083$, $p =$

.003, η_p^2 = .122; there was a genotype difference in bins 1, 7, 8, 9, (smallest $F(1, 209) = 4.67$, $MSE = 20.97$, $p = .031$, for bin 1) and almost in bin 12 $F(11, 209) = 3.73$, $MSE = 20.97$, $p = .054$. The discrimination ratios also increased over bins for both APPswe/PS1dE9 $F(11, 209) = 11.11$, $MSE = .07$, $p < .001$ and wild type mice $F(11, 209) = 19.63$, $MSE = .07$, $p < .001$. Nothing else was significant (smallest $p = .168$).

2.25.3 Extinction – responding in context

Both genotypes decreased their level of responding to context A over the two extinction sessions, whilst response levels remained the same for context B (Figure 2.9 left panel). An ANOVA with genotype, session (2) and context showed a main effect of context, $F(1, 19) = 18.911$, $MSE = 3.39$, $p < .001$, $\eta_p^2 = .499$ and of session $F(1, 19) = 6.43$, $MSE = 1.57$, $p = .02$, $\eta_p^2 = .253$. The ANOVA almost showed a significant interaction between session and context, but this failed to reach significance, $F(1, 19) = 3.73$, $MSE = 1.54$, $p = .068$. No overall differences were observed between the genotypes ($F < 1$). Nothing else was significant (smallest $p = .068$). Thus responding declined to both contexts over the two sessions, reflecting extinction.

2.25.4 Extinction – discrimination ratio

Both genotypes showed a steady decrease in discrimination ratio scores over bins (Figure 2.9 right panel). ANOVA with genotype and bins (1 – 6) revealed only an effect of bins $F(5, 95) = 3.56$, $MSE = .09$, $p = .005$. Nothing else was significant (smallest $p = .537$).

2.26 Discussion

Experiment 3 examined whether or not differences in contextual conditioning or extinction were present in the APPswe/PS1dE9 mouse. APPswe/PS1dE9 mice show impaired discrimination between the reinforced and non-reinforced contexts during acquisition compared to wild types (Figure 2.8). No genotype differences were observed during extinction of responding to the two contexts (Figure 2.9).

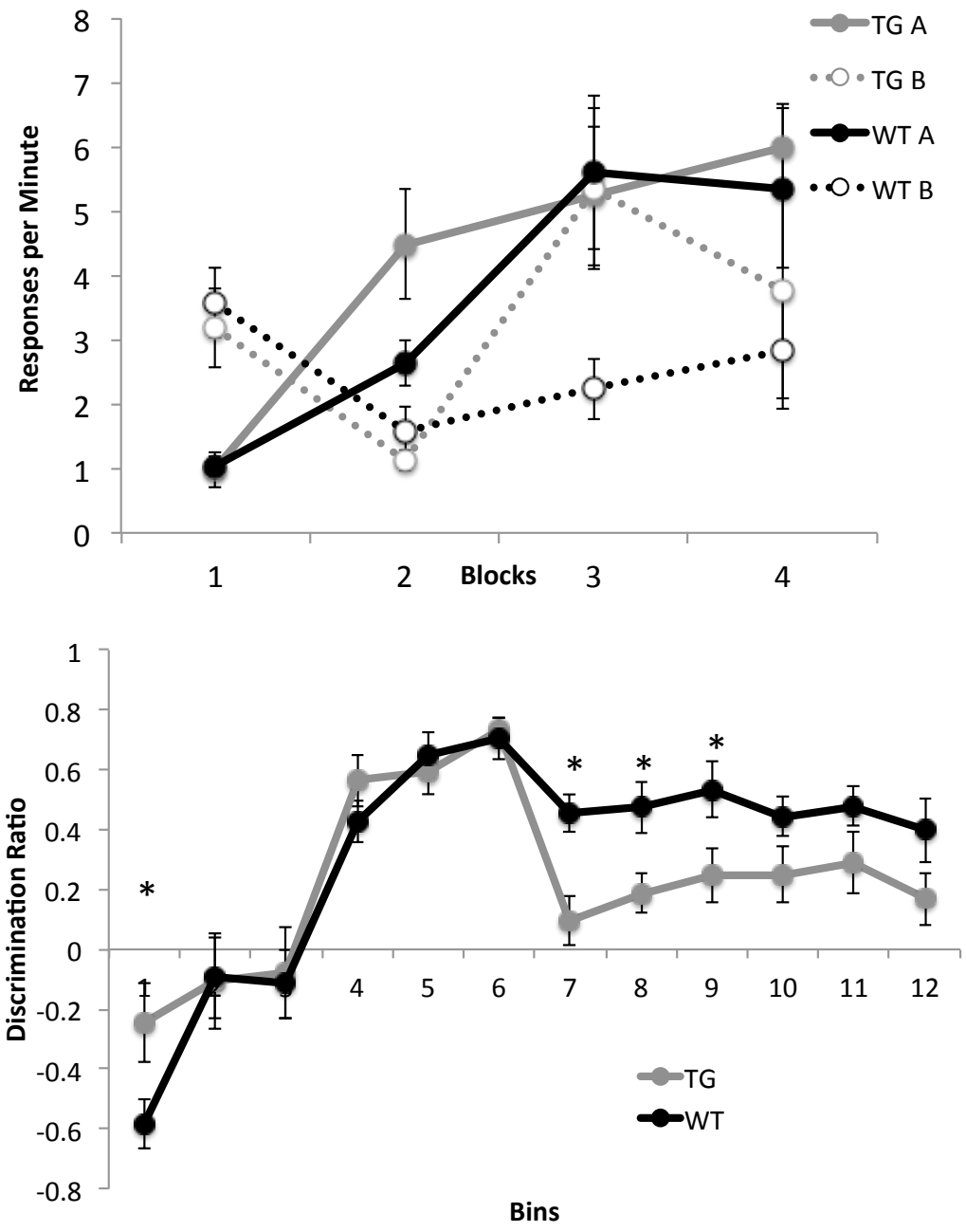


*Figure 2.8. Group mean responding (rpm) during the first pseudo-trial per session in context A and B during acquisition (top panel). Group mean discrimination ratios for both genotypes during bins 1 -12 for the acquisition data (bottom panel). The error bars show the standard error of the mean. * = marks a significant genotype difference.*

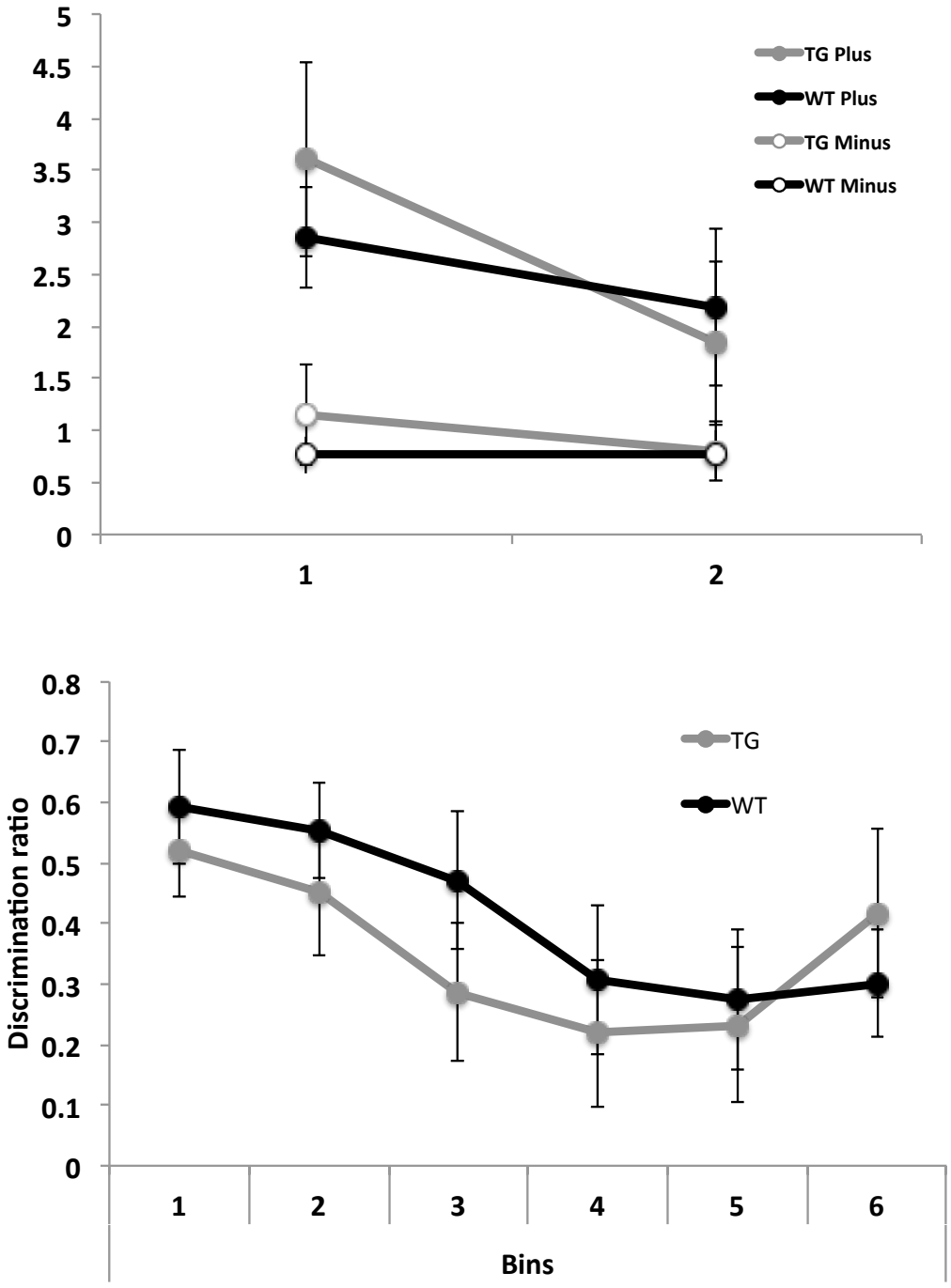


Figure 2.9. Group mean responding (rpm) during the first pseudo-trial for contexts A and B during extinction (top panel). Group mean discrimination ratios during bins 1-6 for both genotypes during extinction.

During acquisition the level of responding to context A was the same in both genotypes; however, APPswe/PS1dE9 mice showed greater responding than the wild types to context B during session 3 (Figure 10 left panel). In the APPswe/PS1dE9 mice, responding to context A and B was almost the same during the third session. The discrimination ratio data also supports the idea of greater responding to the non-reinforced context by the APPswe/PS1dE9 mice during the whole of session 3 (bins 7 - 9) and numerically during session 4 (bins 10 – 12). These data point towards impaired context discrimination in the APPswe/PS1dE9 mice.

Previous research has shown impaired context discrimination in a mouse model of Downs syndrome. The majority of Downs syndrome patients develop AD later in life, and the Ts65Dn mouse has been used to model both Downs syndrome and AD. Hyde et al. (2001) conditioned mice in two different contexts, A and B. Mice received a foot shock in context A but not in context B for consecutive five days. On day 6 mice received a foot shock in context A before later being placed into an extremely novel context (C). The Ts65Dn mouse took longer to show more freezing to context A than context B, but both genotypes were equally able to differentiate contexts A and C. The same impairments have been found in mice which have

received lesions of the dorsal hippocampus (Frankland et al. 1998), which is interesting, given that insoluble amyloid is detectable in the hippocampus as early as 3 months, whilst plaques are consistently seen at around 6 months in the APPswe/PS1dE9 mouse (Pedros et al. 2014). This result supports the role of soluble amyloid in driving the development of AD, and in particular the early cognitive changes that occur.

Unlike the Hyde et al. (2001) experiment, which showed a slower rate of discrimination between the contexts, Experiment 3 showed both genotypes acquired a context preference for the reinforced context by the end of block 2; however, the APPswe/PS1dE9 mice then appeared unable to discriminate between the two contexts during block 3 before showing intact discrimination again during block 4. The discrimination ratio results also support this observation, showing significantly worse discrimination during session 3 and numerically during session 4.

One possible explanation for the genotype difference in context discrimination could be due to the way animals learn about contexts. Whilst the two contexts are distinct from each other in terms of flooring, wall patterns (on two walls and the ceiling) and sounds, they had two identical walls, one of which contained the food cup and

one the house light. Contextual learning can occur via configural learning, in which all the multiple cues in the context are integrated together, and this unified cue is then associated with outcomes. Contextual learning can also occur in an elemental fashion in which each discrete aspect of the context is associated with the outcome separately (Holland & Bouton, 1999; Wagner, 2008). If the APPswe/PS1dE9 mice were using an elemental approach in the context discrimination task, the common elements (wall containing food cup & wall containing house light) would produce conditioned responding in both contexts. These two walls (containing food cup & containing house light) may be more salient to the animals, especially given the house light was the only source of light, whilst the food cup was the source of the sucrose reward. If the APPswe/PS1dE9 mice used an elemental strategy and paid more attention to the salient common elements of the contexts, this could result in increased responding in the non-reinforced context in these mice.

A second alternative explanation for the lack of a clear contextual conditioning deficit in the APPswe/PS1dE9 mouse is that the incremental nature of appetitive context conditioning requires less hippocampal involvement than much fast one-trial learning tasks employed using aversive CFC (Bast et al., 2014; Lehmann et al., 2009;

Wiltgen et al., 2006). As previously discussed, a number of CFC studies have shown no difference in conditioned responding between sham controls and animal with hippocampal lesions when multiple CS-US pairings have been administered (incremental learning), whilst impaired conditioned freezing is seen in the hippocampal animals following a single CS-US pairing (Wiltgen et al., 2006).

The results of Experiment 3 showing some evidence of impaired context discrimination in the APPswe/PS1dE9 mice compared to wild types; however, the evidence is not completely convincing. The main issues surrounds the unusually time course of the impaired context discrimination, and the lack of any impaired discrimination during the extinction test sessions. Further research and investigation is needed to fully establish the results seen in Experiment 3.

2.27 General Discussion

The purpose of the experiments presented in this chapter was to assess appetitive conditioning and extinction to both discrete cues and contexts in 4-5 month-old APPswe/PS1dE9 mice. Experiment 1 found no genotype differences in conditioning or extinction to either discrete cues or contexts, in a novel appetitive preparation. These

results fail to parallel those from the aversive literature, suggesting that young APPswe/PS1dE9 (3-6 month) mice are impaired in extinguishing conditioned freezing to a context (Bonardi et al., 2012; Jacobsen et al., 2006; Kilgore et al., 2010; Lin et al., 2015; Vegh et al., 2014).

Experiment 2 failed to show any genotype differences in appetitive conditioning to discrete auditory cues; it also assessed performance on reversal learning by reversing the reward-contingencies associated with the discrete cues. Previous research has shown impaired reversal learning in young APPswe/PS1dE9 mice (Filali & Lalonde, 2009 & Montgomery et al. 2009). Experiment 2 failed to show any genotype differences when the CS-reward associations were reversed, despite numerical differences between genotypes during the first reversal session suggesting a faster rate of extinction to the previously reinforced CS (Figure 8).

Experiment 3 examined appetitive contextual conditioning and extinction and revealed some signs of impaired contextual discrimination. Impairments in contextual discrimination or conditioning have not been seen before in this mouse model at any age during acquisition, when the CS-US pairings are being presented. A number of studies have shown impaired expression of contextual

fear during test, when the CS is presented in isolation (Jacobsen et al., 2006; Kilgore et al., 2010; Vegh et al., 2014). Experiment 3 found no differences in the extinction of responding to the reinforced context, in contrast to findings by Bonardi et al. (2011) suggesting slower extinction of contextual cues in both aversive and appetitive preparations.

The results presented in this chapter provide little support for the previously reported impairments in the expression of contextual fear seen in CFC procedures; however, Experiment 3, which is the most analogous to those tasks, did find worse contextual discrimination during one of the acquisition sessions that requires further exploration.

3.1 Chapter 3: Trace and Delay Conditioning

Pavlovian cue conditioning can vary in a number of different ways. Two classic variations of this task include: delay conditioning (Figure 3.1, A) in which the US occurs at CS offset, and trace conditioning (Figure 3.1, B) in which a gap is placed between CS offset and the US being delivered (trace interval). Chapter 3 will examine delay and trace conditioning in young APPswe/PS1dE9 and wild type mice, as well as how they learn about the timing of the rewarding US.

3.2 Conditioning and the hippocampus

3.2.1 Human trace and delay conditioning

Eye-blink conditioning (EBC) has been used to assess performance in both delay and trace conditioning. Individuals with medial temporal lobe amnesia show evidence of normal delay EBC (Daum et al., 1989; Gabrieli et al., 1995) but impaired trace conditioning (McGlinchey-Berroth et al., 1997) which worsens as the trace interval increases (for a review see Clark et al., 2002). This is evidence of specific medial temporal lobe involvement in trace conditioning performance; however, damage to the MTL in amnestic patients can vary greatly and is not hippocampal specific, whilst the EBC task itself is also dependent upon other brain regions, including the cerebellum.

Imaging studies in healthy individuals have highlighted the role of the hippocampus in EBC, specifically greater hippocampal activity to reinforced compared to non-reinforced delay and trace CS's, with trace trials eliciting greater activity compared to delay trials. (Blaxton et al., 1996; Buchel et al., 1999; Burger et al., 1976; Cheng et al., 2008; Gilmartin & McEchron, 2005; Knight et al., 2004; Munera et al., 2001).

3.2.2 Trace conditioning in animals

Lesion studies have also implicated the hippocampus in trace conditioning. Hippocampal lesions reduce conditioned responding to a trace CS, in aversive conditioning procedures (Beylin et al., 1999; 2001; Gabrieli et al., 1995; Kim et al., 1995; McEchron et al., 1998; Moyer et al., 1990; Solomon et al., 1986; Weisz et al., 1980, but see, Moyer et al., 1990; Rawlins., 1998; Rogers et al., 2006 for normal conditioned responding), but not in appetitive trace procedures (Kyd et al., 2007; Lin et al., 2011; Ross et al., 1984; Thibaudeau et al., 2007; 2009, but see, Pearce et al., 2005 for impaired performance).

3.2.3 Delay conditioning in animals

The hippocampus also plays a role in conditioning to a delay CS. Lesions of the dorsal portion of the hippocampus (*D*HP) generally have no effect of the level of CR using aversive or appetitive rewards (Bangasser et al., 2006; Benoit et al., 1999; Fox et al., 1998; Good et al., 1998; Holland et al., 2003; Kim & Fanselow, 1992; McEchron et al., 1998; Phillips & LeDoux, 1992; Solomon et al., 1986; and Weiss et al., 1986). However, impaired delay conditioning has been observed under certain conditions, such as when the CS is presented for an extended time (Beylin et al., 2001; Richmond et al., 1999; Solomon et al., 1983; Tam & Bonardi, 2012a; 2012b). Whilst lesions of the

ventral portion of the hippocampus (*V*HP) do impair the level of conditioned responding to discrete cues (Bast et al., 2001; Chen et al., 2016; Maren et al., 1999; Maren & Holt; 2004; Richmond et al., 1999; Zhang et al., 2001; 2014).

3.3 Interval timing and the hippocampus

The level of conditioned responding during the presentation of either a delay or trace CS is not the only measure of learning. The hippocampus can effect the distribution of conditioned responding over the CS duration (Balci et al., 2009; Meck et al., 1984, 1987; Solomon et al., 1986; Tam & Bonardi, 2012; 2012a; Yin & Meck, 2014) Over consecutive conditioning sessions control animals learn to show maximal responding at the end of the CS, close to US delivery. This is a measure of how accurate animals are at timing the arrival of either an appetitive or aversive US. In contrast hippocampal animals appear to show greater responding earlier in the CS (Balci et al., 2009; Meck et al., 1984, 1987; Solomon et al., 1986; Tam & Bonardi, 2012; 2012a; Yin & Meck, 2014).

Another way to examine the timing of US arrival is to use a preparation known as the peak procedure (Meck et al., 1984; Tam & Bonardi, 2012; 2012a). In this task animals are trained on appetitive

delay conditioning task, and once the animals have successfully learned the CS-US association they then receive occasional test trials that are identical to conditioning trials except that they are non-reinforced and the CS is presented for around 3 times as long. During these test trials control animals show the greatest responding at the time point when the US would usually be presented. Animals with hippocampal lesions show maximal responding earlier than controls and prior to when the US delivery would usually occur (an underestimation)(Balci et al., 2009; Buhusi et al., 2004; Meck et al., 1984, 1987; Moyer et al. 1990; Solomon et al. 1986; Tam & Bonardi, 2012, 2012a; Weiss et al. 1999; Yin & Meck, 2014; Yin & Troger 2011).

3.4 Delay and trace conditioning and Alzheimer's disease

Delay and trace conditioning procedures may well be a useful tool to investigate AD, especially given the hippocampal involvement.

3.5 Humans delay and trace conditioning

A limited number of studies have looked at delay and trace conditioning in patients diagnosed with clinical and preclinical AD. Initially aged healthy controls show a lower level of conditioned

responding than young healthy controls in both delay (Bellebaum & Daum, 2004; Cheng et al., 2010; Solomon et al., 1989) and trace (Cheng et al., 2010; Finkniner & Woodruff-Pak., 1991; Knuttinen et al. 2001) conditioning paradigms.

Using EBC a lower rate of conditioned responding to delay (Solomon et al., 1995; 1991; Woodruff-pak et al., 1990; 1996) and trace CSs (Woodruff-Pak 1996) has been observed in patients diagnosed with AD, compared to controls. Interestingly, Moore et al (2005) found impaired delay EBC to an olfactory (but not auditory) US in non-demented patients with a greater genetic risk of developing the disease later in their life (because they possess the apolipoprotein E4 allele). This finding is of particular importance as it shows early disruption of delay conditioning in a population of individuals who only possess an increased risk of developing AD in the future

3.6 Animals delay and trace conditioning

Transgenic mouse models of Alzheimer's disease have been used to assess the effect of early and late pathology on both delay and trace conditioning. Using EBC, Weiss et al. (2002) trained a transgenic mouse line, overexpressing APP (tgV717F), on a delay-conditioning

paradigm. Transgenic mice and wild types received 30 pairings of a 400ms white light with a 100ms co-terminating periorbital shock, for fifteen training days. The animals' hippocampal volume was also measured using magnetic resonance imaging (MRI) after conditioning. Transgenic animals were impaired on delay conditioning, taking significantly longer to reach the criterion of seven conditioned responses per ten trials, than wild types (however see, Ewers et al., 2006; Kishimoto et al., 2012; Ohno et al., 2006 for intact delay conditioning). Transgenic mice also showed significantly smaller hippocampus to brain volume ratios than wild type animals (Weiss et al. 2002).

Trace EBC has been assessed in a number of different AD mouse models. Kishimoto et al. (2012) trained a mouse model, overexpressing APP (Tg2576), on a trace EBC task, in which a 352-ms tone and a 100-ms 0.2mA shock were separated from each other by a 500-ms interval (trace interval). Compared to wild type mice, 6- and 12-month old Tg2576 mice showed lower levels of conditioned responding to the trace CS and took longer to reach criterion of 6 conditioned responses in 10 trials, whilst performance was normal in 3-month transgenic and wild type animals. Guart et al. (2008) compared trace EBC in three different transgenic lines, one

overexpressing APP (APP751SL), one overexpressing PSEN1 (PS1M146L) and a double transgenic mouse with both mutations (APP751SL/PS1M146L). The trace interval between the CS and US lasted 500-ms. The double transgenic mouse showed impaired conditioned responding at 12 and 18 months of age, whilst the single APP and PS1 mice showed lower levels of conditioned responding than wild types only at 18 months. All animals showed normal trace conditioning at 3 months of age (Kishimoto et al. 2012, 2013, Guart et al. 2008; however see Ewers et al. 2006 for intact trace conditioning at 12 months).

Only Ohno et al. (2006) had examined trace conditioning in AD in a task other than EBC, using an auditory fear-conditioning paradigm. They tested mice overexpressing the APP gene (Tg2576) and a double transgenic mouse overexpressing both APP and PS1 (Tg6799) on a trace-conditioning paradigm. A 15s CS was separated from a foot shock by a 30s trace interval. At 5-6 months of age the Tg2576 mice showed a lower level of conditioned responding to the trace CS compared to wild type mice. This impairment was observed prior to plaque development in the brain, which occurs between 9-12 months in the single transgenic mouse model. The result therefore reflects a learning impairment that cannot be attributed to the

deposition of amyloid plaques. The Tg6799 mice also showed a lower level of conditioned responding compared to wild type at the same age point, however plaque deposition in the double transgenic mouse also occurs at this age.

The majority of studies examining delay conditioning in AD mouse models has shown evidence of normal conditioned responding (Ewers et al., 2006; Kishimoto et al., 2012; Ohno et al., 2006), only Weiss et al. 2002 has found evidence of impaired delay conditioning which has been correlated to a reduction in hippocampal volume. Trace conditioning; however, appears to be impaired in AD mice (Guart et al. 2008; Kishimoto et al., 2012; Ohno et al., 2006) from around 5 months of age onwards.

3.7 Interval timing and Alzheimer's disease

Only a small number of studies have looked at timing accuracy in the Alzheimer's patient population. A variety of different tasks have been employed to measure the accuracy, responding at the correct time, and precision, variability in responding, of time estimation in patients with AD. Nichelli et al. (1993) required both AD, elderly, and young subjects to repeatedly reproduce a 1-s interval, by tapping a key. Subjects were also required to read digits of increasing length (5,

10, 20, 40) whilst maintaining the 1-s key-press rhythm. At the end of each trial the participants were instructed to judge the amount of time elapsed (verbal time estimate). AD patients showed a slower response rate for the 1-s tapping rhythm (overestimation) and greater variability (loss of precision) than elderly controls, whilst the overall accuracy was comparable to elderly controls. Severity of AD was also positively correlated with precision in the verbal time estimate task. Carrasco et al. (2000) required AD and age-matched controls to perform a time estimation task, in which participants had to press a key when a particular time interval (5, 10 or 25s) had elapsed. AD participants significantly overestimated the 5s duration compared to controls and showed greater variability in responses for all three durations. Caselli et al. (2009) required mild AD patients to perform a short (100 to 600-ms) and long (1000 to 3000-ms) time bisection task. During the short task, participants were presented with multiple intervals from between 100 and 600-ms, and had to classify them as either short (closest to 100-ms) or long (closest to 600-ms), the same procedure was employed for the long task. The results showed no difference in the accuracy of interval evaluations; however, AD patients were significantly more variable in response (loss of precision) when compared to both elderly and young

controls. Rueda et al. (2009) carried out a prospective verbal estimation paradigm in young healthy, old healthy, MCI and AD patients. During each trial participant's were required to read aloud numbers that were individually presented on a computer screen. Each trial varied in duration and participants were required to estimate the elapsed time at the end of each trial. MCI patients showed a significantly greater absolute error (the discrepancy between actual and estimated time) in comparison to young but not old controls. AD patients showed a significantly greater absolute error than both old and young controls, as well as greater variability in responding than old controls.

In summary, studies looking at the accuracy and precision of time interval estimation in Alzheimer's disease have consistently found increased variability in responding to both short and long time intervals, reflecting a loss of timing precision (Caselli et al., 2009; Carrasco et al., 2000; Hellstrom & Almkvist, 1997; Nichelli et al., 1993; Rueda & Schmitter-Edgecombe, 2009; but see Heinik, 2012).

3.8 Conclusions

The hippocampus has been strongly linked to performance on both delay and trace conditioning procedures, via human imaging and

animal lesion studies. Given that the hippocampus is one of the first areas to be affected during the development of Alzheimer's disease, experimental procedures, such as delay and trace conditioning, which target the hippocampus have often been used in both human and animal AD research. Consistent with this idea, both delay and trace conditioning have been found to be impaired in both humans with AD and transgenic mouse models of the disease.

To date, all the studies looking at delay and trace conditioning in AD mouse models have employed aversive unconditioned stimuli, despite evidence that impairments are also present in appetitive versions in animals with hippocampal lesions. The experiments presented below attempt to examine delay and trace conditioning using an appetitive US during the early development of AD, in the APPswe/PS1dE9 mouse model.

The hippocampal literature also points towards the involvement of the hippocampus in timing the arrival of an unconditioned stimulus, with animal lesion studies showing earlier peak responding (loss of accuracy). Due to the involvement of the hippocampus, timing has been examined in AD. Unlike the animal lesion studies, humans with preclinical, and clinical AD show a loss of precision, but intact accuracy in timing various intervals, with the

majority of the literature showing the same consistent findings. Currently no research has examined timing behaviour in mouse models of Alzheimer's disease, and as a result there is no agreement between human and transgenic animal research. The current experiments also attempt to examine the ability of young APPswe/PS1dE9 mice to time the arrival of food rewards.

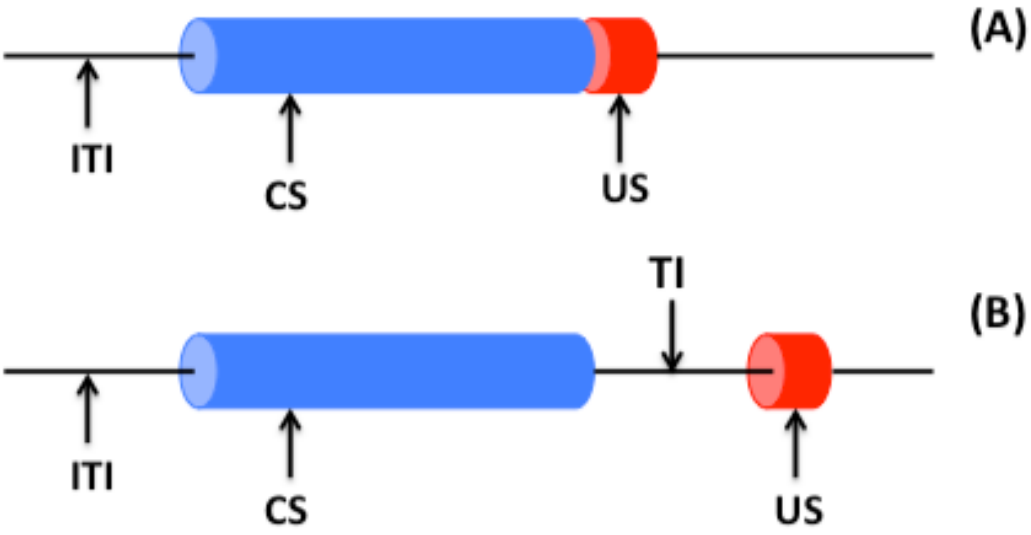


Figure 3.1. A schematic diagram of (A) delay and (B) trace conditioning paradigms, showing inter-stimulus interval (ITI), conditioned stimulus (CS), unconditioned stimulus (US), trace interval (TI).

3.9 Experiment 1

Experiment 1 investigated delay and trace conditioning in 4-month APPswe/PS1dE9 mice. Using an appetitive procedure, transgenic and

control mice were presented with a sucrose pellet after an auditory CS, either at CS offset (delay), or following a short interval after CS offset (trace; Figures 3.1 & 3.2).

Following delay and trace conditioning, Experiment 1 assessed the ability of APPswe/PS1dE9 and wild type mice to time the arrival of the sucrose reward using the peak procedure. This involved presenting the mice with occasional test, delay and trace, trials (Figure 3.2). The delay test trials involved the delay CS being presented for an extended period of time, whilst the trace test trials involved increasing the duration of the trace interval. All test trials were non-reinforced. The level of conditioned responding over the test trials was used to measure how precisely and accurately both genotypes were in timing the arrival of the sucrose reward.

Based on the previous research showing impaired trace conditioning in a variety of AD mouse models, we predicted that 4 month APPswe/PS1dE9 mice would show reduced conditioning responding to the trace CS compared to the wild types, but intact conditioned responding to the delay CS (Gruart et al., 2008; Kishimoto et al., 2012, 2013; Ohno et al., 2006).

We also predicted, based on the human AD literature showing a loss of timing precision (Carrasco et al. 2000; Hellstrom & Almkvist.

1997; Nichelli et al. 1993; Rueda & Schmitter-Edgecombe. 2009), that the APPswe/PS1dE9 mice would show impaired precision in timing the appetitive reward compared to wild types.

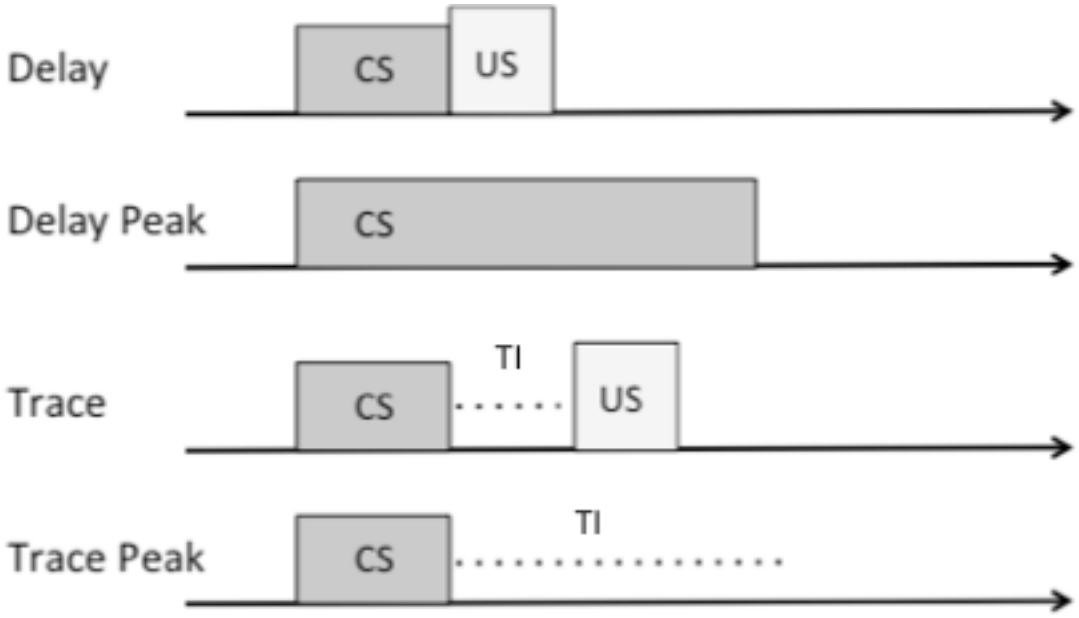


Figure 2.2. Schematic of trial types in Experiment 1.

3.10 Methods

3.10.1 *Animals*

All subjects were bred and housed in the same way as those in in Chapter 2. 24 naïve 16-week-old male mice were used for the experiment, which was conducted in two replications, with 6 APPswe/PS1dE9 mice (ad lib. weight 32.23g: range, 29.9 – 37.6 g) and 6 wild type littermates (ad lib. weight 31.68g: range, 30.7 – 34.2 g) in the first replication, and 6 APPswe/PS1dE9 mice (ad lib. weight

31.35g: range, 27.1 – 34.4 g) and 6 wild type littermates (ad lib. weight 3011g: range, 27.7 – 31.5 g) in the second.

3.10.2 Apparatus

The same apparatus as was used in Chapter 2 was employed in all the experiments in this chapter. The conditioning chambers had the contextual floor and wall inserts removed, whilst the chambers were illuminated throughout the experiments using the house light. The auditory stimuli employed were the click and white noise used in Chapter 2.

3.11 Procedure

3.11.1 Food deprivation

All animals were food deprived in the same way as those in Chapter 2.

3.11.2 Acquisition

Experiment 1 included six acquisition sessions, each comprising 40 trials. Half the trials were delay and the remainder trace trials. A delay trial consisted of a 15-s pre-CS period, immediately followed by a 15-s CS and the immediate delivery of a single sucrose pellet at the end of the CS. Trace trials were identical to delay trials except that a

5-s trace interval was added between CS offset and delivery of the sucrose pellet. The two trial types were presented in a semi-random order during each acquisition session. Half of each genotype received the white noise as the delay CS and the clicker as the trace CS; the remainder received the reverse. The ITI consisted of a fixed 120 seconds and variable portion with a mean of 60 seconds, drawn from an exponential distribution.

3.11.3 *Test – peak procedure*

The acquisition phase was followed by two peak procedure sessions (Figure 2.2). These were identical to the acquisition sessions except that 10 of the delay trials were replaced by non-reinforced peak trials, in which the CS was presented for 45 seconds. 10 of the trace trials were also replaced by non-reinforced peak trials, in which the trace interval was increased to 15 seconds. Both the reinforced and non-reinforced trials were semi-randomly interspersed throughout each peak procedure session.

3.12 Data Treatment

3.12.1 Acquisition

The data treatment for acquisition was the same as Chapter 2. Pre-CS responding was analysed by combining the mean pre-CS scores for

both delay and trace trials during each session. CS and trace interval responding were expressed as a difference score (TI – pre-CS) for each session.

3.12.2 Peak test

One APPswe/PS1dE9 mouse died during the second peak session and was therefore removed from all peak procedure analysis. Interval timing accuracy was assessed following the methods employed by Church, Miller, Meck and Gibbon (1991); see also Tam and Bonardi (2012). Response rates in each 1-second bin of the peak delay CS, peak trace CS and peak trace interval were pooled across the two peak procedure sessions for each mouse. CR distributions were normalized by dividing each bin by the sum total of responding, before being smoothed by finding the average CR for each five consecutive bins. A Gaussian function was fitted to the normalized data. The Gaussian function A*exp(-0.5*((t-C)/B)*((t-C)/B)) has three parameters: A = peak rate, B = spread, C = peak time. Peak rate represents the maximum high of the function and reflects a normalized level of conditioned responding to the CS; peak time refers to the 1-s bin in which the maximum peak rate occurred and indicates the accuracy of CS timing, and the spread is the level of variability around the peak time, representing the level of timing

precision. An R^2 value is also derived from the analysis that reflects how accurate a fit the Gaussian function is to the data.

3.13 Results

3.13.1 Acquisition

Both controls and APPswe/PS1dE9 mice showed an increase in conditioned responding to both the delay and trace CS over the six acquisition sessions, and higher responding to the delay CS than to the trace CS from sessions 4 onwards (Figure 3.3). An ANOVA with genotype (APPswe/PS1dE9 & wild type), trial type (delay & trace) and session (1-6) (Figure 3.3) showed a main effect of sessions, $F(5, 110) = 34.29$, $MSE = 37.87$, $p < .001$, $\eta_p^2 = .609$, trial type, $F(1, 22) = 12.11$, $MSE = 23.57$, $p = .002$, $\eta_p^2 = .355$, and an interaction between these two factors, $F(5, 110) = 5.87$, $MSE = 5.842$ $p < .001$, $\eta_p^2 = .211$; however, no effect of genotype was observed, $F(1, 22) = 2.14$, MSE = 141.74, $p = .158$, $\eta_p^2 = .089$. The interaction showed greater responding to the delay CS in comparison to the trace CS during sessions 3 to 6, the least significant, $F(1, 110) = 9.10$, MSE = 8.79, $p = .003$, $\eta_p^2 = .064$, for session 6. No other main effects or interactions were significant (smallest $p = .149$).

Trace interval differences scores appeared to be larger than zero from session three onwards (Figure 3.4). An ANOVA with genotype and session as factors, revealed only an increase in responding over the six sessions, $F(5, 110) = 44.60$, MSE = 109.39, $p < .001$, $\eta_p^2 = .649$, (Greenhouse-Geisser corrected); no other main effects or interactions were found (smallest $p = .441$).

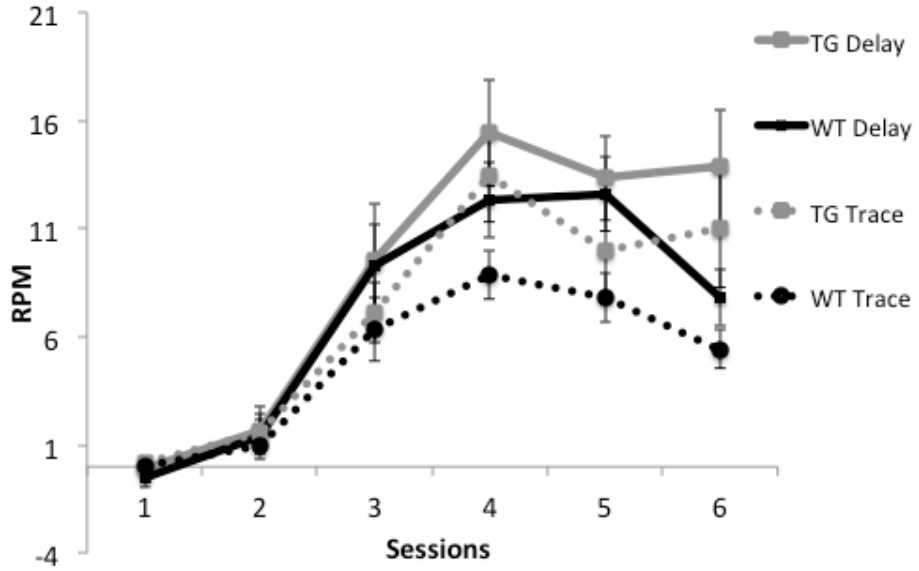


Figure 3.3. Group mean difference scores for responding to the delay and trace CSs during the six acquisition sessions of Experiment. Error bars show standard error of the mean.

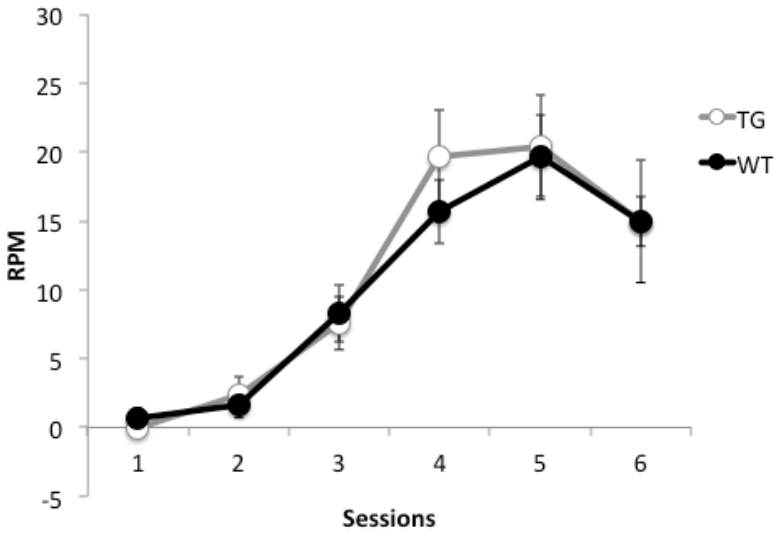


Figure 3.4. Group mean difference scores for responding during the trace interval in the six acquisition sessions of Experiment 1. Error bars show standard error of the mean.

Pre-CS responding levels increased over the first three sessions (Table 3.1), before slowly decreasing for both genotypes. The analysis revealed a main effect of sessions, $F(5, 110) = 24.59$, MSE = 16.80, $p < .001$, $\eta_p^2 = .528$ (Greenhouse-Geisser corrected). No other main effects or interactions were observed (smallest $p = .161$).

Pre-CS responding						
	Session					
	1	2	3	4	5	6
TG	2.04	7.06	6.87	2.39	2.39	1.95
	(.46)	(.79)	(1.0)	(.34)	(.34)	(.31)
WT	2.03	0.79	6.03	2.85	1.80	2.09
	(.40)	(1.36)	(1.07)	(.49)	(.44)	(.36)

Table 3.1. Mean level of pre-CS during the six acquisition sessions of Experiment 1. Brackets show standard error of the mean.

3.13.2 Peak test

Levels of responding to the delay and trace CS peak trials are presented in Table 3.2. The APPswe/PS1dE9 mice appeared to show greater responding during session one of the peak sessions for both the delay and trace CSs. An ANOVA with genotype, trial type and session (2) revealed only a main effect of trial type, $F(1, 21) = 8.87$, MSE = 10.50, $p = .007$, $\eta_p^2 = .297$. No other effects or interaction was significant (smallest $p = .121$).

The same analysis on the trace interval (Table 3.2) revealed no significant main effects or interactions (smallest $p = .244$).

Test – Peak Sessions		
Peak Trials		
	Test 1	Test 2
TG Delay	10.03 (2.0)	7.47 (2.2)
WT Delay	7.15 (1.3)	6.077 (1.2)
TG Trace	7.52 (3.0)	6.8 (3.0)
WT Trace	4 (.95)	5.06 (1.3)
TG Trace Interval	12.36 (2.6)	9.27 (2.7)
WY Trace Interval	4 (1.9)	9.06 (1.9)

Table 3.2. Mean responding to the delay CS, trace CS and trace interval during the two test sessions of Experiment 1. Brackets show standard error of the mean.

Pre-CS responding during the two test sessions showed no real difference between genotypes. APPswe/PS1dE9 mice responded on average 1.8rpm (.55 SE) and 1.4rpm (.24 SE), whilst wild types responded at 1rpm (.23 SE) and 1.1rpm (.31 SE) during test sessions 1 and 2. The analysis found no main effects or interactions (smallest p = .104; Table 3.3).

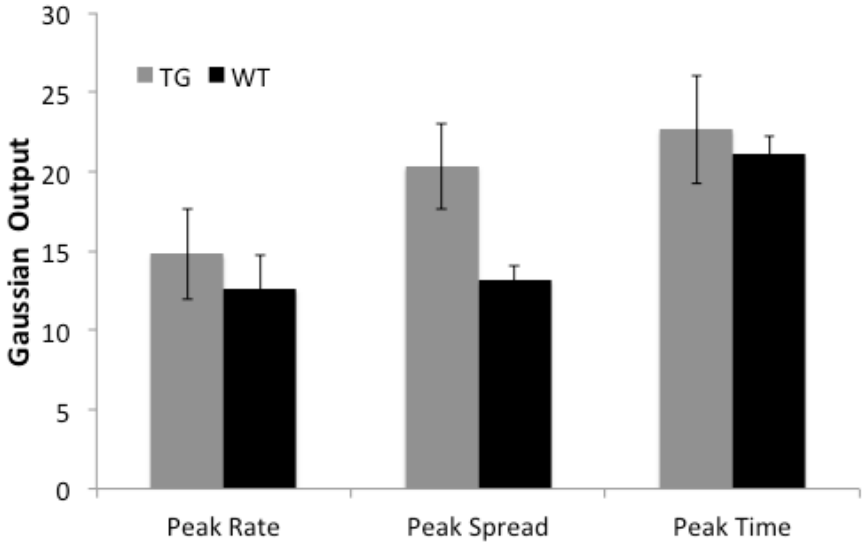
Figure 3.4 shows the normalized responding during both the delay and trace interval peak sessions from which the Gaussian function was calculated. The Gaussian parameters for the delay CS (Figure 3.5) appeared to be similar in the two genotypes for peak rate and time, but peak spread appeared to be greater in the APPswe/PS1dE9 mice. All three Gaussian parameters for the trace interval (Figure 3.6) appear similar between genotypes. R^2 scores are presented in figure 3.7.



Figure 3.4. Group mean level of responding, in 1-second bins, to the delay (D) CS and trace interval (T) peak trials during the two test sessions of Experiment 1.

Delay CS: Gaussian scores for each parameter were analysed using a one-way ANOVA. There was a main effect of genotype for spread, $F(1, 21) = 6.71$, MSE = 44.19, $p = .017$, but not for peak rate or peak time $Fs < 1$. Analysis of the R^2 score also showed no genotype difference, $F(1, 21) = 1.32$, MSE = .002, $p > .05$, $\eta_p^2 = .19$

Trace interval Gaussian scores for each parameter revealed no genotype differences $Fs < 1$. Analysis of the R^2 score also showed no genotype difference $F < 1$.



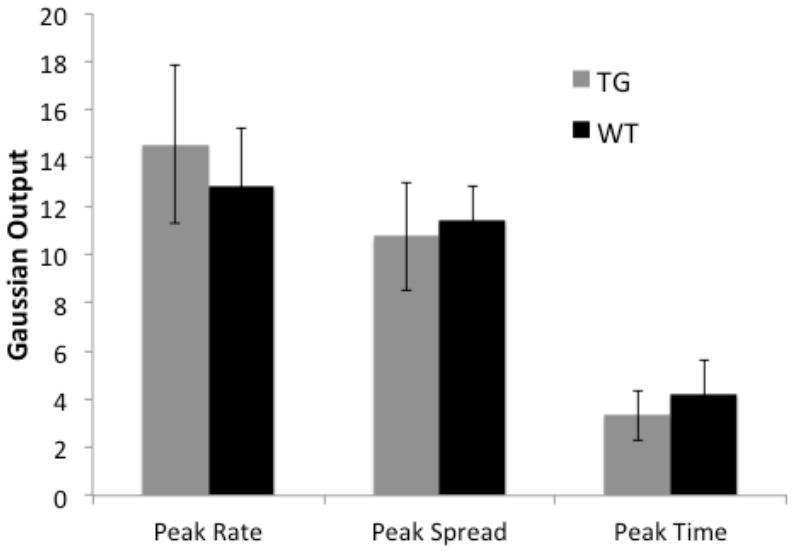


Figure 3.5. Group mean Gaussian parameter values (peak rate, spread, peak time) for the delay CS (top panel) and trace interval (lLower panel) during Experiment 1. Error bars show standard error of the mean.

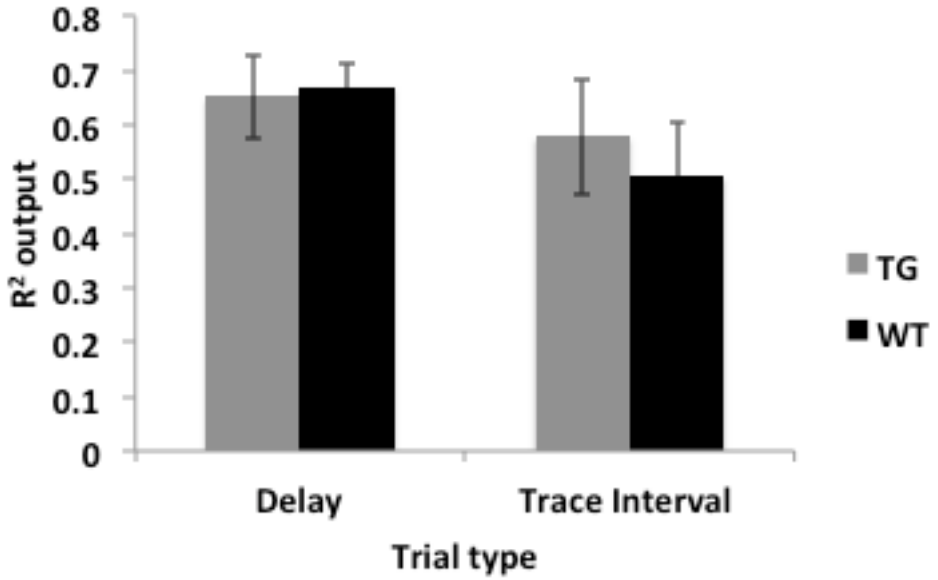


Figure 3.7. Group mean R^2 values for the delay CS and trace interval during Experiment 1. Error bars show standard error of the mean.

3.14 Discussion

3.14.1 Acquisition

Experiment 1 found no difference between genotypes in the level of conditioned responding to a delay CS, consistent with previous experiments using appetitive US's (Bonardi et al., 2011; Chapter 2). Two studies have examined delay conditioning using an aversive US, with Lin et al. 2015 showing a lower level of conditioned responding to the CS between 2 and 4 months of age in the APPswe/PS1dE9 mice in comparison to wild types; however, Cheng et al. (2014) found no genotype difference in aversive delay conditioning in 6 month mice.

Experiment 1 also showed no differences between genotypes in the level of conditioned responding to the trace CS using an appetitive reward. This is a novel finding, as currently no previous studies have examined appetitive trace conditioning in the APPswe/PS1dE9 mouse. Trace conditioning using an aversive US's has also not been assessed in the APPswe/PS1dE9 mouse; however, different APPswe mouse models of AD have shown evidence of a reduction in conditioned responding to the trace CS compared to wild types (Guart et al., 2008; Kishimoto et al., 2012, 2013; Ohno et al., 2006).

The level of conditioned responding was lower during the trace CS than the delay CS in both genotypes, which is consistent with trace CS – US association being harder to learn (Bangasser et al. 2006, Benoit et al. 1999, Fox et al. 1998, Han et al. 1996, Holland et al. 2003, McEchron et al. 1980, Solomon et al. 1986, Weiss et al. 1999).

One possible explanation for the lack of a selective trace-conditioning deficit in the transgenic mice is the length of the trace interval in Experiment 1. A number of studies have only found impaired trace conditioning with longer trace intervals. Chowdhury et al. (2005) conditioned mice with post-training dorsal hippocampal lesions on a trace fear conditioning paradigm consisting of a 20-s CS and either a 1-s, 3-s or 20-s trace interval before the US. Hippocampal lesions only reduced the level of conditioned freezing in the 20-s trace test. Misane et al. (2005) used NMDA receptor blockade of the dorsal hippocampus before trace conditioning with a 30-s CS, and either a 1-s, 3-s, 5-s, 10-s, 15-s, 30-s and 45-s trace interval. Hippocampal disruption impaired conditioned freezing during test in the 15-s and 30-s trace interval group, whilst the 45-s trace interval group failed to show conditioned freezing compared to shams. Ohno et al. (2006) found impaired auditory trace fear conditioning using a 15-s CS and a 30-s trace interval. The trace

procedure employed in Experiment 1 consisted of a 15-s CS, which is comparable to Ohno et al; however, the trace interval was only 5-s which is much shorter than previous studies. The cumulative CS duration (CS + trace interval) was also much shorter in Experiment 1.

3.14.2 Test

Experiment 1 also showed that APPswe/PS1dE9 mice had a greater spread score in comparison to wild type mice, during the delay test trials. The greater spread is believed to reflect increased variability or loss of precision in estimating the arrival of a food reward. This result parallels findings from human Alzheimer's disease literature that shows greater variability in estimating time intervals using a number of different procedures (Carrasco et al., 2000; Caselli et al., 2009; Nichelli et al., 1993; Papagno et al., 2004; Rueda & Schmitter-Edgecombe, 2009). This loss of precision, but no shift in peak time, has also been observed in rats with lesions of the ascending serotonergic system (dorsal & median raphe nuclei; Morrissey et al., 1994), whilst lesions of the dopaminergic system in both rats and mice produce an increase in variability and a shift in the peak time (Drew et al., 2003; 2007; Matell et al., 2003). Experiment 1 showed no shift in peak time, which suggests that both genotypes were still able to learn the point at which a food reward was delivered. Lesions

of the hippocampus have often resulted in an underestimation of the arrival of a timed food reward (Balci et al., 2009; Meck et al., 1984, 1987; Moyer et al. 1990; Solomon et al. 1986; Tam & Bonardi, 2012, 2012a; Weiss et al. 1999; Yin & Meck, 2014). Whilst this was not observed in Experiment 1, it seems plausible that older mice with greater levels of AD pathology in the hippocampus may well show a disruption in peak time as well as spread, due to hippocampal lesions causing early shifts in peak time.

No differences were observed between genotypes in the timing of food reward during the trace test trials. The lack of an observed timing impairment may well be due to manipulating the trace interval length as opposed to increasing the duration of the CS itself. The trace interval is a poor predictor of food, compared to the CS itself, so manipulation of the trace interval may well have had little effect on timing the food reward.

3.15 Experiment 2

Experiment 1 found impaired performance when APPswe/PS1dE9 mice were required to estimate the time of US delivery from a previously learned CS-US association (delay conditioning) using the peak procedure. To further examine this finding Experiment 2 gave 4-5 month APPswe/PS1dE9 mice appetitive conditioning to two

delay CSs of different lengths (10s & 20s). Following acquisition the temporal distribution of conditioned responding was examined once again by introducing peak test sessions. Experiment 2 also introduced a third non-reinforced auditory stimulus, which was presented at both the 10s and 20s duration. This was done to increase the level of attention to the two reinforced delay CSs during acquisition, as pilot studies without the third stimulus showed a lack of conditioned responding to the reinforced stimuli during the test sessions.

Having two different stimulus lengths allowed Experiment 2 to assess whether or not timing behaviour in APPswe/PS1dE9 and wild type mice obeys Weber's Law (scalar property). Weber's law in interval timing states there is a linear relationship between the objective time and the variability or error in time estimation. As the peak time increases, peak spread or variability also increases (Buhusi et al., 2009; Gibbon, 1977). This appears to hold true in normal wild type animals, whilst a lack of scalar property has been observed after lesions of the ventral hippocampus and in clinical populations (Parkinson's & Alzheimer's disease; Caselli et al., 2009; Yin & Meck., 2014). Weber's law should be a constant regardless of the interval lengths being timed, for example, Caselli et al (2009) computed the

coefficient of variation (CV), a measure of Weber's law, for short (100 – 600 ms) and long (1000 – 3000ms) estimated durations for young, healthy old and AD participants. The results showed a significant difference in CV scores between the short and long interval estimates in the AD participants, whilst young controls showed the same CV at both durations.

We predict that APPswe/PS1dE9 mice will once again show normal appetitive conditioning to both the 10s and 20s CS, whilst showing impaired precision in timing during the peak procedure to both CSs.

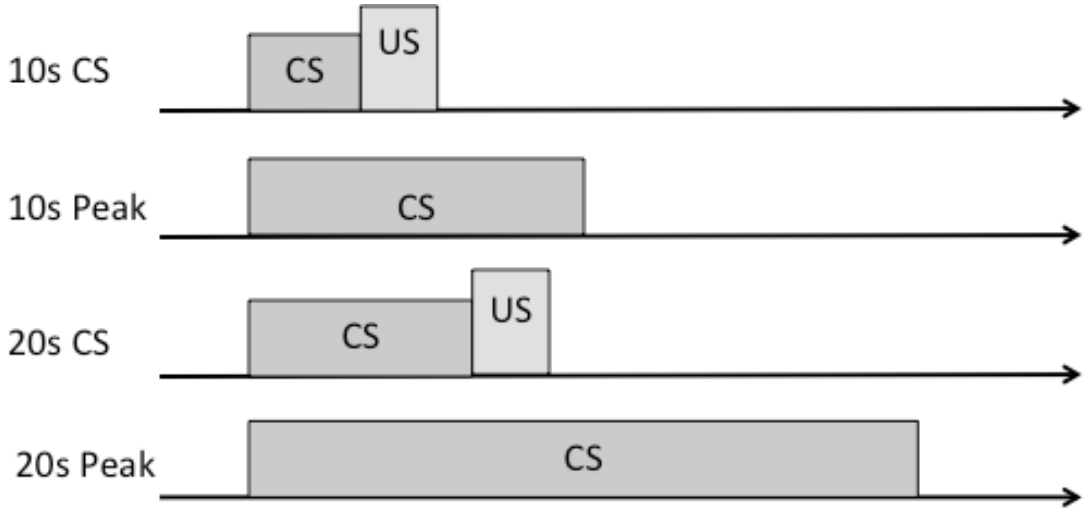


Figure 3.8. Schematic of Experiment 2.

3.16 Methods

3.16.1 Animals

24 young (16 and 20 week old) APPswe/PS1dE9 mice were used in two replications. 12 naïve 16-week-old male mice were used for the

first replication: 6 APPswe/PS1dE9 (ad lib. weight 32.33g: range, 27.4 – 35.9 g) and 6 wild type littermates (ad lib. weight 32.783g: range, 30.6 – 33.1 g). 12 naïve 20-week-old male mice were used for the second replication: 6 APPswe/PS1dE9 (ad lib. weight 33.3g: range, 26.7 – 34g) and 6 wild type littermates (ad lib. weight 30.25g: range, 27.4 – 39.45g).

3.16.2 Apparatus

The same apparatus was used as in Experiment 1, but with the addition of a third auditory stimulus, a 75-dB, 2-Khz tone.

3.17 Procedure

Experiment 2's procedures were identical to those of Experiment 1 unless specifically stated.

3.17.1 *Acquisition*

Three different trial types were presented during the acquisition phase (Figure 3.8). A 15-s pre-CS period was used for all of the three trial types. Short delay trials consisted of the pre-CS period, immediately followed by a 10-s CS presentation before a single sucrose pellet was delivered at CS termination. Long delay trials were identical to short delay trials except that the CS lasted 20s. The non-reinforced trials consisted of the same pre-CS period, followed

immediately by either a 10s or 20s CS (half the trials were 10s CS presentations, the remainder lasted 20s). A sucrose pellet did not follow the non-reinforced CSs. Each session consisted of 42 trials: 14 short delay, 14 long delay and 14 non-reinforced trials. The 75-dB, 2-Khz sine wave was the non-reinforced - CS for all the animals.

3.17.2 Peak test

The acquisition sessions were followed by six peak sessions. Each session was identical to the acquisition sessions, except that three of the short and three of the long trials were replaced with peak trials. The short peak trial was composed of a 30s CS presentation, and the long peak trial of a 60s CS period. No sucrose reward was delivered during the peak trials.

3.18 Data treatment

3.18.1 Acquisition

Head entry responding during the non-reinforced trials was recorded in the same way as during the reinforced trials in Experiment 1.

3.18.2 Peak test

The test session data was split into two three-session blocks, before being analyzed in the same way as in Experiment 1 with the addition of block as a factor.

One wild type mouse was excluded from the Gaussian analysis; this animal produced a spread score (136.86) over four standard deviations (SD 26.47) larger than the mean (mean 17.92) and was associated with a R^2 score of .003, the lowest of all animals.

2.18.3 CV

The coefficient of variation (CV) was calculated for each mouse by dividing the spread by the peak time (spread/peak time). CV gives a measure of whether or not the spread of responding is related to the CS length being timed. According to Weber's law the CV score should be the same between both genotypes and CS durations. 3 APPswe/PS1dE9 mice were removed from the CV analysis due to producing peak times of zero, which would yield a meaningless value of CV (infinity). CVs were analyzed using mixed repeated measures ANOVA with genotype, CS length and block as variables.

3.19 Results

3.19.1 Acquisition of Pavlovian conditioning

During acquisition both genotypes show discrimination between the reinforced and non-reinforced CSs from session three onwards (Figure 3.9). An ANOVA with genotype, trial type (10s, 20s & non-reinforced CS) and session revealed a main effect of session, $F(5,$

110) = 30.15, MSE = 65.90, p <.001, η_p^2 = .578 (Greenhouse-Geisser corrected) and trial type, F(2, 44) = 41.33, MSE = 57.87, p < .001, η_p^2 = .653. Significant interactions were found between session and trial type, F(10, 220) = 10.75, MSE = 22.51, p <.001, η_p^2 = .328, and also between session and genotype, F(5, 110) = 5.703, MSE = 65.90, p =.002, η_p^2 = .206 (Greenhouse-Geisser corrected).

The session and trial type interaction showed differences between trial types from session two onwards (smallest, F(2, 220) = 9.29, MSE = 20.33, p = .001, η_p^2 = .065 for session 2). The analysis also showed increased responding over the six sessions for the 10s CS, F(5, 220) = 31.42, MSE = 21.41, p <.001, η_p^2 = .32 and the 20s, CS F(5, 220) = 28.58, MSE = 21.41, p <.001, η_p^2 = .30, whilst responding to the non reinforced CS decreased, F(5, 220) = 7.20, MSE = 21.41, p <.001 , η_p^2 = .098

To analyze responding between trial types during the six sessions, individual one-way ANOVA were carried out for each session with trial type as a factor. Post hoc pairwise comparison (Bonferroni corrected) was used to assess the differences between trial types. Regardless of genotype, mice showed no difference in responding between trial types during session 1 (F <1). Responding to the 10s was greater than the 20s CS during session 2 (p = .024),

but not sessions 3-6 (smallest p = .32, during session 3). Both the 10s and 20s CS's showed greater responding than the non-reinforced CS between sessions 2-6 (10s – Non, largest p <.001, 20s – non, largest p = .026).

Analysis of the session by genotype interaction showed a difference in response rates between genotypes only during session two, $F(1, 110) = 6.684$, MSE = 71.28, p = .01, η_p^2 = .048, reflecting greater responding by the wild type mice. Overall increase in responding across the six sessions for both the wild types, $F(5, 110) = 16.53$, MSE = 38.58, p <.001, η_p^2 = .42 and the APPswe/PS1dE9 mice, $F(5, 110) = 19.32$, MSE = 38.58, p <.001, η_p^2 = .46.

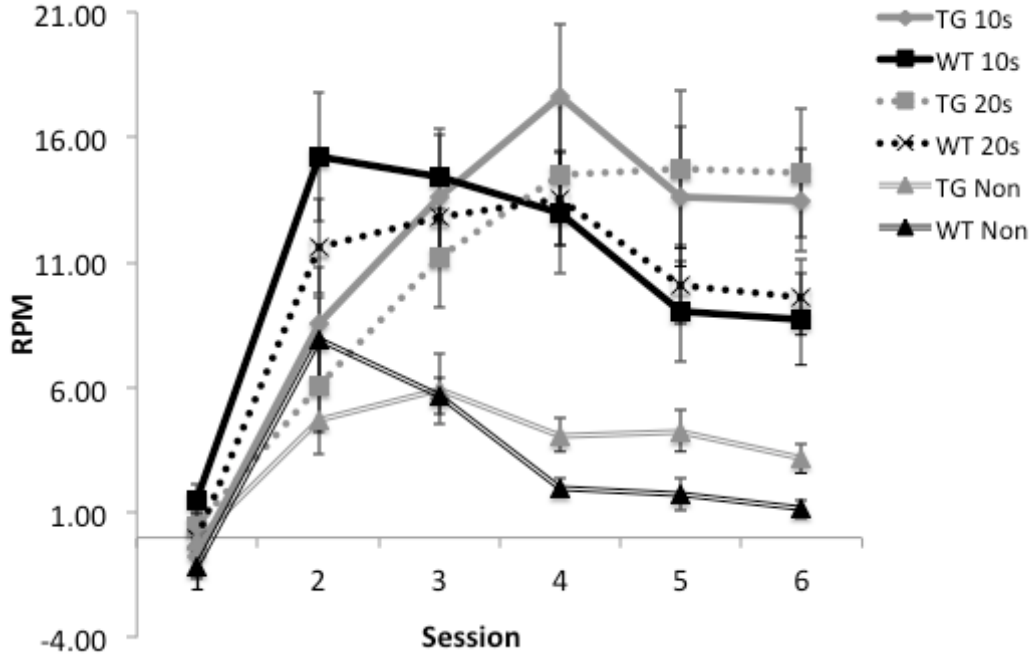


Figure 3.9. Mean difference scores for the short (10s), long (20s) and non-reinforced CSs during the six acquisition sessions in Experiment 2. Error bars show standard error of the mean.

APPswe/PS1dE9 mice show slightly greater pre-CS responding than wild types, across all sessions (Table 3.3). The analysis revealed a main effect of session, $F(5, 110) = 33.63$, MSE = 5.304, $p < .001$, $\eta_p^2 = .605$ (Greenhouse-Geisser corrected), and a main effect of genotype, $F(1, 22) = 7.90$, MSE = 3.26, $p = .01$, $\eta_p^2 = .264$. No other main effects or interactions were significant observed (smallest $p = .076$, for session by genotype interaction).

Pre-CS responding						
	Session					
	1	2	3	4	5	6
TG	5.15	5.19	2.82	2.48	1.42	1.33
	(.53)	(.45)	(.42)	(.44)	(.29)	(.33)
WT	5.45	3.01	2.01	1.11	0.87	0.84
	(.92)	(.35)	(.28)	(.24)	(.24)	(.15)

Table 3.3. Mean level of pre-CS responding during the six acquisition sessions in Experiment 2. Brackets show standard error of the mean.

3.19.2 Peak Procedure

Responding to the 10s CS was greater than to the 20s CS during these trials (Figure 3.10). The analysis found a main effect of session, $F(5, 110) = 8.113$, MSE = 31.99, $p < .001$, $\eta_p^2 = .269$ (Greenhouse-Geisser corrected) reflecting a decrease in responding for both trial types. The analysis also found a main effect of trial type, $F(1, 22) = 33.33$, MSE = 49.04, $p < .001$, $\eta_p^2 = .602$ reflecting a lower rate of responding for the 20s than the 10s CS. No other main effects or interaction were significant (smallest $p = .063$, session * trial type interaction).

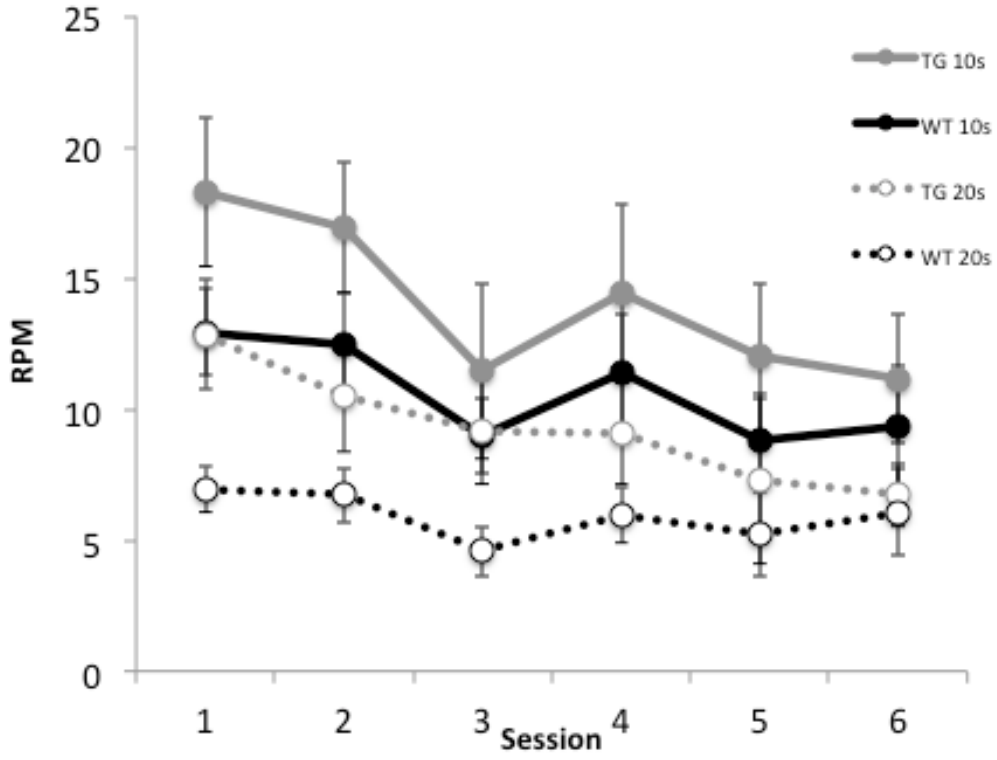


Figure 3.10. The mean discrimination ratio to the short (10s) and long (20s) delay CSs during the six test sessions of Experiment 2. Error bars show standard error of the mean.

Figure 3.11 shows the normalized responding during both the 10s and 20s CS from which the Gaussian function was calculated. The group mean scores for peak rate, spread and peak times are presented in Figure 3.12, and R^2 in Figure 3.13. The APPswe/PS1dE9 mice appear to have greater peak rate and spread scores than wild types in both the 10s and 20s CS trials during the first block. Higher peak rates are also observed in the APPswe/PS1dE9 mice during the second block for both CS types.

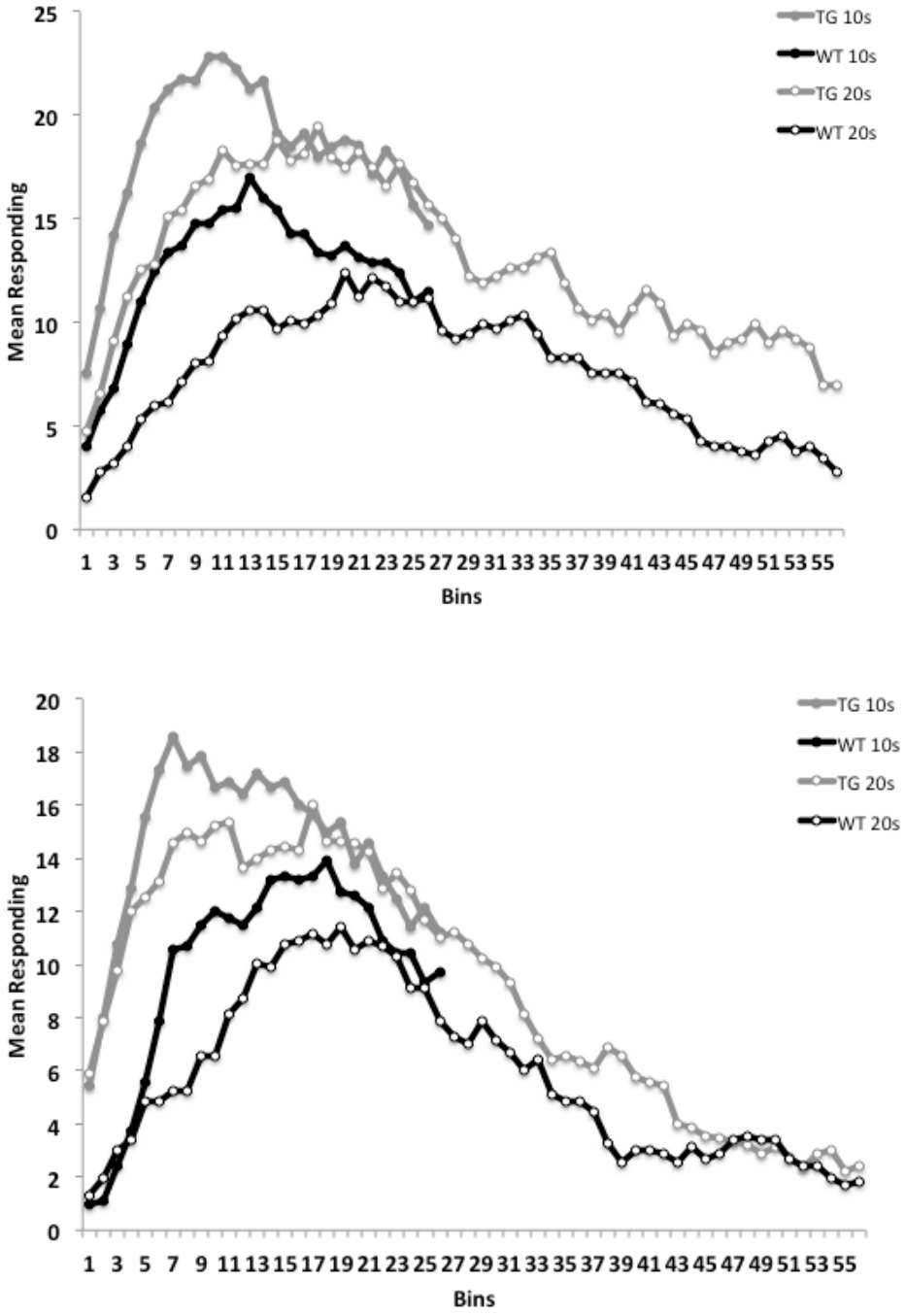


Figure 3.11. Mean level of responding, in 1-second bins, to the 10s (short) and 20s (long) delay peak trials, split between block 1 (top panel) and 2 (bottom panel) during the six test sessions of Experiment 2.

3.19.3 Peak Rate

Each Gaussian parameter was analyzed separately using an ANOVA with genotype, CS length (10s or 20s) and block (block 1 & block 2) as factors. The peak rate revealed only a difference in CS length, $F(1, 21) = 14.910$, MSE = 23.32, $p = .001$, $\eta_p^2 = .415$, with higher peak rates associated with the short 10s CS. No other main effects or interactions were observed (smallest $p = .068$ for the CS length * block interaction).

3.19.4 Spread

Spread scores revealed a main effect of genotype, $F(1, 21) = 8.863$, MSE = 48.22, $p = .007$, $\eta_p^2 = .297$, reflecting higher spread scores in the APPswe/PS1dE9 mice, a main effect of CS length, $F(1, 21) = 17.288$, MSE = 66.55, $p < .001$, $\eta_p^2 = .308$, reflecting greater spread in the longer 20s CS, and a main effect of block, $F(1, 21) = 9.35$, MSE = 37.66, $p = .006$, $\eta_p^2 = .452$, showing greater spread scores in the first block. The interaction between block and genotype almost reached significance $F(1, 21) = .409$, MSE = 66.55, $p = .053$, $\eta_p^2 = .166$; analysis of this interaction showed greater spread scores for the APPswe/PS1dE9 mice than wild types during block 1 $F(1, 21) = 12.85$, MSE = 42.94, $p < .001$, $\eta_p^2 = .23$ but not block 2 $F < 1$. The interaction also showed a reduction in spread scores from block 1 to

2 in the APPswe/PS1dE9 mice $F(1, 21) = 13.62$, MSE = 37.66, $p = .001$, $\eta_p^2 = .39$, but no difference between blocks for the wild types $F < 1$.

3.19.5 Peak Time

Peak time scores revealed a main effect of CS length, $F(1, 21) = 16.20$, MSE = 47.63, $p = .001$, $\eta_p^2 = .436$ and an interaction between CS length and block, $F(1, 21) = 6.68$, $MSE = 29.82$, $p = .017$, $\eta_p^2 = .242$. The interaction revealed a significant difference in peak time between CS lengths during the first block, $F(1, 21) = 22.42$, MSE = 38.72, $p < .001$, $\eta_p^2 = .34$, reflecting accurate differentiation between the short and long CSs, but no difference during the second block, $F(1, 21) = 2.35$, MSE = 38.72, $p > .05$. The interaction also showed a significant change in peak time between block 1 and 2 only in the 20s CS peak trials, $F(1, 21) = 6.14$, MSE = 40.52, $p = .017$, $\eta_p^2 = .12$; the peak time in block 2 (mean = 19.5s) was much closer to the target duration (20s) than that in block 1.

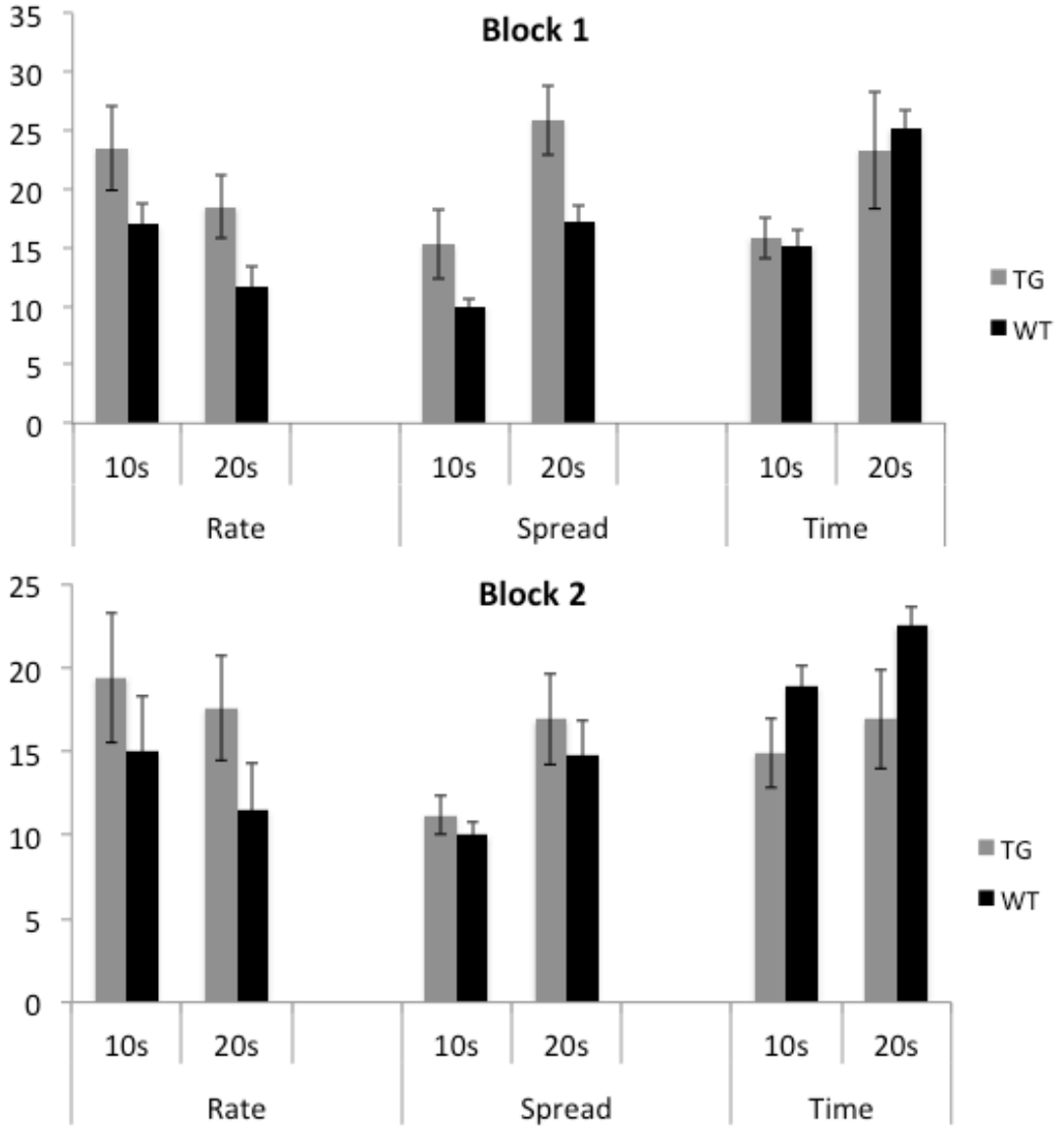


Figure 3.12. Values of peak rate, spread and peak time for the 10s (short) and 20s (long) delay CSs during blocks 1 (top panel) and 2 (bottom panel) of the test of Experiment 2. Error bars show standard error of the mean.

R²

The R^2 results showed a main effect of CS length, $F(1, 21) = 8.19$, MSE = .036, $p = .009$, $\eta_p^2 = .201$, and of block, $F(1, 21) = 5.28$, MSE = .033, p

= .032, η_p^2 = .281, and also an interaction between block and genotype, $F(1, 21) = 5.16$, MSE = .033, $p = .034$, $\eta_p^2 = .197$. The simple main effects revealed higher R^2 scores for the APPswe/PS1dE9 than wild types during block 1, $F(1, 21) = 10.92$, MSE = 0.03, $p = .003$, $\eta_p^2 = .34$. No other main effects or interactions were significant (smallest $p = .433$).

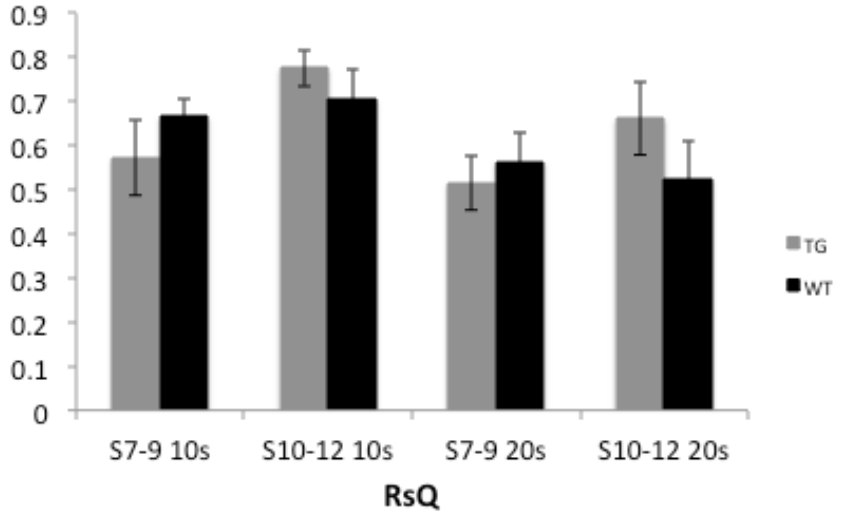


Figure 3.13. Mean R^2 scores for the 10s (short) and 20s (long) delay CSs during blocks 1 and 2 of the test sessions of Experiment 2. Error bars show standard error of the mean.

3.19.6 CV

The APPswe/PS1dE9 mice appear to show a numerically larger value of CV for the longer CS duration than the shorter, which would violate Weber's law (Figure 18). However, there was no significant

main effects or interactions (smallest p = .142). The lack of an effect of CS length or an interaction between genotype and CS length on CV scores gives support for the intact timescale-invariance of interval timing in the APPswe/PS1dE9 mice.

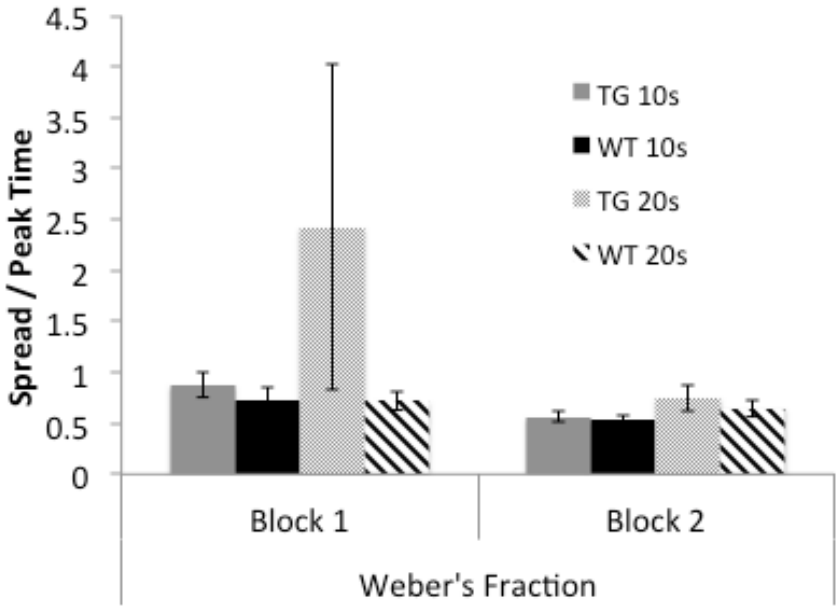


Figure 3.14. The coefficient of variation (CV) scores for the 10s (short) and 20s (long) delay CSs, for blocks 1 and 2 of the test sessions in Experiment 2. Error bars show standard error of the mean.

3.20 Further analysis

One simplistic explanation for the greater spread scores in the APPswe/PS1dE9 mice is that they are being produced by the higher rates of responding in the transgenic mice. For this explanation to be the cause, peak rate and spread should be correlated. To test this

hypothesis, correlations were performed between peak rate and spread for both the 10s and 20s delay CSs (Table 3.4, Figure 3.15), split by genotype. Table 3.4 shows the Pearson's r correlations between peak rate and spread, whilst Figure 3.15 shows the relationship between the two variables. For the APPswe/PS1dE9 mice there was a significant negative correlation between peak rate and spread during block 1 of the 10s CS condition (r = -0.581, n = 12, p = .045). The results of the correlations show no evidence of a strong positive correlation between peak rate and peak spread.

		10s CS		*20s CS*	
Block 1		*R*	*P value*	*R*	*P value*
	TG	*-0.58*	*0.048**	*-0.34*	*0.276*
	WT	*0.21*	*0.518*	*-0.27*	*0.411*
Block 2					
	TG	*0.13*	*0.682*	*-0.44*	*0.145*
	WT	*-0.57*	*0.062*	*-0.50*	*0.113*

*Table 3.4. Pearson R correlations between peak rate and peak spread, for both the 10s (short) & 20s (long) CS in blocks 1 and 2 during Experiment. * Designates p <.05, Error bars show standard error of the mean.*

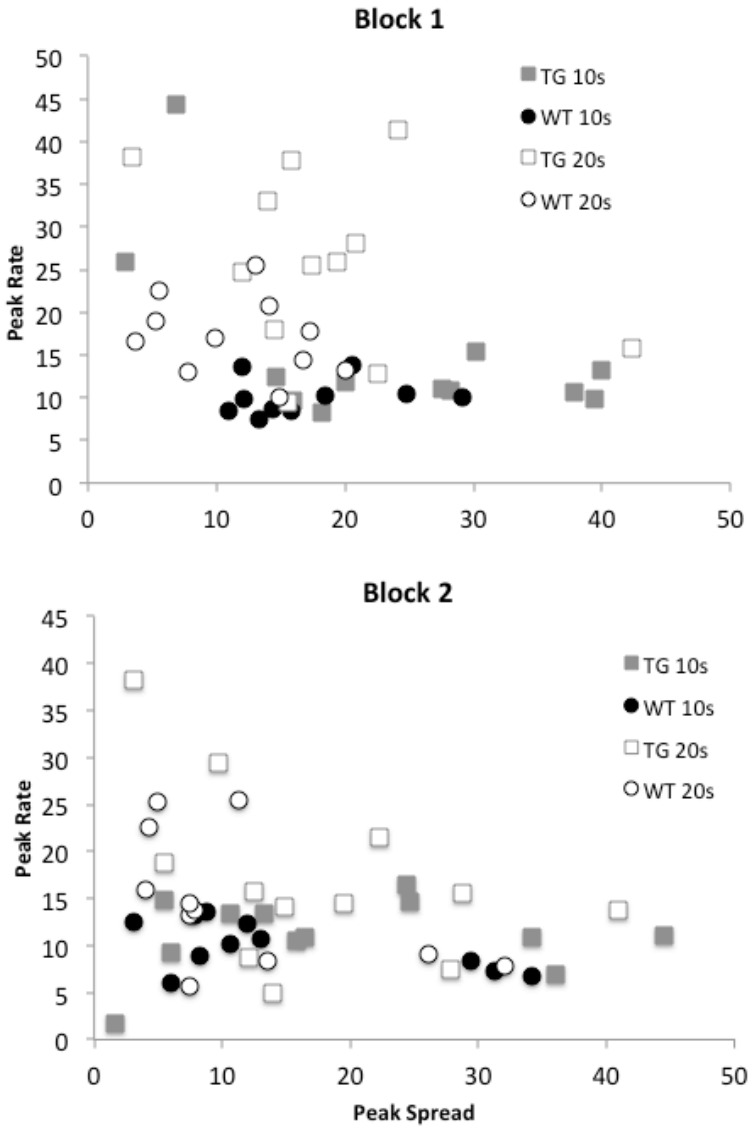


Figure 3.15. Scatter graph of the relationship between peak rate and spread. The top panel shows the relationship during block 1, whilst the bottom panel shows the relationship during block 2 for the two CS lengths and genotypes (TG: APPswe/PS1dE9 & WT: wild type).

3.21 Discussion

Experiment 2 found evidence of impaired timing precision, via increased variability in conditioned responding, in 4-5 month old

APPswe/PS1dE9 mice. No genotype differences were observed in the level of conditioned responding to two different delay CS's using an appetitive CS.

3.21.1 *Acquisition*

Experiment 2 failed to show any differences between genotype in the level of conditioned responding to both the 10s and 20s CS's using an appetitive reward. APPswe/PS1dE9 mice did show a higher rate of pre-CS (background) responding than wild types during acquisition, possibly reflecting the observed hyperactivity found by previous research in the APPswe/PS1dE9 mouse (Bonardi et al., 2011; Filali et al., 2011, Maroof et al., 2013). However, pre-CS responding was very low and unlikely to have had any major influence on the results presented.

3.21.2 Test

Experiment 2 did show evidence of a loss of precision of timing in terms of greater spread scores in the APPswe/PS1dE9 mice than wild types. This lack of temporal precision was both a replication of the results from Experiment 1 and supports findings from the human AD literature, which has also shown the loss of timing precision (Carrasco et al., 2000; Caselli et al., 2009; Nichelli et al., 1993; Papagno et al., 2004; Rueda & Schmitter-Edgecombe, 2009).

The analysis of the spread scores also revealed an almost significant interaction between block and genotype (p = .053). This interaction showed greater spread scores for the APPswe/PS1dE9 mice during the first block of three sessions but not the second. Instead of absolute disruption of temporal precision in the young APPswe/PS1dE9 mouse, it is possible that the APPswe/PS1dE9 mice may just take longer to gain temporal precision during the test trials, compared to wild types.

Experiment 2 also aimed to test the scalar nature of interval timing in these animals by testing the conformity of our data to Weber's law. The results found intact scalar property in young (4-5 month old) APPswe/PS1dE9 mice; despite showing impaired precision (greater spreads) in timing intervals, timing accuracy (peak time) and precision (spread) were linked in a linear relationship (Buhusi et al., 2009 Gibbon et al., 1997, 1984). This scalar property of interval timing has been shown to be stable over lesion and pharmacological manipulation, despite changes in peak time and spread (Balci et al., 2008; Buhusi et al., 2002; Meck et al. 1987; Tam et al., 2014).

Experiment 2 also examined the possibility that the greater spread scores observed in the APPswe/PS1dE9 mice were actually

being caused by the greater response rate seen in these animals. The correlational analysis failed to show any evidence of a positive correlation between peak rate and spread, which suggests that greater spread scores in the APPswe/PS1dE9 mice reflect a loss of timing precision rather than higher values of peak rate.

3.22 Experiment 3

Experiment 3 aimed to build on the results of Experiments 2, by carrying out an identical replication of Experiment 2 with older (8 month old) APPswe/PS1dE9 mice and wild types. Given the progressive nature of the AD, early changes in cognitive ability would be expected to worsen as the neuropathology increases.

By 8 months of age APPswe/PS1dE9 mice show an abundance of amyloid plaques in both the cortex and hippocampus. APPswe/PS1dE9 mice at 8 months old also show consistent impairments in a large number of cognitive tasks, such as the Morris water maze, radial arm water maze, Barnes maze, novel object recognition & conditioned taste aversion (Arendash et al., 2001; Cheng et al., 2014; Donkin et al., 2010; Frye et al., 2008; Lalonde et al., 2005; Pistell et al., 2008; Jardanhazi-Kurutz et al., 2010; Li et al., 2014; Ramirez-Lugo et al., 2009; Savonenko et al., 2004; Stover & Brown., 2012). The increased pathological burden and impaired

performance on a variety of cognitive tasks by 8 months suggests that APPswe/PS1dE9 at this age are more likely to show impaired performance on appetitive delay conditioning, which has not been assessed at this age, and timing US arrival via the peak procedure.

We predict that 8 month APPswe/PS1dE9 mice will show normal Pavlovian appetitive conditioning to the 10s and 20s delay CS, as observed in the younger transgenic mice in Experiment 2. We also predict that older, 8-months, APPswe/PS1dE9 mice will show increased peak spreads replicating Experiment 1 and 2 results, but significantly more pronounced.

3.23 Methods

3.23.1 Animals

Twenty-three 32-week-old APPswe/PS1dE9 mice were used in this experiment, which was carried out in two replications. 12 naïve 32-week-old male mice were used for the first replication: 6 APPswe/PS1dE9 (ad lib. weight 36.02: range, 30.4 – 40.75 g) and 6 wild type littermates (ad lib. weight 39.25g: range, 35.95 – 42.9 g). 11 naïve 32-week-old male mice were used for the second replication: 4 APPswe/PS1dE9 (ad lib. weight 34.95g: range, 33.1 – 37.15g) and 7 wild type littermates (ad lib. weight 40.06g: range, 34.25 – 44.45g).

Experiment 3's procedures were identical to those of Experiment 2 unless specifically stated.

3.24 Data treatment

3.24.1 *Peak test*

During the Gaussian analysis one APPswe/PS1dE9 mouse was removed, due to producing a peak spread score of 46290, that was more than four standard deviations (13.88) over the mean (13.7), that was associated with an R^2 score of zero. During the CV analysis four (1TG, 3WT) mice were removed due to peak times of zero.

3.25 Results

3.25.1 Acquisition

The data appears to show discrimination between the reinforced and non-reinforced CSs from session 3 onwards (Figure 3.16). The ANOVA found a main effect of session, $F(5, 105) = 27.82$, MSE = 97.90, $p < .001$, $\eta_p^2 = .570$ (Greenhouse-Geisser corrected), trial type, $F(2, 42) = 28.48$, MSE = 37.34, $p < .001$, $\eta_p^2 = .647$, and an interaction between session and trial type, $F(10, 210) = 18.59$, MSE = 20.67, $p < .001$, $\eta_p^2 = .470$, genotype and session, $F(5, 105) = 5.69$, MSE = 97.90, $p = .003$, $\eta_p^2 = .213$ (Greenhouse-Geisser corrected) and a three way interaction between session, trial type and genotype, $F(10,$

210) = 4.20, MSE = 20.67, p = .002, η_p^2 = .167 (Greenhouse-Geisser corrected).

The three-way interaction was split by genotype and reanalyzed, the APPswe/PS1dE9 mice showed a main effect of trial type, $F(2, 18) = 27.22$, MSE = 33.11, p <.001, η_p^2 = .752 , a main effect of sessions, $F(5, 45) = 22.59$, MSE = 46.85, p <.001, η_p^2 = .715, and an interaction between trial type and session, $F(10, 90) = 13.69$, MSE = 26.41p = .000, η_p^2 = .603. The interaction revealed differences between the CSs from session 3 onwards, $F(2, 18) = 3.64$, MSE = 33.11, p = .047, η_p^2 = .28. Pairwise analysis found no difference between CS's during sessions 1 and 2 (Fs < 1). Greater responding to the 10s verses 20s CS was found only during session 4 (p = .003). The 10s CS showed greater responding compared to the non-reinforced CS during sessions 3 – 6 (largest p = .031), whilst greater responding to the 20s CS compared to the non-reinforced was only seen between sessions 5 – 6 (largest p = .004). Increased responding over the six sessions was observed for the 10-s, $F(5, 45) = 17.11$, MSE = 46.85, p <.001, η_p^2 = .65 and 20-s, $F(5, 45) = 9.91$, MSE = 46.85, p <.001, η_p^2 = .52, CSs, while responding to the non-reinforced CS showed no change over the sessions, $F(5, 45) = 1.15$, MSE = 46.85, p = .34.

The wild type mice also revealed a main effect of trial type $F(2, 24) = 13.94$, MSE = 40.51, $p < .001$, $\eta_p^2 = .537$, session $F(5, 60) = 10.50$, MSE = 52.25, $p < .001$, $\eta_p^2 = .467$ and a trial type session interaction, $F(10, 120) = 8.17$, MSE = 28.06, $p < .001$, $\eta_p^2 = .405$ (Greenhouse-Geisser corrected). A difference between CSs from session 3 onwards, $F(2, 24) = 4.78$, MSE = 40.51, $p = .017$, $\eta_p^2 = .28$. Analysis of the difference in responding between CS showed no differences between sessions 1 and 2 ($Fs < 1$). No differences were found between the 10s and 20s CS during any of the sessions ($Fs < 1$), whilst greater responding to the 10s and non-reinforced, and the 20s and non-reinforced CS found between sessions 4 – 6 (largest $p = .003$ & $p = .005$). The analysis also showed an increase in responding over sessions for the 10-s, $F(5, 60) = 6.40$, MSE = 52.25, $p < .001$, $\eta_p^2 = .34$ and 20-s, $F(5, 60) = 6.45$, MSE = 52.25, $p < .001$, $\eta_p^2 = .34$, CSs, but not the non-reinforced CS $F < 1$. No other main effects or interactions were significant (smallest $p = .154$).

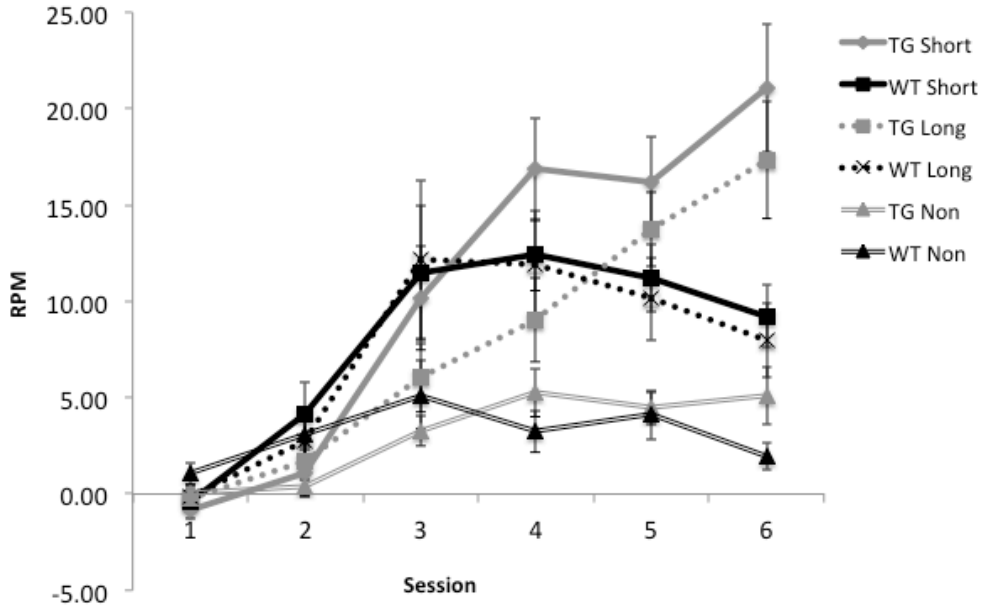


Figure 3.16. Mean difference scores for the 10s (short), 20s (long) and non-reinforced CSs during the six acquisition sessions of Experiment 3. Error bars show standard error of the mean.

APPswe/PS1dE9 mice show greater pre-CS responding (Table 3.5), which was confirmed with a significant genotype effect, $F(1, 21) = 4.93$, MSE = 3.00, $p = .037$, $\eta_p^2 = .297$ main effect of session $F(5, 105) = 10.73$, MSE = 3.03, $p < .001$, $\eta_p^2 = .283$, confirming a reduction in responding over the six sessions. No other main effects or interactions were significant (smallest $p = .343$).

Pre-CS responding						
	Session					
	1	2	3	4	5	6
TG	1.88	2.77	2.19	2.09	1.47	0.82
	(.41)	(.42)	(.36)	(.48)	(.34)	(.22)
WT	2.04	2.90	2.31	1.61	1.41	1.07
	(.35)	(.34)	(.30)	(.28)	(.22)	(.18)

Table 3.5. Mean level of pre-CS responding during the six acquisition sessions in Experiment.. Error bars show standard error of the mean.

3.25.2 Peak Procedure

Responding to the 10-s CS was higher than to the 20-s CS, and the APPswe/PS1dE9 mice appeared to show a higher rate of responding to both (Figure 3.17) than controls. The ANOVA showed a main effect of session, $F(5, 105) = 13.81$, MSE = 17.20, $p < .001$, $\eta_p^2 = .39$, trial type, $F(1, 21) = 48.57$, MSE = 48.11, $p < .001$, $\eta_p^2 = .698$, and genotype, $F(1, 21) = 6.76$, MSE = 442.85, $p = .017$, $\eta_p^2 = .244$. An interaction between session and trial type was also observed, $F(5, 105) = 6.75$, MSE = 9.83, $p < .001$, $\eta_p^2 = .243$, showing greater responding to the 10-s CS than the 20-s CS from session 1 onwards (smallest $F(1, 126) = 48.98$, MSE = 16.21, $p < .001$, $\eta_p^2 = .27$). As well

as a reduction in responding over the sessions for both the 10-s, $F(5, 105) = 18.72$, MSE = 13.51, $p < .001$, $\eta_p^2 = .30$ and the 20-s CS, $F(5, 105) = 2.63$, MSE = 13.51, $p = .024$, $\eta_p^2 = .059$. No other significant main effects or interactions were observed (smallest $p = .113$).

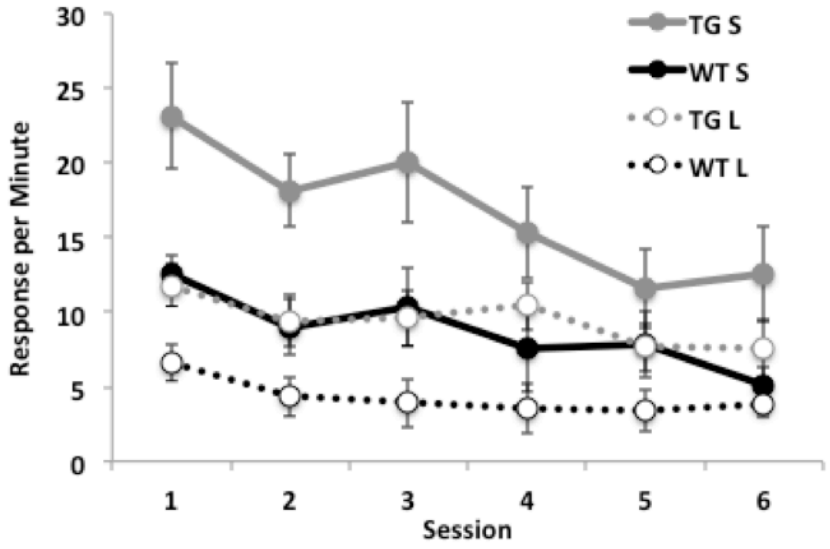


Figure 3.17. Mean difference scores to the 10s (short) and 20s (long) delay CSs during the six test sessions of Experiment 3. Error bars show standard error of the mean.

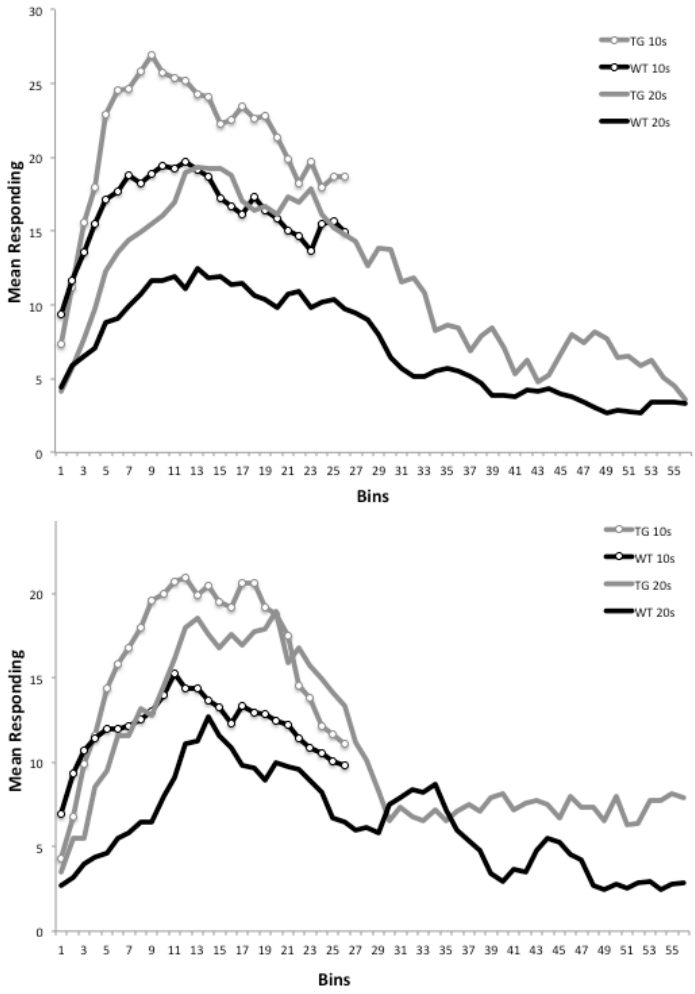


Figure 3.18. The mean level of responding, in 1-second bins, to the 10s (short) and 20s (long) delay peak trials, split between block 1 (top panel) and 2 (bottom panel) during the six test sessions of Experiment 2 for the TG (APPswe/PS1dE9) and WT (wild type) mice.

3.29.3 Peak Rate

APPswe/PS1dE9 mice appear to show higher peak rates than wild types during both blocks. The ANOVA showed a main effect of block, $F(1, 20) = 27.47$, MSE = 10.67, $p < .001$, $\eta_p^2 = .579$, CS $F(1, 20) = 30.44$,

MSE = 26.66, p <.001, η_p^2 = .604 and genotype, F(1, 20) = 4.82, MSE = 394.54, p =.04, η_p^2 = .194, and an interaction between block and CS, F(1, 20) = 22.53, MSE = 8.56, p <.001, η_p^2 = .530, showing greater values of peak rate with the 10-s CS in block 1 than in block 2, F(1, 20) = 48.01, MSE = 9.61, p <.001, η_p^2 = .54. The analysis also showed greater values of peak rate for the 10-s CS than the 20-s CS during block 1, F(1, 20) = 48.31, MSE = 17.61, p <.001, η_p^2 = .54. An interaction between CS and genotype F(1, 20) =4.467, MSE = 26.66, p = .047, was also seen; showing greater peak rates in the APPswe/PS1dE9 mice than the wild types during the 10-s CS, F(1, 20) = 7.06, MSE = 210.60, p = .011, η_p^2 = .15, whilst peak rates were greater for the 10-s than the 20-s CS for both the APPswe/PS1dE9, F(1, 20) = 26.69, MSE = 26.66, p <.001, η_p^2 = .57 and the wild types, F(1, 20) = 6.37, MSE = 26.66, p <.02, η_p^2 = .24. No other significant main effects or interactions were found (smallest p = .152; Figure 3.19).

3.25.4 Spread

Analysis revealed a main effect of CS, F(1, 20) = 5.08, MSE = 154.66, p = .036, η_p^2 = .203, reflecting greater spreads for the 20-s CSs, but not for genotype F < 1. An interaction between CS and genotype was also observed F(1, 20) = 5.93, MSE = 154,66, p = .024, η_p^2 = .229. Simple

main effects showed a greater spreads for the 20-s than the 10-s CS in the APPswe/PS1dE9 mice, $F(1, 20) = 10.08$, MSE = 154.66, p = .004, $\eta_p^2 = .33$. No other main effects or interactions were significant (smallest $p = .28$; Figure 3.19).

3.25.5 Peak Time

A significant different between CS lengths, $F(1, 20) = 7.37$, MSE = 88.15, $p = .013$, $\eta_p^2 = .269$, reflecting discrimination between the short (10s) and longer (20s) CSs. No main effect of genotype, $F(1, 20) = 2.03$, MSE = 107.82, $p > .05$, $\eta_p^2 = .093$, and nothing else significant (smallest $p = .15$; Figure 3.19).

3.25.6 R^2

The R^2 scores (Figure 3.20) revealed no significant main effects or interactions (smallest $p = .087$, for CS length).

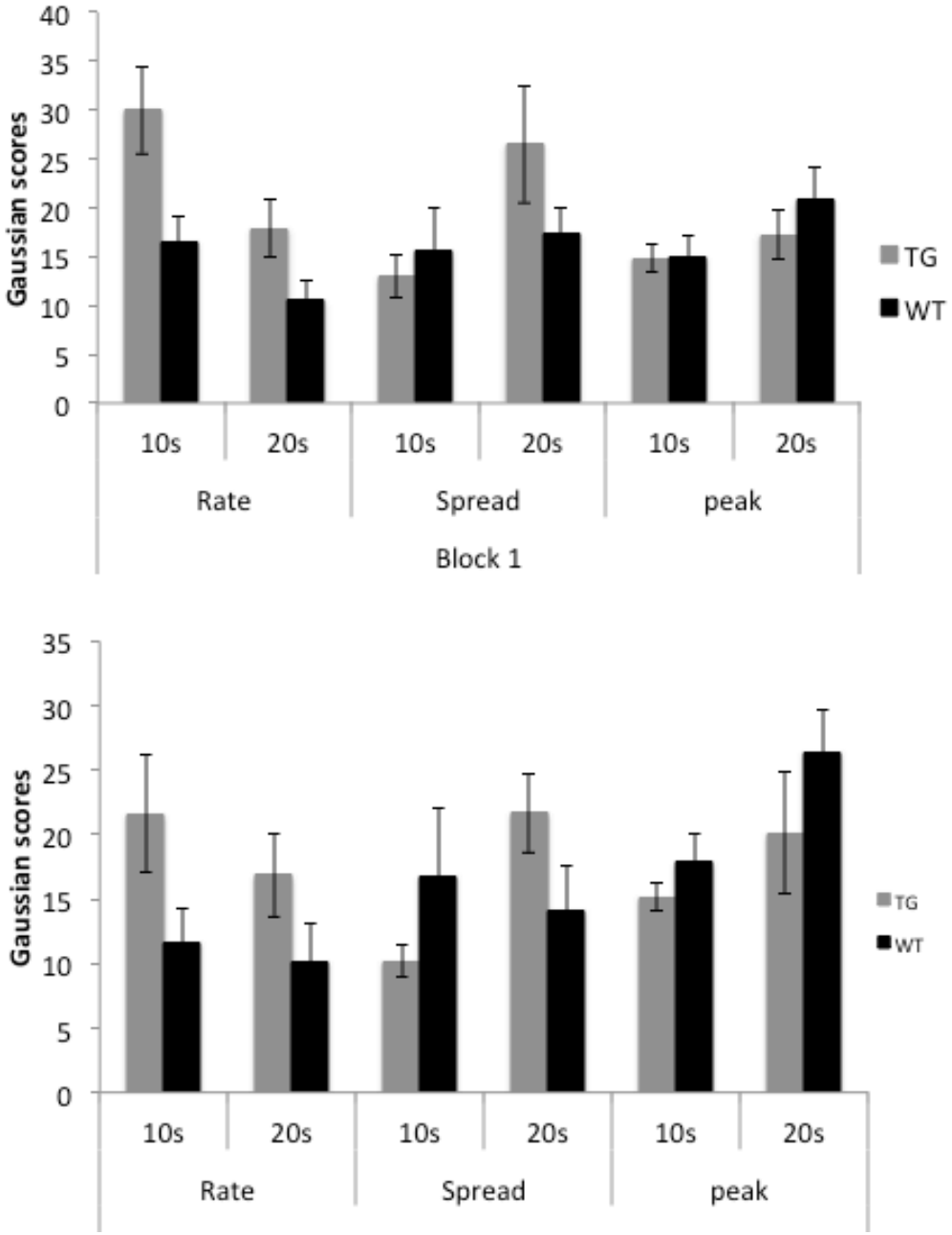


Figure 3.19. Gaussian parameter values (peak rate, spread and time) for the 10s (short) and 20s (long) delay CS's during blocks 1 and 2 during Experiment 3. Error bars show standard error of the mean.

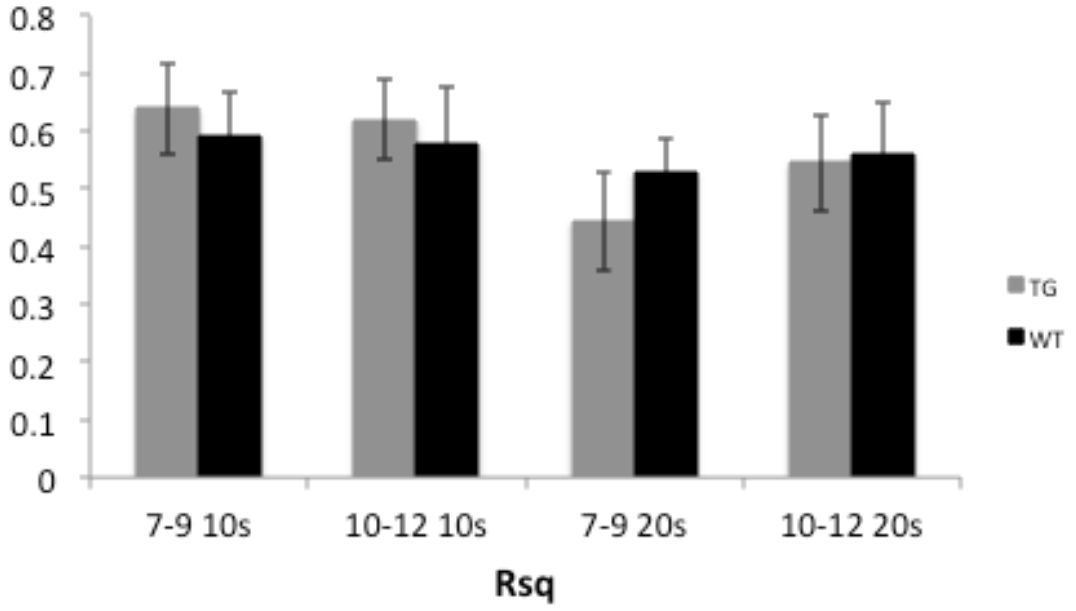


Figure 3.20. Mean R^2 scores for the Gaussian model for S7-9 (block 1) and S10-12 (block 2) and the 10s (short) and 20s (long) delay CS's during the test sessions for Experiment 3. Error bars show standard error of the mean.

3.25.7 CV

Once again the APPswe/PS1dE9 mice appeared to show numerically large CV values during the longer duration (Figure 3.21), however no significant effects or interactions (smallest p = .084 for the main effect of genotype) were found.

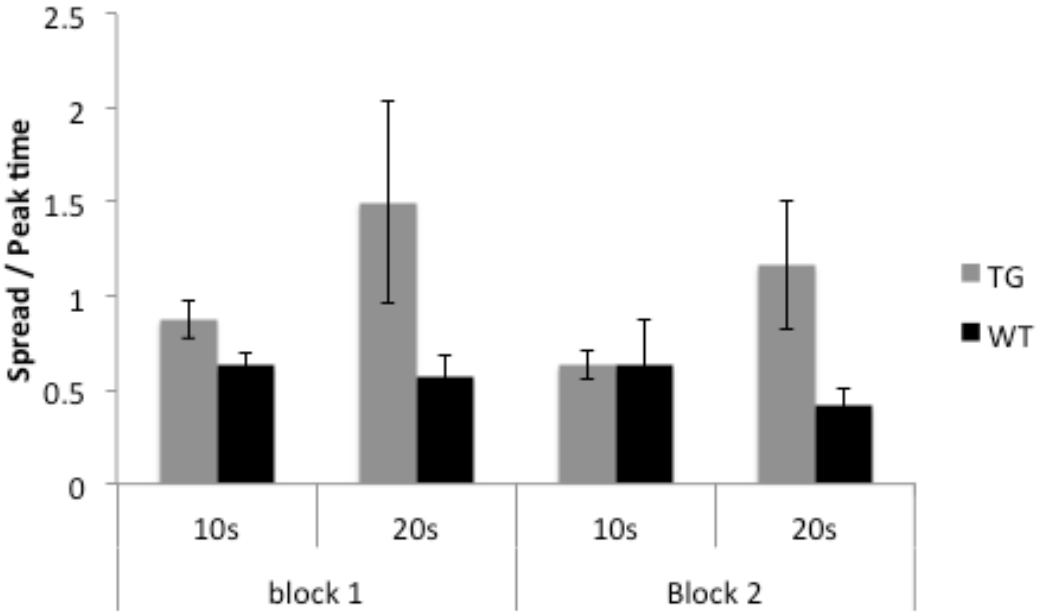


Figure 3.21. The coefficient of variation (CV) scores for blocks 1 and 2 and the 10s (short) and 20s (long) delay CS's during the test sessions in Experiment 3. Error bars show standard error of the mean.

3.26 Further analysis

3.26.1 R^2 between Exp2 and 3

R^2 scores for the animals in Experiments 2 and 3 were analysed together to see if the fit of the Gaussian function decreased as the animals became older. The younger animals appear to shower greater R^2 values. However, an ANOVA with genotype, age (Experiment 2 & 3), CS type and block as factors only revealed a main effect of CS type, $F(1, 41) = 10.35$, MSE = 41.00, $p = .003$, $\eta_p^2 = .202$, reflecting higher R^2 scores for the shorter 10s CS. There was no

significant main effect of age, $F(1, 41) = 1.34$, MSE = .120, $p > .05$, $\eta_p^2 = .032$ or an interaction between age and genotype ($F < 1$).

3.26.2 Gaussian Spread between Exp2 and 3.

Spread scores for the animals in Experiments 2 and 3 were analysed together to assess whether or not the level of precision changed with age between genotypes. An ANOVA with genotype, age, CS type and block was carried out on the data, showing a main effect of CS $F(1, 41) = 17.47$, MSE = 109.53, $p = <.001$, $\eta_p^2 = .299$, and block $F(1,41) = 6.32$, MSE = 70.75, $p = .016$, $\eta_p^2 = .134$. An interaction was also seen between CS and genotype $F(1,41) = 5.85$, MSE = 109.53, $p = .02$, $\eta_p^2 = .125$. Simple main effects revealed greater spreads in the TG compared to the WT mice during the 20s CS $F(1,41) = 6.68$, MSE = 3231.60, $p = .013$, $\eta_p^2 = .14$ but not the 10s CS ($F>1$). The TG mice also showed greater spreads during the 20s CS compared to the 10s CS $F(1, 41) = 21.24$, p <.001, Wilk's $\lambda = .659$, $\eta_p^2 = .341$, no difference between CS's was seen in the WT mice ($F>1$).

No genotype by age interaction was observed ($F>1$) in the main ANOVA, suggesting that the spreads scores did not change between ages in either the wild types of APPswe/PS1dE9 mice. The analysis

did, however add some more tentative support towards impaired timing precision in the 8 month APPswe/PS1dE9 mice.

3.27 Discussion

Experiment 3 showed that both genotypes were able to quickly acquire conditioned responding to 10-s and 20-s duration CSs and discriminate between reinforced and non-reinforced stimuli. Unlike in Experiment 2, APPswe/PS1dE9 mice showed greater responding to the reinforced cues during the last acquisition session than wild types, possibly reflecting greater motivation to respond. Also both genotypes showed greater responding to the shorter 10-s CS than to the 20-s CS, which was not seen in Experiment 2.

The APPswe/PS1dE9 mice once again showed increased pre-CS responding during the acquisition sessions, similar to Experiment 2, possibly reflecting increased hyperactivity (Bonardi et al., 2011; Filali et al., 2011; Maroof et al., 2013).

We predicted greater peak spreads in the 8-month APPswe/PS1dE9 mice; however, this was not found. APPswe/PS1dE9 mice showed significantly higher peak rates than wild type, possibly suggesting greater motivation. No genotype differences in spread or peak time were found, which reflects normal

accuracy and precision in timing. The Gaussian scores for the 20-s CS showed numerically greater spreads and earlier peak times for the APPswe/PS1dE9 mice, which would be consistent with impaired hippocampal function; however, these differences were not significant.

One possible explanation for the lack of an observed effect in the APPswe/PS1dE9 mice, at 8 months, is possible increased variability in responding in both genotypes due to normal age-related decline in performance. Shoji et al. (2016) has examined age related behaviour in the C57BL/6J mouse, the backcrossed strained used in the APPswe/PS1dE9 mouse, between the ages of 2-3, 4-5, 6-7 and 8-12. Following cue and contextual fear conditioning, 8-12 month mice showed reduced freezing to both context and cue during test compared to all three other age groups. This does seem unlikely as the analysis of the Gaussian spread scores, a measure of variability between Experiment 2 and 3 found no age related increased in variability in the 8 month mice, regardless of genotype.

The animals in Experiment 3 may well have shown poorer learning about the CS-US association compared to the younger animals in Experiment 2, as a result possibly masking any genotype effect in the older animals. The animals in Experiment 3 did appear

to take longer to discriminate between the reinforced and non-reinforced CS's, from session 3 onwards (Figure 3.9), whilst the animals in Experiment 2 discriminate between them from session 2 (Figure 3.16). This assumption was tested by analyzing the acquisition data (Figure 3.22), using the reinforced CS's, for both Experiments 2 and 3 together. An ANOVA with age (Experiment 2 and 3), session, and CS were carried out. The ANOVA found main effects of session $F(5,225) = 40.85$, $MSE = 100.32$, $p<.001$ $\eta_p^2 = .476$, and CS $F(2,90) = 77.84$, $MSE = 54.61$, $p < .001$ $\eta_p^2 = .634$. Two-way interactions were seen between session and age $F(5,225) = 5.78$, $MSE = 100.32$, $p < .001$ $\eta_p^2 = .114$ and session and CS $F(10,450) = 23.53$, $MSE = 471.14$, $p < .001$ $\eta_p^2 = .343$. A significant three-way interaction between session, CS and age was also observed $F(10,450) = 2.11$, $MSE = 20.01$, $p = .022$ $\eta_p^2 = .045$. The three-way interaction was subsequently analysed further by carrying out ANOVA with age and CS on each individual session. For session 1, an interaction between CS and age was found $F(2,90) = 5.08$, $MSE = 3.47$, $p = .008$ $\eta_p^2 = .102$, with simple main effect showing only greater responding by the older animals to the non-reinforced CS compared to the younger one ($p = .026$). Session 2 found an overall main effect of age $F(1,45) = 16.45$, $MSE = 94.68$, $p < .001$ $\eta_p^2 = .268$, reflecting greater responding

by the animals in Experiment 1 compared to 2, a main effect of CS $F(2,90) = 13.86$, $MSE = 9.94$, $p < .002$ $\eta_p^2 = .236$ and an interaction between the two $F(2,90) = 7.29$, $MSE = 9.94$, $p = .001$ $\eta_p^2 = .139$. Animal in Experiment 1 showed greater responding to the 10s versus 20s ($p = .002$) CSs, 10s and non-reinforced CS ($p < .001$) and the 20s and non-reinforced CS ($p = .004$). Experiment 2 failed to discriminate between the three CS types (Fs < 1). Experiment 1 also showed greater responding compared to Experiment 2 for the 10s ($p < .001$), 20s ($p = .001$) and non-reinforced ($p = .002$) CSs. Session 3 onwards only showed main effects of CS (smallest $F(2,90) = 49.08$, MSE – 26.43, $p < .001$ $\eta_p^2 = .522$ for session 5), no effects relating to age were observed.

The results show that the younger animals from Experiment 2 are quick to discriminate between the reinforced and non-reinforced CSs (from session 2 onwards) compared to the older mice from Expeirment 3 (from session 3 onwards). This supports the notion that older mice are slower to learn the CS-US association; however comparable levels of responding and discrimination are observed from session 3 onwards. There is no evidence in an overall reduction in responding or discrimination in the older animals.

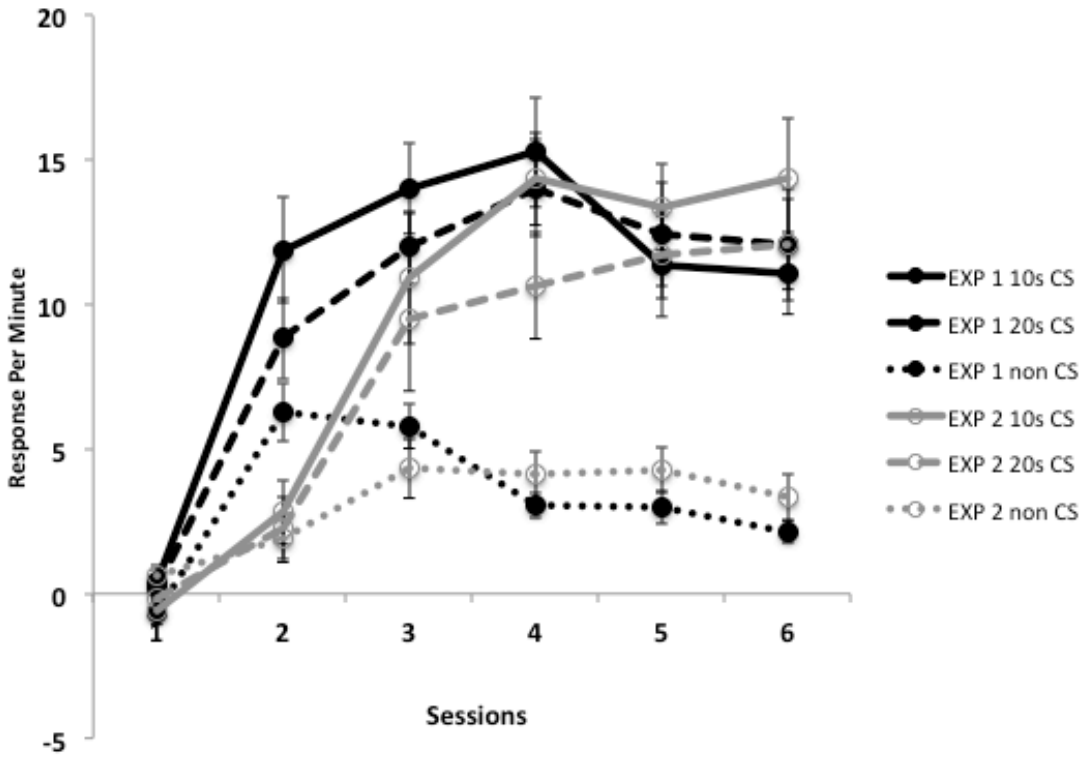


Figure 3.22: The acquisition data split by Experiment (2 and 3) over the six sessions and three trial types. The error bars show the standard error of the mean.

Interestingly, Montgomery et al. (2009) has shown evidence of impaired reversal learning at 3 months, but intact performance at 12 months of age in the APPswe/PS1dE9 mice. The authors argued for some sort of compensatory mechanism that improved performance at 12-months when the pathological burden was much greater.

3.28 General Discussion

Theoretical models have been developed to explain the processes that govern interval timing.

3.28.1 Scalar expectancy theory

The scalar timing theory (STT; Gibbon et al., 1984) is an information-processing model that has been used extensively to explain timing behaviour in the peak procedure and impairments in timing associated with lesion studies. The model is made up of a pacemaker, accumulator and a memory store. The pacemaker emits pulses at a constant rate, when a CS is encountered the pacemaker pulses are moved into the accumulator that stores the pulses emitted during the CS presentation. When a US is encountered the total number of pulses stored between CS onset and US delivery are transferred to the memory store, and multiplied by a constant *K*. When the same stimulus is encountered for a second time the number of pulses in the accumulator is compared to that in the memory store, and when the values are sufficiently close conditioned responding occurs.

The observed increased spreads can be explained in terms of the STT. One possible explanation is that AD pathology is affecting the criteria that define when the current accumulator value is close enough to the value in the memory store to initiate a response,

resulting in the APPswe/PS1dE9 mice responding earlier and stopping later, resulting in increased spreads (loss of precision) seen in Experiments 1 and 2 (Tam et al., 2014; Machado et al., 2009; Meck et al., 1984; 2013).

Temporal-difference model

Associative learning explanations of how animal time behaviour have also been developed. The temporal-difference (TD) model (Brandon et al. 2003) of learning is a prediction error model and an extension of the Rescorla-Wagner (RW) model of learning (Rescorla & Wagner 1972). The TD model argues that when a CS is presented it is perceived as broken up into individual elements for every moment of the CS before the US is administered (micro-stimulus). Each micro-stimulus is subject to an eligibility trace, which defines how much each element can be modified by events. Eligibility is higher for recently activated micro-stimuli compared to less recently activated elements.

Lets imagine an example of a CS-US association, in which the CS is made up of 10 micro-stimuli (MS). During the first trial the US occurs at the same time as the last MS10, as a result MS10 gains associative strength due to the association. During trial two, MS10 once again gains further associative strength and MS9 also gains

some due to the close temporal proximity between its self and MS10 (higher eligibility), and generalization. With repeated trials this process repeats until MS10 reaches asymptote and its level of associative strength matches the US intensity, whilst the earlier micro-stimuli have gained associative strength associated with their temporal proximity to the US. In Pavlovian conditioning animals will show the greatest level of conditioned responding at the end of the CS, closest to the US than at the start.

The loss of timing precision seen in Experiments 1 and 2 could also be explain in terms of the TD model, via greater generalization in the APPswe/PS1dE9 mice. Given that the MS temporally closest to the US gain the most associative strength, increased generalization between the MS would result in greater associative strength to the MS around the time of the US, resulting in increased conditioned responding and a greater spread.

3.28.2 Experiment summary

This chapter looked to assess the ability of APPswe/PS1dE9 mice to acquire conditioned responding during both delay (Experiments 1, 2, 3) and trace (Experiment 1) conditioning procedures, using an appetitive reward. The chapter also sought to examine the accuracy of the APPswe/PS1dE9 mice on timing CS durations following

conditioning in both young (Experiment 1, 2) and old (Experiment 3) mice.

The main findings from the chapter are that 4-5 month APPswe/PS1dE9 mice show impaired precision in being able to time the delivery of a food reward after extensive training (Experiments 1 & 2). This effect appears not to be evident in older (8 month) transgenic mice, despite presumably a greater degree of AD pathology. The observed greater spreads (loss of precision) found in Experiments 1 and 2 are similar to the increased variability in making time estimation judgments seen in human Alzheimer's patients (Carrasco et al., 2000; Hellstrom & Almkvist, 1997; Nichelli et al., 1993; Rueda & Schmitter-Edgecombe, 2009). The precision in measuring time intervals may well be a useful tool for detecting early AD.

The initial hypothesis predicted lower levels of conditioned responding to the trace CS in the APPswe/PS1dE9 mice than wild types, given impaired trace conditioning has been seen in other mouse models of AD (Guart et al., 2008; Kishimoto et al., 2012; 2013; Ohno et al., 2004). Our findings of intact appetitive delay conditioning in APPswe/PS1dE9 mice in all three experiments is consistent with previous literature (Bonardi et al., 2011; Chapter 2),

whilst impairments in both delay and trace conditioning have been seen when the unconditioned stimulus has been aversive (Guart et al., 2008; Kishimoto et al., 2012; 2013; Lin et al., 2015; Ohno et al., 2004). This difference in performance on delay and trace conditioning between appetitive and aversive USs possibly reflects a difference in processing unconditioned stimuli (aversive or appetitive), as discussed in Chapter 2.

4.1 Chapter 4. Recognition memory

Recognition memory is often defined as the ability to evaluate a previously experienced stimulus as being encountered before (Squire et al., 2007). Recognition memory is a specific component of memory that is impaired in patients with Alzheimer's disease (AD) (Dalla Barba et al., 1997; Didic et al., 2013; Hart et al., 1985; Westerberg et al., 2006).

4.2 Recognition memory in Alzheimer's disease

The visual paired comparisons (VPC) task has been used to assess recognition memory in patients with both clinical and preclinical AD. The task involves participants watching pictures presented on a screen whilst their eye movements are tracked. Subjects are not

required to make any conscious judgments about the images. During a standard trial, two identical pictures are presented side by side for 5-seconds before the screen goes black for a variable interval. During test participants see the same display; however, one of the familiar pictures has been replaced with a novel picture. Participants show a preference for the novel stimulus, as assessed by increased viewing time (Crutcher et al., 2009; Zola et al., 2012). The VPC task can differentiate between normal controls and patients with mild cognitive impairment (MCI) (Crutchner et al., 2009; Zola et al., 2012). Crutchner et al. (2009) found that with a 2-minute delay between sample and test, MCI patients viewed the novel picture for 53% of the time, whilst normal controls spent 70% of the test viewing the novel stimulus. The VPC task can also differentiate between MCI patient who went on to be diagnosed with AD and those that did not (Zola et al. 2012). In Zola et al. (2012) all but one participant who spent less than 50% of the time viewing the novel stimuli converted from MCI to AD or from healthy to MCI within three years of the experiment. MCI participants who viewed the novel stimuli for 67% of the time showed no further cognitive decline. As a result of these findings visual recognition memory could be used a diagnostic marker for preclinical Alzheimer's (Didic et al., 2010).

What neurobiological changes could be account for these impaired VPC performance in MCI and AD patients. Both MCI and AD patients show evidence of hippocampal volume loss, with AD patients showing greater loss than MCI (Henneman et al. 2009; Schuff et al., 2009; for MCI meta-analysis see Shi et al., 2009). This loss of hippocampal volume could well have an effect of VPC performance, as monkeys with lesions of the hippocampus also show impaired performance on the VPC task (Pascalis et al., 1999; Zola et al. 2009).

4.3 Recognition memory in rodents

An analogue to the VPC task is carried out in rodents - the spontaneous novel object recognition (sNOR) task. sNOR takes advantage of rodents' innate preference for exploring novelty. During a sample phase rodents are presented with a pair of identical junk objects (X) and allowed to explore them for a period of time. Following pre-exposure the rodents undergo the test phase where one copy of X and a novel junk object Y is presented. Rodents typically explore Y more than the familiar X (Ennaceur & Delacour 1988).

4.4 Recognition memory in APPswe/PS1dE9

This task has revealed impairments in a number of different AD mouse models, for a review see Grayson et al. 2015. The APPswe/PS1dE9 mouse is impaired in the classic sNOR task from 6 months onwards (Cheng et al. 2014, Donkin et al. 2010, Jardankhazi-Kurutz et al. 2010, Li et al. 2014, Pedros et al. 2014, Yan et al. 2009, Yoshiike et al. 2009; but see Barbero-Camps et al. 2014, Frye & Walf. 2008, Jardankhazi-Kurutz et al. 2010). Some studies have looked at sNOR performance in young (2-6) pre-plaque APPswe/PS1dE9 mice, and both failed to find any impairment (Bonardi et al. 2011, Lin et al., 2015; Pedros et al. 2014).

4.5 Standard operating procedures model

An associative learning account has been proposed to explain recognition memory and its variations. Wagner's *standard operating procedures* model of memory (SOP; Wagner, 1981) can provide a framework for explaining performance on the sNOR task (Honey & Good 2000; Robinson & Bonardi., 2015; Tam et al., 2014; Whitt & Robinson., 2013). SOP asserts that stimuli are encoded in the brain as distinct elements. Each element can be represented in one of three possible activation states: inactive (I), primary state (A1) and secondary state (A2). The presentation of a stimulus results in the

elements of that stimulus entering the A1 state. Activity in A1 quickly decays into the A2 state, before slowly decaying back into the inactive state. A number of rules govern how the SOP model works. First, elements in A1 will elicit higher rates of responding than those in A2. Second, elements in A2 must first decay into the inactive state before reactivated into A1. Finally, A1 has a limited capacity, and only a certain number of elements can be in A1 at any one time.

Both excitatory and inhibitory learning can be explained in terms of the SOP model. Excitatory learning occurs between a CS and US when their elements are both in the A1 state. Following learning the presentation of the CS (elements into A1), produces associative activation of the US elements directly into the A2 state, bypassing A1. Inhibitory learning occurs when elements of the CS are in A1, whilst the US is in A2.

Both Sanderson & Bannerman (2011) and Robinson et al. (2015) have argued that sNOR task performance can be explained using two possible *SOP* mechanisms: stimulus-generated priming and retrieval-generating priming (Robinson et al., 2009; Robinson & Bonardi 2015; Sanderson & Bannerman. 2011; Tam et al., 2014; 2015; Whitt & Robinson. 2013).

Stimulus-generated priming (SGP) involves stimulus elements being in A2 via the recent presentation of the stimulus. During the sNOR sample phase the presentation of X allow its elements to enter the A1 state, but they will quickly decay into A2. Thus when X is presented again at test most of its elements are in A2 and cannot be activated into A1; in contrast all of the elements of the novel object Y are activated into the A1 state, resulting in greater exploration of Y.

Retrieval-generated priming (RGP) involves the activation of stimulus elements via the associative activation of other elements. During the sample phase of sNOR, object (X) and the environmental context are both activated into A1, resulting in an association between them. During test, being exposed to the context results in X's elements being associatively activated into the A2 state. When the rodent encounters object X, its elements are already in the A2 state, stopping exploration, whilst the novel Y object has not been encountered so is activated in A1 and elicits exploration.

4.6 SOP and APPswe/PS1dE9 mice

Robinson et al.'s (2015) theory of dual mechanisms underpinning sNOR performance can ask questions of the sNOR data in animal models of AD.

In the APPswe/PS1dE9 mouse normal sNOR performance has been observed between 2-5 months (Bonardi et al., 2011; Lin et al., 2015; Pedros et al., 2014). The multiple explanations of sNOR performance according to SOP raise the possibility that one of the mechanisms, SGP or RGP, underlying recognition memory could be impaired; however, sNOR performance would remain intact if the other possible mechanism was still functioning.

Impaired sNOR performance has been observed in the APPswe/PS1dE9 mouse from 6 months onwards (Cheng et al. 2014, Donkin et al. 2010, Jardankhazi-Kurutz et al. 2010, Li et al. 2014, Pedros et al. 2014, Yan et al. 2009, Yoshiike et al. 2009), no attempt has been made to establish whether this is due to impairments in either SGP, RGP or both.

4.7 Stimulus-generated priming vs Retrieval-generated priming

To test SGP or RGP separately, two variations of the classic sNOR have been developed: the relative recency (RR) task and the object in context (OIC) task.

4.7.1 Stimulus-generated priming & relative recency

Mitchell & Laiacona (1998) first carried out the RR procedure (Figure 4.1, b) using two sample stages and test phase. During the first

sample phase rodents are exposed to one set of identical objects (W). After an interval (usually 1 hour) rodents then received a second sample phase in which they are exposed to a second set of identical objects (Z), followed by another interval. The rodents are then tested with W and Z. Rodents typically show a preference for the first experienced object W relative to the more recently encountered object Z.

According to the *SOP* model, the RR task is a reasonably pure measure of SGP. During the test phase of the task, elements of object W have had more time than the elements of object Z to decay from A1 to A2 and back into I. Object W has decayed back into I whilst object Z is still represented in A2, resulting in the elements of object W being able to activated back into A1, resulting in exploration, whilst the elements of object Z are active within A2 and unable to enter into A1.

RGP in most circumstances does not explain the results seen in the RR task. According to RGP, both the elements of object W during sample phase 1 and object Z during sample phase 2 become associated with the same surrounding context (A), so during test when objects W and Z are presented in the same context, both

objects' elements will be activated into A2, stopping either object's elements entering A1 and eliciting exploration.

4.7.2 Retrieval-generated priming & object in context

In one version of the OIC task (Dix & Aggleton 1999; Robinson & Bonardi 2015) (Figure 4.1, c) rodents undergo a sample phase where they are presented with four different objects (W, X, Y, Z) in a context. After an interval rodents are placed back into the context with the same four objects, however; two of the objects have swapped positions (displaced) whilst the other two (static) are in the sample phase position. Rodents show an exploration preference for the displaced objects. The SOP model is able to explain this effect using RGP. During the sample phase the elements of each object and the context in closest proximity enter A1 together forming an association. The contextual cues are therefore able to directly activate the objects elements into the A2 state. During the test phase non-displaced objects (W & Y) become activated in the A2 state due to the context-object association, whilst the displaced objects will command more A1 activity, due to reduced priming between the context and objects, resulting in increased exploration (Robinson & Bonardi. 2015; Tam, Bonardi & Robinson. in review). The important aspect of the OIC task is that all the objects are familiar to the rodents

during test and have all been experienced as recently as each other, meaning performance cannot be explained in terms of differential SGP.

4.7.3 Mouse models of Relative Recency and Object in Context

Currently no other studies have performed an in-depth analysis of sNOR performance in the APPswe/PS1dE9 mouse. However, this is not true for all transgenic models of AD. Hale & Good (2005) tested a single mutation APP mouse (tg2576) at 14 months of age, when plaques are abundantly found in the cortex, hippocampus and parahippocampal regions. The tg2576 mice showed normal sNOR performance with sample-test intervals of 2, 30 minutes, 24 hours and normal RR performance with a 2-minute sample-sample interval. Interestingly tg2576 mice were significantly impaired on the OIC task, failing to show a novelty preference for the objects that have switched locations. The results of this experiment, in terms of the SOP model, support the idea of impaired RGP but intact SGP in AD; however, due to the age of the animals and level of AD pathology no conclusions can be drawn about early cognitive impairments.

Davis et al. (2012; 2013) performed a detailed recognition memory analysis using a triple mutation mouse model of AD,

including APP, PS1 and Tau mutations. Davis et al. (2012; 2013) has shown that young Tg mice showed intact sNOR, RR and OIC performance, whilst showing impairments in a '*what-where-which*' task which the authors define as analogous to episodic-like memory. In terms of the SOP model Davis's results do not support impairments in either RGP or SGP, but rather point towards impaired episodic-like memory.

4.8 Chapter overview

The experiments carried out in this chapter firstly aim to assess performance of young (4-5 month old) APPswe/PS1dE9 mice on the spontaneous novel object recognition task; the chapter will assess the two possible mechanisms, SGP and RGP.

4.9 Experiment 1

Experiment 1 examined sNOR performance (Figure 4.1, A) in young (4 month) APPswe/PS1dE9 mice. This experiment set out to confirm previous research showing intact sNOR in this age group (Bonardi et al., 2011; Jardanhazi-Kurutz et al., 2010; Lin et al., 2015; Pedros et al., 2014), prior to plaque development. Experiment 1 then carried out the RR task (Figure 4.1, B),

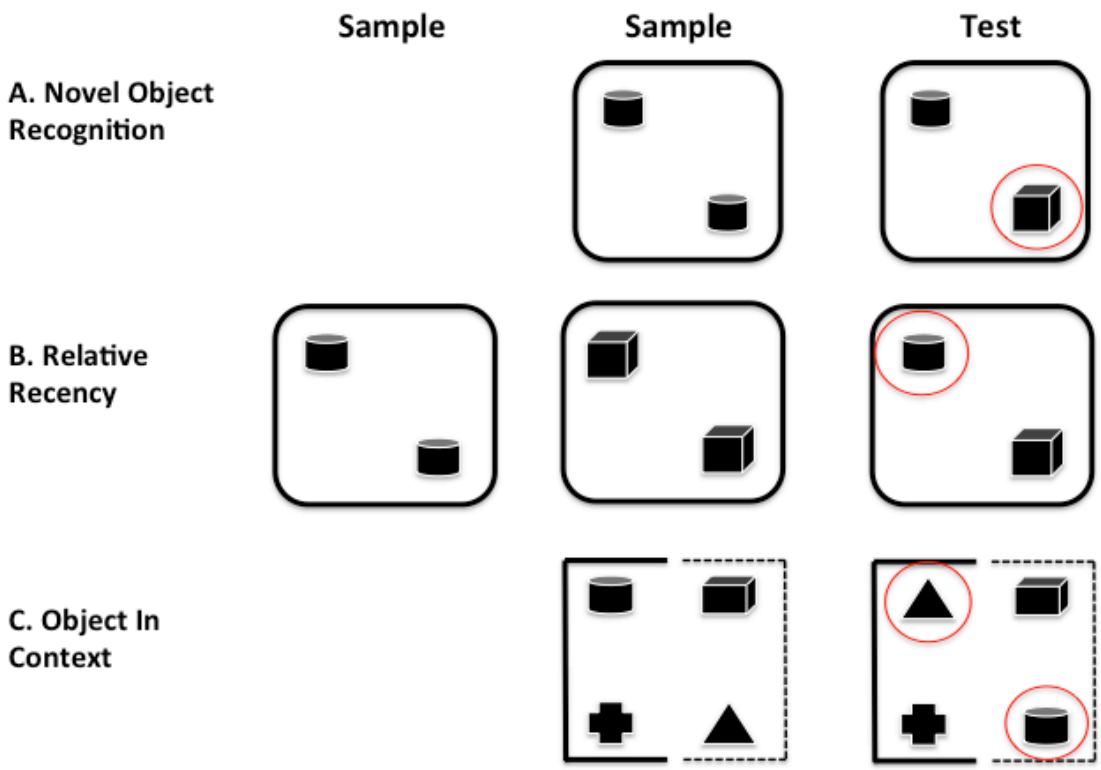


Figure 4.1. Diagram of three variations of the classic object recognition task: A; the novel object recognition task (sNOR), B; the relative recency task (RR), C; the object in context (OiC) task. The red circle indicates which objects would produce increased exploration compared to the non-circled objects.

4.10 Methods.

4.10.1 Animals.

All animals were bred and housed in the same way as those in Chapter 2. Fourteen 16-week old mice (7 APPswe/PS1dE9 & 7 wild type) served as subjects. All the animals were used in a previous experiment and had received two weeks of food deprivation before

the start. Five days prior to the start of the experiment, all animals were placed back onto ad libitum access to food. Mice had prior experience of the experimental room from the previous experiment, but not the experimental apparatus.

4.10.2 Apparatus.

The apparatus used was a single white rectangular walled high-density polyethylene box (Mini Mobile, supplied by Slingsby, Shipley, UK (length × width × height: 60cm x 40cm x 45cm). A black wooden frame was used to support a FireWire camera (Fire-I, Unibrain, Athens, Greece), which was placed 90cm above the centre of the arena floor. The camera captured the entire arena floor, and was connected to a laptop, which ran the AnyMaze video tracking software (Stoelting Co., Illinois, USA). Two lights, each consisting of six light-emitting diodes (LEDs) in a circular pattern, were positioned on the wooden frame, one on either side of the camera. Both the arena lights and the room lights were on throughout the experiment.

Two sets of junk objects were used for the different tasks in Experiment 1 (Appendix 6.5, 6.6). The first comprised a blue eggcup containing a red Christmas bauble (a), two translucent star-shaped candleholders attached to each other (b), a white multi adapter plug (c), and a black cylinder paperweight (d); the second consisted of a

small black and white hexagon patterned ball (e), a random shape made from lego blocks (f), a glass salt shaker filled with brown sand (g), and a silver tin can with a black stripe around the outside (h). Each object (average dimension of circular objects: range: diameter 3.5-7.5cm, height 2.5-14cm. Average dimension of rectangular objects: range: Length 4-7cm, width 3.4-8cm & height 5-10cm) was secured to the base of the arena floor with Blu-Tack (Bostik, Stafford, UK.)

Experiment	*Objects*
1a & 1b	*A B C D*
1c & 1d	*E F G H*

Table 4.1. Objects used during each experiment. Note: A, B, C, D, E, F, G, H = stimuli, objects.

4.11 Procedure.

The mice received two 10-minute habituation sessions in the apparatus prior to the start of the experiment, to familiarize them with the contextual surroundings.

4.11.1 General experimental procedure

Throughout Experiment 1 all sample phases lasted five minutes whilst all test phases lasted three minutes. All sNOR experiments employed a five-minute interval between sample and test phases, whilst the RR task used a 3-hour interval between sample 1 and 2, and a five-minute interval between sample and test.

4.11.2 Experiment 1a

Using the first set of objects, the mice underwent the sNOR task (Figure 4.1, A; Table 4.1). The sample phase involved placing each mouse in the apparatus with a pair of identical objects (X), one in the top left and one in the bottom right. Each mouse was then returned to his home cage. The arena and objects were cleaned with ethanol solution between each trial and mouse. Each mouse was then placed back into the apparatus, which now contained a copy of the sample object (X) and novel object (Y).

For half the experimental animals (8 mice: 4tg & 4wt), the objects used were a, b, c & d with each object being used as the novel test object (Y). The remaining animals (6 mice: 3tg & 3wt) used objects e, f, g & H with each object being used as the novel test object (Y) (Appendix 6.3).

The position of the novel object Y at test was counterbalanced between genotype (Appendix 6.3); half the mice the novel object Y was positioned in the top left corner, whilst the remainder in the bottom right corner. The mice were run in a counterbalanced double alternation order (TG, WT, WT, TG, WT, TG, TG, WT etc).

4.11.3 Experiment 1b

Using the first set of four junk objects (Appendix 6.3), the mice underwent a relative recency task (Figure 4.1, B; Table 4.2). The first sample phase was identical to the sNOR procedure. After the first sample phase each mouse was returned to its home cage for a 3-hour interval. The mice then received the second sample phase, which was identical to the first, using the second pair of identical objects. The mice were placed back into the apparatus for the test phase, which was identical to Experiment 1a. The mice were run in the same order as Experiment 1a.

4.11.4 Experiment 1c

Using the second set of four junk objects (Appendix 6.3), the mice underwent a replication of the RR task. The task was carried out in an identical manner to Experiment 1b, with the arena positions being moved to the top left and bottom right corners for objects W and Z.

4.11.5 Experiment 1d

Using the second set of four junk objects (Appendix 6.3), the mice underwent a replication of the sNOR task carried out in Experiment 1a. The task was carried out in an identical manner to Experiment 1a, with the arena positions being moved to the bottom left and top right corners for objects X and Y.

4.12 Data treatment

The measurement used to analyze the data was the total duration of time the mice spent in a pre-specified zone (9cm length/10cm width) that was placed around the objects in AnyMaze. The zone size was based on maximize the level of mouse exploration of the objects whilst reducing the amount of accidental recorded exploration. Accidental exploration consists of the animal being within the object zone without exploring the object. Ennaceur & Delacour (1988, p.49) defined object exploration as "directing the nose at a distance of <2 cm to the object and/or touching it with the nose". The experiments in this study used an automated software method for capturing exploration. A target spot was placed on the mouse's nose, and the software recorded the amount of time the spot was within the target zones. This method may not be as sensitive as human observation scoring; however, it has been shown that automated software is as

reliable as human observations (Rutten et al. 2008, Silvers et al. 2007), whilst also being immune to unconscious bias.

The results are presented as the actual amount of time spent in the zones. During the sample phases the amount of exploration (seconds) of the novel/least recently encountered (object Y/W) object during test is compared to the familiar/most recently encountered (object X/Z) object. This was done to check for any positional bias.

The test phase analysis during the sNOR tasks compared exploration between the novel (Y) object and the familiar (X) objects. The RR test phase compared exploration of the least recently encountered (W) object and the most recently encountered (Z) object.

The sample and test phase data for the sNOR tasks (Experiments 1a & 1d) were pooled and analyzed together using a between-subjects factor of experiment. The same was done for the RR task (Experiments 1b & 1c). The results were then analyzed the same as Chapter 1.

4.13 Results

4.13.1 Novel object recognition (Experiments 1a & 1d)

4.13.2 Sample

Experiment 1d showed reduced sample phase exploration compared to Experiment 1a (Table 4.3). APPswe/PS1dE9 mice showed slightly higher levels of object exploration compared to wild types, but no bias towards one object position.

An ANOVA with genotype (APPswe/PS1dE9 & wild type), experiment (1a & 1d) and test position (X & Y) confirmed only a main effect of experiment, $F(1, 12) = 11.67$, $MSE = 86.72$, $p = .005$, $\eta_p^2 = .493$, reflecting greater exploration during Experiment 1a compared to 1d. No other main effects or interactions were significant (smallest $p = .370$).

	Sample Phase	
Experiment 1a	*Test position of X*	*Test position of Y*
APPswe/PS1dE9	*20.2s (5.1s)*	*24.5s (7.2s)*
Wild Type	*17.9s (2.5s)*	*17s (2.3s)*
Experiment 1d		
APPswe/PS1dE9	*11s (2s)*	*13.1s (2.2s)*
Wild type	*10.3s (1.7s)*	*11.1s (2.9s)*

Table 4.3. Object exploration levels during the sample phase, based on their locations during test. Object exploration is presented in seconds, whilst standard error of the mean is presented in brackets.

4.13.3 Test

The test phase (Figure 4.2), revealed that APPswe/PS1dE9 mice in Experiment 1a were unable to discriminate between the novel and familiar objects, whilst the wild types showed a strong preference for object Y. Interestingly both genotypes showed a strong novelty preference in the second replication (Experiment 1d), presented in Figure 4.3. An ANOVA with familiarity (X & Y), genotype and replication revealed a significant main effect of familiarity, F (1, 12) =

27.88, *MSE* = 55.66, $p < .001$, $\eta_p^2 = .699$, and a three-way interaction between familiarity, experiment and genotype, F (1, 12) = 8.56, *MSE* = 33.31, p = .013, $\eta_p^2 = .416$; no other main effects or interactions were significant (smallest $p = .214$).

To analyze the interaction an ANOVA with familiarity and genotype as factors was conducted on the data from Experiments 1a and 1d separately. For Experiment 1a (Figure 4.2) this revealed a main effect of familiarity, $F(1, 12) = 15.76$, *MSE* = 32.94, $p = .002$, $\eta_p^2 = .568$, and an interaction between familiarity and genotype, $F(1, 12) = 10.79$, *MSE* = 32.94, $p = .007$, $\eta_p^2 = .474$. A greater preference for the novel object in the wild type mice, $F(1, 12) = 26.33$, *MSE* = 32.94, $p < .001$, but not in the APPswe/PS1dE9 mice, $F < 1$ was found. The same analysis of Experiment 1d (Figure 4.2) only showed a significant main effect of familiarity, $F(1, 12) = 19.34$, *MSE* = 56.03, $p = .001$, $\eta_p^2 = .617$. The main effect of genotype revealed no differences, $F(1, 12) = 2.85$, *MSE* = 43.57, $p > .117$, $\eta_p^2 = .192$

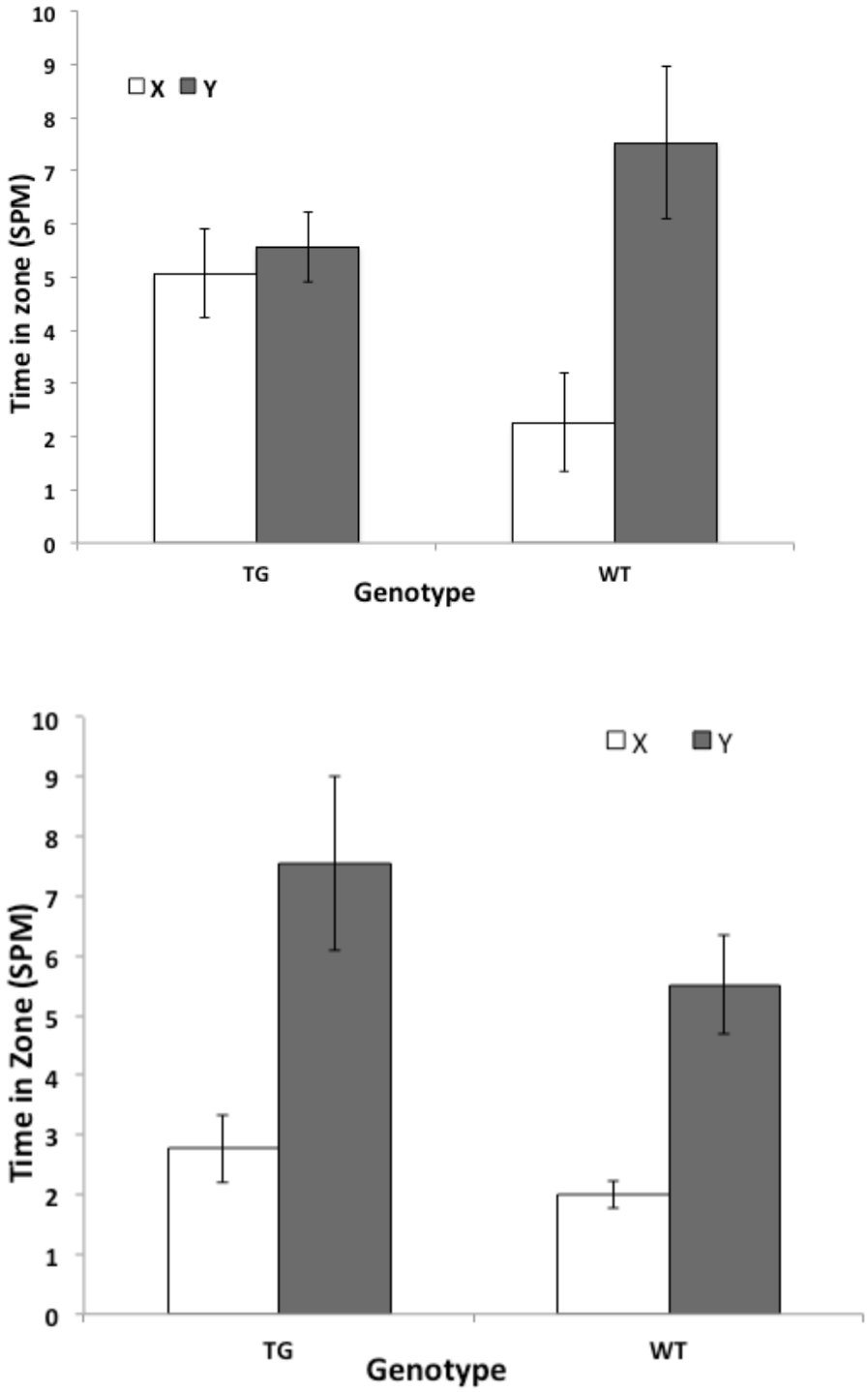


Figure 4.2. Test phase exploration during Experiment 1a (top panel) and Experiment 1d (bottom panel). Group mean time (seconds per minute) spent exploring object X (familiar) and Y (novel) pooled over

the three minutes of test. Error bars show the standard error of the mean.

4.13.4 Relative recency (Experiments 1b & 1c)

4.13.5 Sample

During the sample phase, both genotypes showed a similar level of object exploration; however, less exploration was observed during sample phase 2 of Experiment 1c compared to sample phase 1 (Table 4.4). An ANOVA carried out on the sample phase data, using experiment (1b & 1c), genotype, sample phase (1 & 2), and test position (W & Z) as factors, revealed only an interaction between experiment and sample, $F(1, 12) = 6.38$, $MSE = 43.84$, $p = .027$, $\eta_p^2 = .347$. Less exploration was observed during sample phase 2 compared to 1 during Experiment 1c, $F(1, 12) = 9.56$, $MSE = 54.69$, $p = .005$, whilst greater exploration was observed during sample phase 2 of Experiment 1b compared to Experiment 1c, $F(1, 12) = 10.73$, $MSE = 41.22$, $p = .003$. In particular there was no effect of genotype, or interaction between genotype ($Fs < 1$) or any other significant main effects or interactions (smallest $p = .053$ for a experiment * sample * test position interaction).

	Sample 1		*Sample 2*	
	Test positions		*Test positions*	
Experiment 1b	*Z (s)*	*W (s)*	*Z (s)*	*W (s)*
APPswe/PS1dE9	*17.6 (4.2)*	*10.1 (2.3)*	*15.2 (2.1)*	*15.9 (3.2)*
Wild type	*19.3 (2.5)*	*13.5 (2.9)*	*16.1 (5.5)*	*14.2 (2.3)*
Experiment 1c				
APPswe/PS1dE9	*16.5 (2.7)*	*17.8 (3.1)*	*14.4 (5)*	*9.2 (1.5)*
Wild type	*14.3 (3.4)*	*14.8 (3.8)*	*8.2 (2.9)*	*7 (2.2)*

Table 4.4. Object exploration levels (sec) during the sample phase, based on their locations during test for Experiment 1b and 1c. Standard error of the mean is presented in brackets.

4.13.6 Test

Analysis of the test phase (Figure 4.3) for both Experiments 1b & 1c revealed a preference for object Z - the opposite of the normal tendency. An ANOVA with genotype, experiment, and familiarity (W & Z) revealed a main effect of experiment, $F(1, 12) = 13.75$, $MSE = 44.38$, $p = .003$, $\eta_p^2 = .534$ confirming greater responding during Experiment 1c than 1d. A main effect of familiarity, $F(1, 12) = 6.69$, $MSE = 37.06$, $p = .024$, $\eta_p^2 = .358$, was also observed confirming the

apparent preference for the most recently seen object (object Z). No other significant main effects or interactions were observed (smallest $p = .081$).

4.14 Discussion

4.14.1 Novel object recognition (Experiments 1a & 1d)

The experiments tested whether or not 4-month-old APPswe/PS1dE9 mice are capable of discriminating between novel and familiar stimuli (Ennaceur & Delacour 1998). Wild type mice showed a significant preference for the novel (Y) object in both experiments, whilst the novelty preference was abolished during Experiment 1a in the APPswe/PS1dE9 mice, but intact during Experiment 1d.

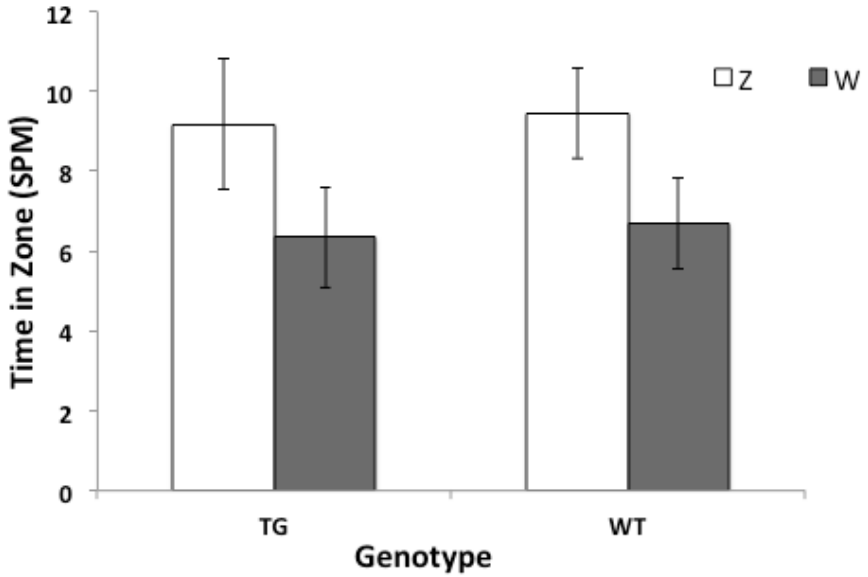


Figure 4.3. Test phase relative recency exploration pooled between Experiment 1b and c. Time (seconds per minute) spent exploring object W (least recently encountered) and Z (most recently encountered). Error bars show the standard error of the mean.

There is no clear explanation of the discrepancy between the two experiments, such as a significant difference in sample phase exploration. During the test phase of Experiment 1a, APPswe/PS1dE9 mice showed almost the same amount of exploration for objects X and Y, whilst wild type mice explored Y more than X. Increased anxiety to novel stimuli has been previously observed in the APPswe/PS1dE9 mouse (Chen et al., 2013) but does not explain the findings, as the transgenic mice do not show less exploration of the novel stimuli Y.

A hypothetical explanation of Experiment 1a's results comes from a possible object location bias during the sample phase. APPswe/PS1dE9 mice showed numerically greater exploration of object Y's location during the sample phase, reducing the location's novelty. This may have reduced the exploration of the APPswe/PS1dE9 mice to object Y's location during test and increased the novelty of object X's location during test; however this difference was not significant.

Experiment 1a may well be a more accurate measure given it was the first task carried out. The mice had no previous experience of the task, or any effect of interference from similar experiments being carried out before. Experiment 1d, in contrast, was carried out after the RR tasks so may well have familiarized the mice more with the procedure. An increase in familiarization and decrease in anxiety may produce a more accurate measure of behaviour.

In conclusion, Experiment 1 has failed to conclusively show intact or impaired sNOR performance in 4-month-old APPswe/PS1dE9 mice. Further experiments would be required to establish whether or not young APPswe/PS1dE9 mice show impaired object recognition memory.

4.14.2 Relative recency (Experiments 1b & 1c)

The experiments assessed 4 month APPswe/PS1dE9 mouse performance on, the RR task (Barker et al. 2007, Hannesson et al. 2004). Both genotypes were unable to show a preference for the least recently encountered object (W). Both genotypes in both experiments showed a preference for object Z, which was the most recently encountered object, the opposite result to what should occur. RR has been consistently demonstrated in rats; however, fewer studies have managed to replicate the finding in the mouse (Barker et al. 2007, Sanderson et al. 2011). Both Sanderson et al. (2011) and Hale & Good (2005) have shown intact RR in mice using two 10-minute sample phases, a 2-minute interval between phases and a 5-minute test phase.

Experiments 1b & c differed from those in the literature by having shorter, 5-minute sample exploration phases and a longer (3-hour) interval between the two sample phases. The longer sample-sample interval should theoretically make the RR task easier as the greater interval makes the recency judgment easier to make. In light of the literature the results of Experiment 1b & c are hard to understand. The shorter sample phase length in Experiments 1b & c, compared to the literature, gives less time for the mice to explore the

objects and for that information to be remembered. Albasser et al. (2009) showed that increasing the same phase duration results in improved novelty preference in sham animals in the sNOR task. In contrast, Mumby et al. (2007) have found normal novelty preference in perirhinal cortex lesioned rats following five successive sample phases. The same lesioned animals tested with only one sample phase failed to show the novelty preference seen in sham animals.

Differences in sample phase exploration were observed during Experiment 1c: greater exploration of sample phase 1 objects was observed compared to sample phase 2 in both genotypes, which could have increased exploration of Z, resulting in a lack of a RR effect. Interestingly this sample phase difference was observed only during Experiment 1c, yet the same pattern of results during test was observed in both Experiment 1b and 1c, suggesting that the sample phase difference has little effect on the results.

Due to the failure to show RR in either genotype, it is impossible to establish whether or not APPswe/PS1dE9 mice are capable of showing a preference for novelty using SGP.

4.15 APPswe/PS1dE9 mice & recognition memory

Other data collected in our laboratory (Bonardi et al., 2016) has involved almost identical replications of Experiments 1a, b, c, d;

however, the main procedural difference involved increasing the number of habituation sessions, performed prior to testing, from two to seven, to increase apparatus familiarity and decrease anxiety.

4.15.1 Spontaneous novel object recognition

Experiment A by Bonardi, Pardon & Armstrong (2016) was a replication of Experiment 1a in 5-month APPswe/PS1dE9 and wild type mice. The test phase data revealed a significant preference for the novel object (Y) in both genotypes (Figure 4.4). This result is consistent with the results from Experiment 1d, showing intact novelty preference in both genotypes at 5 months of age.

The second set of experiments (B1 & B2) by Bonardi et al. (2016) was a replication of Experiment 1a; however, the only difference was a 24-hour time interval between the sample and test phase. The results of the experiments showed a significant preference for the novel object (Y) in both genotypes (Figure 4.4), confirming normal novelty preference in the APPswe/PS1dE9 mice.

Taking into account Experiments 1a/d and the other data collected in our laboratory (Bonardi et al., 2016), the majority of the evidence presented so far in this chapter supports the notion of

intact sNOR performance in young (4 to 5 month old) APPswe/PS1dE9 mice and wild types.

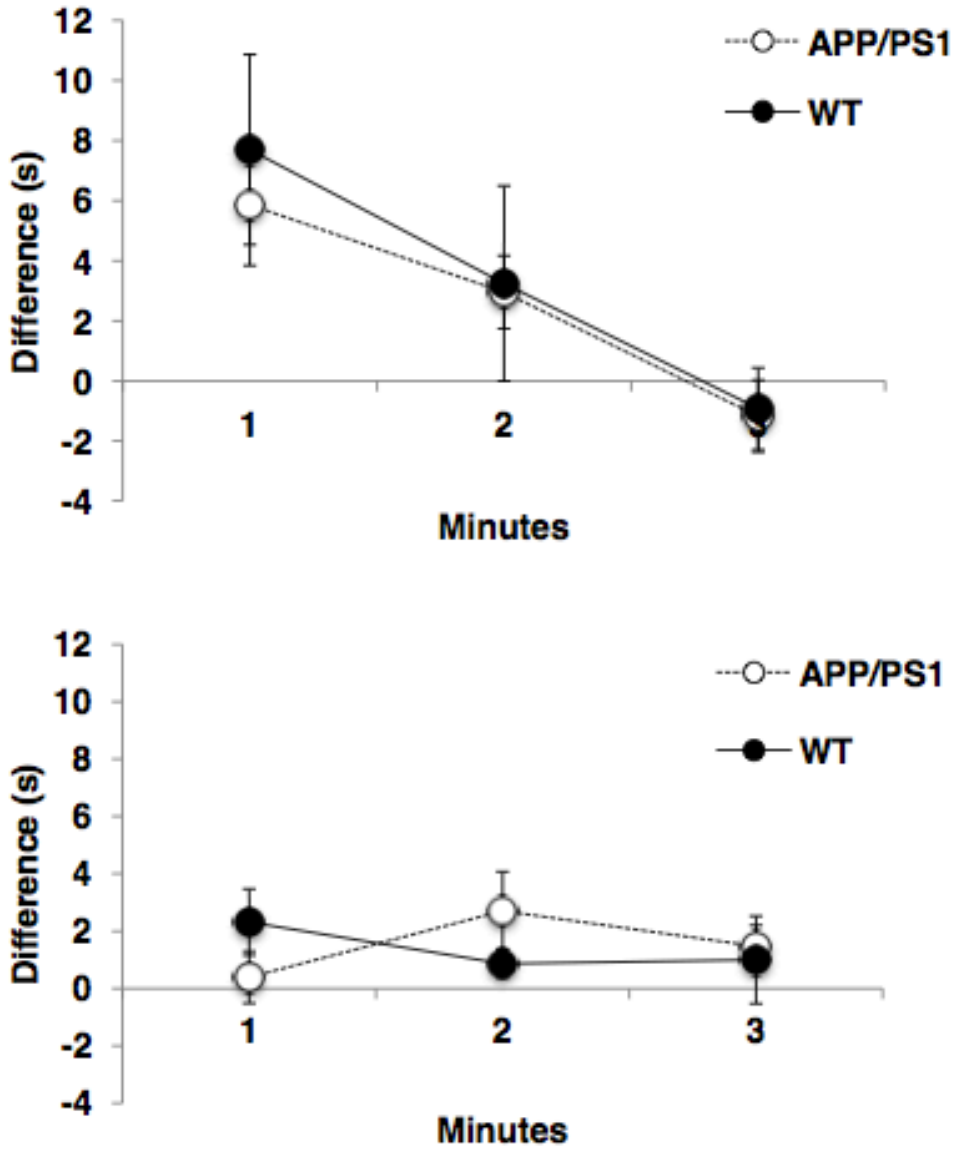


Figure 4.4. Difference scores (novel – familiar exploration of objects) on the sNOR task in 5 month old wild type (WT) and APPswe/PS1dE9 (APP/PS1) mice using either a five minute (upper panel) or 24 hour (lower panel) sample-test interval, graphs taken from Bonardi, Pardon & Armstrong (2016).

4.15.2 Relative recency

Bonardi et al. (2016) also carried out a replication of the RR tasks, Experiments 1b & c. The replication (Experiments C1 & C2) was carried out identically, but with a 24-hour interval between samples. Increasing the interval between sample phases actually makes the RR task easier to complete. As the RR task is a relatively pure measure of SGP, increasing the interval between the sample phases allows more time for the sample 1 objects to decay from A2 back into the I state.

The results found that both 5-month APPswe/PS1dE9 and wild type mice showed a preference for the least recently encountered object (W), reflecting intact RR memory with a 24-hour delay between sample phases (Figure 4.5). The wild type mice did show a numerically larger effect, with more time spent exploring object W than object Z compared to the APPswe/PS1dE9 mice. This may well reflect poorer RR performance in the APPswe/PS1dE9 mice; however, this difference was not significant.

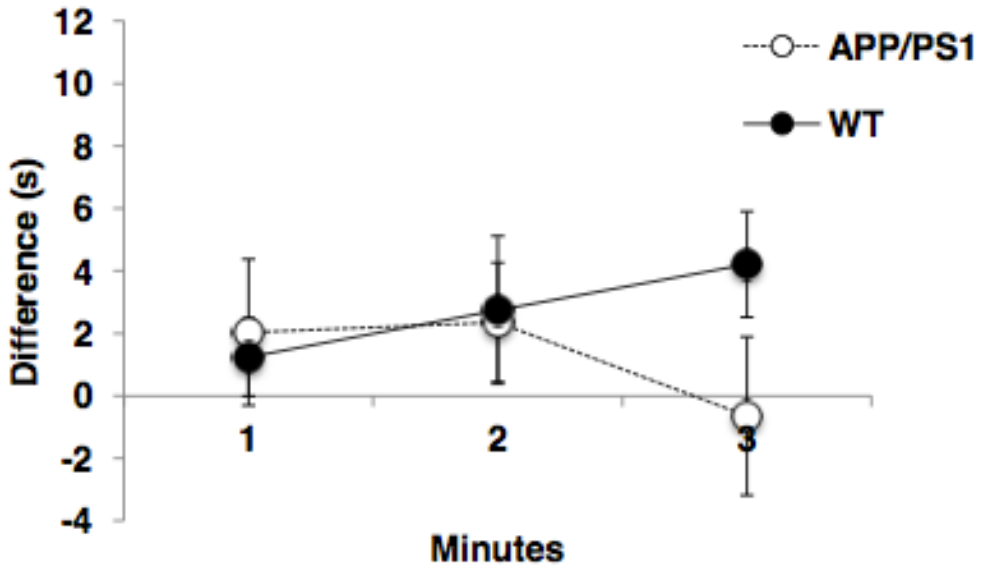


Figure 4.5. Difference scores for the relative recency task carried out by Bonardi et al. (2016) for 5 month old wild type and APPswe/PS1dE9 mice, using a 24 hour sample-sample interval.

4.16 Experiment 2

The majority of the evidence presented in this chapter supports the notion of intact sNOR and RR performance in young (4 to 5 month old) APPswe/PS1dE9 mice and wild types. According to SOP account both the 5-minute (Experiment 1d) and 24-hour interval versions (Bonardi et al., 2016) of the sNOR task can be explained by both the SGP and RGP mechanism, whilst the RR experiments by Bonardi et al. (2016) generally support the notion of intact SGP in young (4-5) month) APPswe/PS1dE9 mice.

4.17 Object in context – retrieval generated priming

Bonardi et al. (2016) has also specifically examined the role of RGP in young (5 month) APPswe/PS1dE9 mice. Bonardi et al. carried out the OIC version of the classic sNOR task (Dix & Aggleton 1999; Robinson & Bonardi 2015) (Figure 4.1, c). Bonardi et al. found that wild type mice showed a significance preference for the displaced objects, whilst the APPswe/PS1dE9 mice did not (Figure 4.6).

According to SOP account, OIC performance can only be explained in terms of RGP, suggesting a specific impairment in RGP in young (5 month) APPswe/PS1dE9 mice.

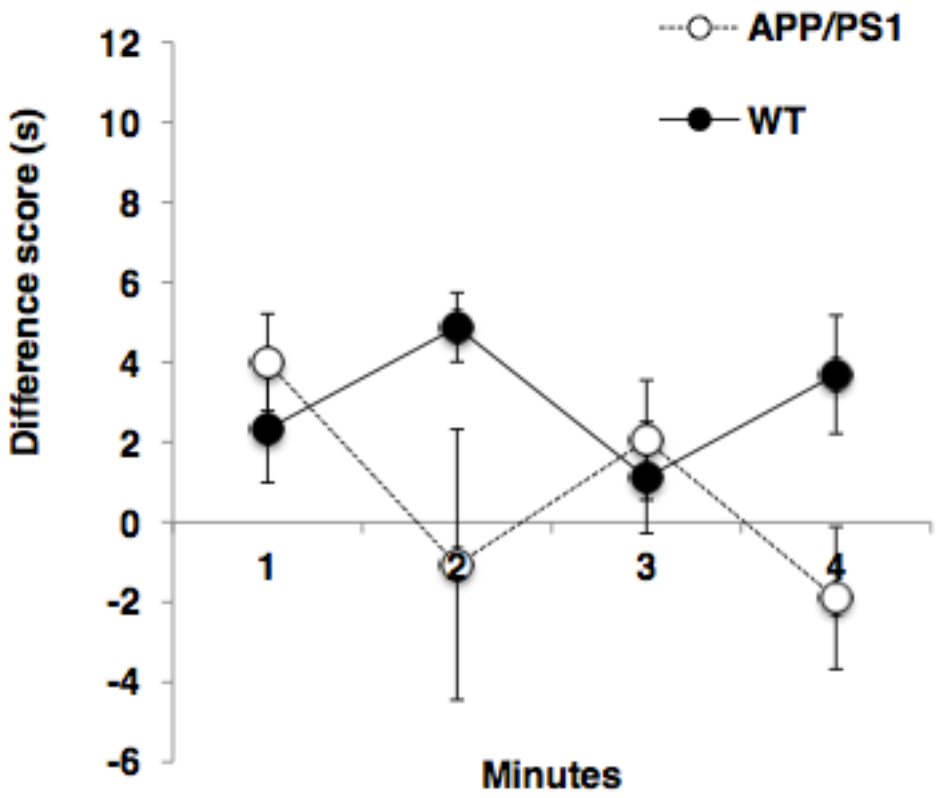


Figure 4.6. OIC task performed by 5 month old wild type and APPswe/PS1dE9 mice, taken from Bonardi, Pardon & Armstrong (2016).

4.18 Object in context – generalization decrement

The OIC task can also be explained in terms of generalization decrement (Hall & Honey 1989; Lovibond et al., 1984). Which could account for the impaired performance seen in APPswe/PS1dE9 mice. During the sample phase of the OIC task (Figure 4.7), the four objects (A, B, C, D) become associated with the contextual position (P & O) they are in (PA, PC, OB, OD). During the test phase the displaced objects (A & D) are perceived differently (AO & DP) than during sample (PA & OD). They then act as a partial novel stimulus during test, resulting in approach behaviour.

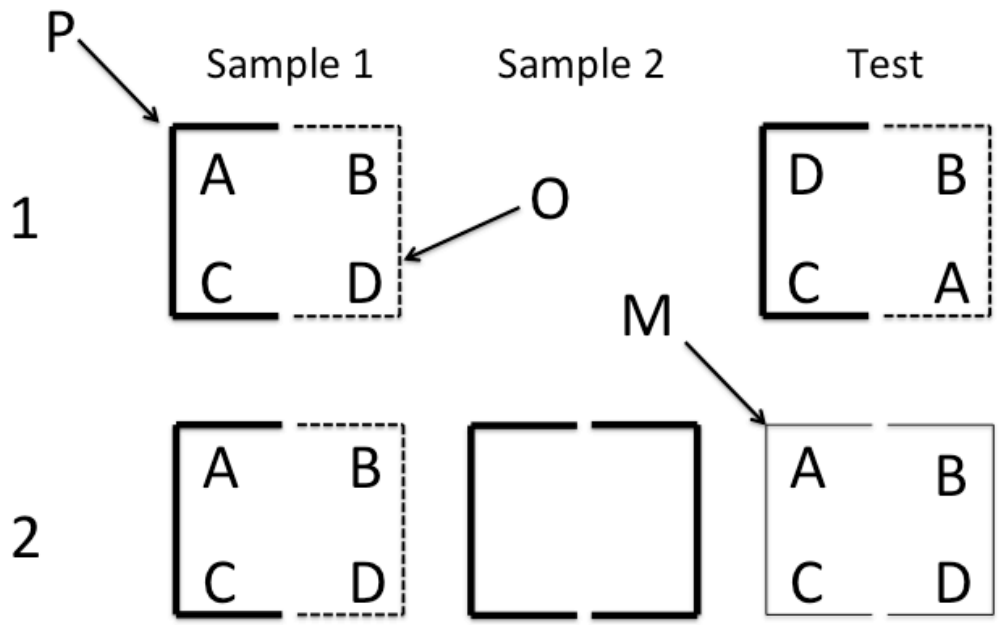


Figure 4.7. A diagram of associations that occur during the object in context (panel 1) and context priming (panel 2) experiments.

4.19 Context-priming

To deal with generalization decrement, Experiment 2 carried out a variation of the OIC task, using the *context-priming task* (Whitt et al., 2012), which does not suffer, to the same degree, from the effects of generalization decrement (Figure 4.7) and also specifically measures RGP. The context-priming task (Figure 4.8) involves placing mice into a context with four different junk objects (A, B, C, D). The context is made up of two distinct wall inserts (P & O) that cover the chamber. During sample phase two, no objects are presented; mice are

exposed to one wall insert pattern (P or O). During the test phase, the context inserts are removed leaving the original white arena (M). The objects and object positions during sample phase 1 are used during test. Mice will typically show reduced exploration of the objects associated with the context presented in sample phase 2 (primed), and increased exploration of the remaining objects (non-primed) (Figure 4.8).

	Sample phase 1	*Sample phase 2*	*Test Phase*
Experiment 2	*PA OB* *PC OD*	*PP*	*MA MB* *MC MD*

Table 4.5. Experimental design for Experiments 2: context priming. Note: W, X, Y, Z = stimuli, objects. P, O, M = stimuli, contexts

Whilst the OIC task assesses novelty based on changing the position of objects in relation to contextual cues, the context-priming task assesses the same mechanism, but affects novelty by priming a particular context-cue association.

4.20 Context-priming & SOP

According to SOP model, RGP, but not SGP, can explain this particular finding (Whitt & Robinson 2013). During sample phase 1, both the objects and the nearby contexts cue become associated together in A1, before decaying into A2 and then the inactive state. During the context-priming phase, the presentation of certain contextual cues places the objects associated them back into the A2 state (primed). During test in a neutral context, the primed objects are already in A2, whilst the non-primed objects are newly activated into A1, producing greater responding.

4.21 Context-priming & generalization decrement

The context-priming task does not suffer from the effects of generalization decrement (Figure 4.7). During the sample phase the objects and contextual locations become associated in the same way as in the OIC task (PA, PC, OB, OD); however, during the context-priming test phase, all the objects are placed in a new novel context. According to generalization decrement all the objects are then perceived differently (MA, MC, MB, MD) and would produce the same amount of novel exploration.

Experiment 2 aimed to replicate the context-priming task, previously shown in rats (Whitt et al., 2012) and assess the

performance of young APPswe/PS1dE9 mice. We predict that APPswe/PS1dE9 mice will be impaired on the context-priming task, based on their impaired performance on the OIC task, both of which are reliant on RGP.

4.22 Methods.

4.22.1 Animals.

Experiment 2 used 15 naïve (7 APPswe/PS1dE9 and 8 wild type) mice as test subjects.

4.22.2 Apparatus.

The apparatus used in Experiment 2 was almost identical to that used in Experiment 1. The only difference was the inclusion of contextual wall inserts during the sample phases. The inserts were added to improve the ability of the mice to form context-stimulus associations. Each context insert was made up from three wooden boards, which were hinged together and covered in linoleum. The large centerboard was 32.0 x 45.0 cm, and the two smaller boards were 21.0 x 45.0 cm. All boards were hinged together along the 45.0 cm edge. Each context insert covered half of the inner wall area of the arena. Half of the arena contained one context insert pattern, whilst the other half contained the other pattern. The linoleum attached to

each context insert was made of two distinct patterns. The first context insert had a white and black pattern (large white squares, 16.0 x 16.0 cm, with interspersed black diamonds, 4.0 x 4.0 cm); the second had a blue tiled pattern (small blue diamond shapes, 1.5 x 1.5 cm). The objects and locations used during Experiment 2 were: a Tabasco bottle (w) in the top left corner, battery (x) in the top right corner, bulb (y) in the bottom left and white ping pong ball (z) in the bottom right of the chamber.

4.23 Procedure

Sample phase 1 involved placing the mice in the arena, with a black and white context insert on the left (P) and a blue context insert on the right (O). Four different novel objects (w, x, y, z) were placed in the top and bottom left and right corners of the arena. Half the animals had the objects placed in one set position, whilst the remaining animals the objects were placed into another set position (Table 4.6). The sample phase lasted 10 minutes in total, after which the mice were returned to their home cages for 5 minutes. Sample phase 2 involved no objects being presented; however, the mice were exposed to one context insert pattern (P or O), on all sides of the arena, for a 10-minute period. Following sample phase 2 the mice

were returned to their home cages again for another 5 minutes. During the test phase, all the context inserts were removed leaving the original white arena (M). During test, the same objects and positions during sample phase 1 were used, which lasted 5 minutes (Table 4.8). Half the mice received context priming (sample phase 2) with the black and white inserts and the remainder with the blue diamond insert.

Context Priming object positions				
	Top Left	Bottom Left	Top Right	Bottom Right
3tg & 4wt	w	x	y	z
4tg & 4wt	y	z	w	x

Table 4.6. The location of the novel objects during sample phase 1 and test; during the context-priming task.

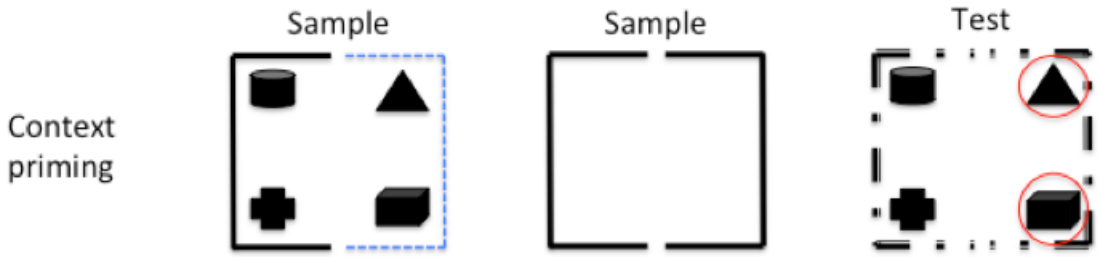


Figure 4.8. Diagram of the context priming variation of OIC task. The red circles indicate the objects that should elicit the greatest level of responding.

4.24 Data treatment

Data treatment was very similar to that employed in Experiment 1. The amount of time exploring each object was analysed for the 10-minute sample phases and the full 5-minutes during test. The level of mouse activity was used as a measure of arena exploration during the full 10 minutes of sample phase 2.

4.25 Results

4.25.1 Context Priming (Experiment 2)

4.25.2 Sample

Sample 1 data showed no difference in object location exploration between genotypes (Table 4.7). An ANOVA with genotype, and novel position (primed & non-primed) revealed no significant main effect of genotype, familiarity or an interaction between the two (*Fs* < 1).

Experiment 4	*Non-Primed*	*Primed*
APPswe/PS1dE9	*46.60 (3.38s)*	*45.10 (1.79s)*
Wild type	*44.27 (3.71s)*	*43.65 (1.82s)*

Table 4.7. Shows the sample phase exploration rate for both genotypes during Experiment 2.

4.25.3 Priming

APPswe/PS1dE9 mice travelled on average 17.2 cm (SE = 1.4cm), whilst wild types traveled on average 17.7cm (SE = 1.9cm). A one-way ANOVA found no difference between the groups, $F(1, 14) = .981$, $MSE = .981$, $p = .83$.

4.25.4 Test

Wild type mice show greater responding to the non-primed objects than to the primed objects, reflecting the expected performance, whilst APPswe/PS1dE9 mice showed numerically the opposite effect (Figure 4.8). An ANOVA with genotype and primed position showed no significant main effect of genotype ($F < 1$), nor a main effect of novel position ($F < 1$) or an interaction between the two, $F(1, 13) = 3.46$, MSE = 13.00, $p = .08$, $\eta_p^2 = .210$. Given the interaction was almost significant it was analysed further. The simple main effects showed no difference between genotypes in exploration of the non-primed ($F < 1$) or primed, $F(1, 13) = 1.79$, $MSE = 1.11$, $p = .19$ positions. APPswe/PS1dE9 mice showed no difference in exploration between the primed and non-primed positions ($F < 1$), whilst the wild types almost showed significantly greater exploration of the non-primed location, $F(1, 13) = 3.72$, $MSE = .83$, $p = .075$.

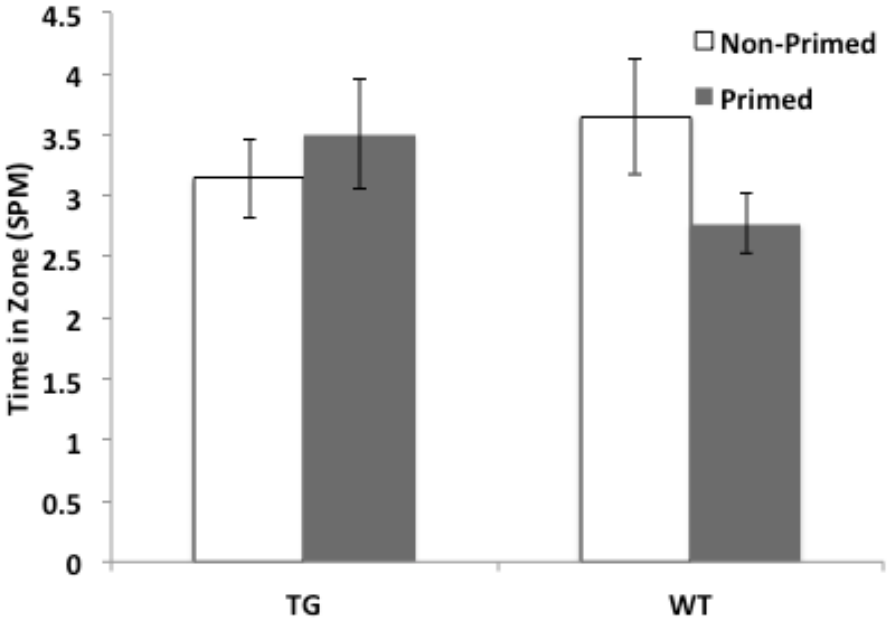


Figure 4.6. Data from the test phase of the context-priming task showing time spent exploring the non-primed (Novel) or primed (Familiar) experienced object, collapsed across the 5 minutes of the test.

4.26 Discussion

Experiment 2 failed to find significant evidence of contextual priming in wild type and APPswe/PS1dE9 mice. However, the wild type mice did show numerically greater exploration of the non-primed compared to primed objects, whilst APPswe/PS1dE9 mice very slightly showed the opposite result.

This particular task has only been carried out once, in any species (Whitt et al., 2012). More experiments are needed to

establish the context priming effect, observed in rats, in normal wild type mice, before any conclusions can be drawn about the performance of transgenic animals. As a result generalization decrement cannot be ruled out as a simpler explanation of the impaired OIC performance in APPswe/PS1dE9 mice, found in Bonardi et al. (2016). More research will be needed to establish the true involvement of RGP in young APPswe/PS1dE9 mice.

4.27 General Discussion

The first aim of this chapter was to examine sNOR performance in a young (4-5) month mouse model of AD. Previous research has shown sNOR performance in 2-5 month APPswe/PS1dE9 mice (Bonardi et al., 2011; Lin et al., 2015; Pedros et al., 2014). Experiment 1a did find impaired novel object performance in the APPswe/PS1dE9 mouse; however, Experiment 1d and Experiments A, B1 & B2 from Bonardi et al. (2016) supports the idea that sNOR performance is intact.

The second aim of this chapter was to examine the two theoretical mechanisms, SGP via the RR task, and RGP via the OIC task, which some believe underpin sNOR performance. Experiments 1b & c were unable to establish the RR effect in either wild type or APPswe/PS1dE9 mice using a 3-hour sample phase interval. Whilst

Experiments 1b & c failed to show RR performance in either genotype, research carried out in our laboratory (Bonardi et al., 2016) has shown an intact RR effect in 5-month APPswe/PS1dE9 and wild type mice, using a 24-hour interval between sample phases. These experiments support the idea that SGP is not disrupted in young APPswe/PS1dE9 mice.

Bonardi et al. (2016) has found that 5-month APPswe/PS1dE9 have a deficit in performance on the OIC task, a measure of RGP. The results of the OIC task are unfortunately ambiguous, as performance can also be explained by generalization decrement. Experiment 2 aimed to measure RGP, using the context-priming procedure (Whitt et al., 2012; Whitt & Robinson 2013), which cannot be explained in terms of generalization decrement, and would give support towards impaired RGP in APPswe/PS1dE9 mice. Experiment 2 failed to show normal performance on the task in either genotype. Experiment 2's attempt to rule out the involvement of generalization decrement was inconclusive, so it remains an alternative explanation for the impaired OIC performance (Bonardi et al., 2016).

4.27.1 SOP issues and implications

The intact sNOR seen in Experiment 1 and Bonardi et al., (2016) is more likely reliant on SGP processes. However, while this assumption seems perfectly plausible in a sNOR task employing a short sample-test interval (5 minutes), it does seem less likely when the interval is increased to 24 hours. SGP becomes a less likely process, as the sample phase objects have much more time to decay from A1 to A2 and even back into the I state. RGP, on the other hand does not suffer from temporal decay. The context-stimulus (object-contextual environment) associations survive the interval (Tam et al., 2014) and could result in decreased exploration of the sample phase objects. However, the time it takes for elements to decay from A2 back into the I state is unknown.

The nature of the arena used during the 24-hour sNOR task may well implicate SGP in performance. During Experiment 1d and Bonardi et al. (2016) the context used was the normal experimental arena, to which the mice had already received extensive habituation. Habituation to the chamber according to Wagner (1979) would reduce the ability for context – object associations to form (RGP), thus relying on SGP to perform the object discrimination (Robinson et al., 2009). To investigate this hypothesis, it would be prudent to

carry out two replications of the NOR task using a 24 hour interval using either a context to which the animals have had larger amounts of habituation and a novel context. Impaired performance in the APPswe/PS1dE9 mice may well be observed in latter case due to a greater demand on RGP. More research is needed to establish the involvement of SGP or RGP in APPSwe/PS1dE9 mice performing the sNOR task with a 24-hour sample-test interval.

Intact RR performance seen in young APPswe/PS1dE9 mice (Bonardi et al., 2016) has also been attributed toward intact SGP. Unfortunately the RR task is not a completely clean measure of SGP, and cannot rule out the involvement of RGP. The SGP explanation of RR relies on the context-W and context-Z associations between sample phases being equal (Table 4.2). However, during the second sample phase when Z is presented in the context, the context is presented without W possibly resulting in some extinction of the context-W association, thus during test increasing exploration of object W.

4.27.2 Theories of recognition memory

Aggleton & Brown's (1999) dual model of recognition memory is based on the dissociation of separate brain regions that govern the subjective experiences of familiarity (novelty) and recollection. The

model argues that context-dependent recollection but not familiarity is based on intact hippocampal function. Good et al. (2007) have shown evidence of impaired OIC performance (recollection), but intact sNOR and RR (familiarity) in hippocampal lesioned rats (Fortin et al., 2004; Good et al., 2007), whilst Langston & Wood (2010) have also shown intact sNOR and RR performance in hippocampal lesioned rats, but impaired performance on an object-place-context (OPC) task. The OPC task involves two sample phases and a test phase. During sample phase one animal are exposed to context A and two different objects (a & b), during sample two animal are exposed to context B with the same objects in the opposite spatial position (b & a) before the test session in context A and a pair of identical objects (a & a). Animal will show a preference for the object location that did not match the location of that object in sample phase one (context A). The result supports the argument that the hippocampus is essential for the context-dependent nature of recognition.

In relation to the results in this chapter, given that neurotoxic soluble Aβ is detected as early as 3 months of age in the hippocampus of APPswe/PS1dE9 mice (Hu et al., 2010; Shemer et al., 2006), this disruption of normal hippocampal function could have resulted in impaired OIC performance (Bonardi et al. 2016). Further support

comes from young 3-4 month APPswe/PS1dE9 mice showing impaired performance on other hippocampal dependent tasks (Morris water maze & contextual fear conditioning; Vegh et al., 2014; Zhang et al., 2012).

4.27.3 Representational-hierarchical (RH) model

The results can also be interpreted in terms of the representational-hierarchical (RH) model (Cowell et al., 2010; McTight et al., 2010). The RH model is a single process model of recognition memory, which argues that recognition memory is dependent on a functional brain pathway termed the ventral visual-perirhinal-hippocampal connection. The ventral visual stream (primary visual cortex, V1, V2, V3, V4) deals with simple feature stimuli, whilst the perirhinal cortex deals with more complex configural stimuli; finally the hippocampus is thought to be involved in combining complex stimuli with other environmental information (spatial and temporal information). Lesion studies have confirmed the involvement of these brain regions in various recognition memory tasks. Perirhinal lesions disrupt performance on the sNOR task (Mumby et al., 1994; Ennaceur et al., 1996; Bussey et al., 1999; Norman & Eacott 2004), but leave simple recognition memory tasks based on simple comparisons (colour & size) and location-based comparison intact

(Lee et al., 2005a; 2005b). Lesions of the hippocampus have been shown to disrupt OIC performance only (Barker & Warburton, 2011; DeVito & Eichenbaum, 2010; Good et al., 2007; Tam et al., 2014, but see Langston & Wood for normal performance using the same start position during sample and test). The experiments presented in this chapter would support impaired hippocampal function in the APPswe/PS1dE9 mouse that results in a specific impairment in combining complex stimuli with allocentric and environmental information (Langston et al., 2010; Langston & Wood 2010).

4.27.4 Object in context & human AD

Our results showed impaired OIC performance in young pre-plaque APPswe/PS1dE9 mice. Human experiments have also been carried out which measure the association between different objects and contexts. Tasks that required patients to make judgments based on paired associations have revealed impairments in AD patients (Fowler et al. 2001, Lindeboom et al. 2002, Parra et al. 2009). For example, Parra et al. (2009) carried out a task that measured the ability of participants to bind different elements together (faces, colours, shapes etc). Participants were presented with two stimuli, which could differ on two features: shape (S) and colour (C). Three

variations of this task were presented, either with stimuli differing in one feature: colour (S1C1 / S1C2), or shape (S1C1 / S2C1) or both features (S1C1 / S2C2). During the test phase for the single feature tasks (colour or shape) participants had to discriminate between a familiar stimulus (S1C1) and a novel one (S3C1 or S1C3), which is analogous to the classic sNOR task. In the combined feature task participants had to discriminate between the sample (S1C2) and when the two feature of stimuli had been rearranged (S2C1). They had to discriminate between equally familiar features (colour & shape) in either familiar (sample phase) or unfamiliar combinations. Which is comparable to the OIC task. The results revealed that patients with a familiar form of AD and non-symptomatic relatives with the disease were impaired on the combined stimulus task, but not on the single stimulus tasks. This result shows AD patients are impaired on a human parallel to the OIC task, but show intact performance on the sNOR parallel.

4.27.5 in-depth analysis of sNOR in transgenic animals

Davis et al. (2012; 2013) experiments mentioned in the chapter introduction has shown that young Tg mice showed intact sNOR, RR and OIC performance, whilst showing impairments in a '*what-where-*

which' task. The results show inconsistencies with the results presented in this chapter; however, there are two possible reasons for the inconsistencies. Firstly the mouse model of AD used by Davis et al (2012; 2013) is significantly different to the APPswe/PS1dE9 mouse. Between mouse models of AD, there are substantial variations in the expressed pathology (for example, the amount, onset, structure and location of pathology). The second reason is the procedural differences in the OIC task between experiments. Davis et al. employ a version of the task, where animals were pre-exposed to two different objects (A & B) during sample phase 1 and then presented with two identical objects in the same locations during test (A & A). The differences in results could therefore be due to the task difficulty (four objects in Bonardi et al. vs two objects in Davis et al).

The overall results from this Chapter and Bonardi et al. (2006) support the results previously seen by Hale & Good (2005) mentioned above. Hale & Good (2005) found the same pattern of results in a much older (14 month) single mutation APP mouse model of AD. Together the results suggest that OIC performance is impaired from an early stage of the disease and persists throughout. The results together also point towards normal sNOR and RR performance throughout the disease, and does not become impaired

with increase age and pathology. These assumptions would still need to be established in older APPswe/PS1dE9 mice.

5.1 Chapter 5. Immunohistochemistry

Over the last 10 years research into the role of brain inflammation in AD has grown rapidly. Experiments have found that an inflammatory response occurs in the brains of AD patients, specifically around the insoluble aggregated form of amyloid-beta, amyloid plaques (Gasic-Milenkovic et al., 2003; Kummer et al., 2011; Lue et al., 2001; McGeer & McGeer, 1995).

5.2 Chronic inflammation

According to the amyloid cascade-inflammatory hypothesis, the inflammatory response to the increased aggregation of amyloid and deposition of plaques increases the development of further AD pathology (McGreer & McGreer, 2013).

Beta amyloid accumulation in the brain results in the activation of microglia and astrocytes in a site-specific manner around the amyloid depositions. Glia activation around the plaques results in the release of pro-inflammatory proteins to clear away Aβ deposits (Akiyama et al., 2000; Cooper et al., 2000), as a result of the constant aggregation and accumulation of Aβ in the brain, a chronic pro-

inflammatory response develops. Chronic stimulation of the immune response due to constant Aβ deposition can lead to an overall harmful effect on the brain; as the chronic production of pro-inflammatory responses by microglia and astrocytes in high concentrations can result in them becoming neurotoxic and causing further cell death (Blasko et al., 2004).

Recent research has shown that elimination of microglia does appear to have a positive effect on AD pathology. Dagher et al. (2015) treated a triple transgenic AD mouse (3xTG) with PLX5622 that inhibits the dial colony-stimulating factor 1 receptor and the c-kit kinase, which results in an elimination of microglia in the CNS. Following 3 month of PLX5622 treatment, 15-month-old 3xTG mice showed faster escape latencies on the MWM and faster latencies to the platform location during the probe trial compared to untreated 3xTG mice. 3 month treated 3xTG mice also showed a 30% reduction in microglia compared to untreated TG mice and a reduction in microglia clusters around amyloid plaques compared to untreated TG mice. No difference in plaque load was seen between the TG mice. A similar study by Spangenberg et al. (2016) treated a 5xfAD mouse model with PLX3397, a more effective inhibitor than PLX5622, for 28 days in 10-month-old 5xfAD mice. 5xfAD mice showed impaired

contextual fear conditioning compared to controls; however a trend towards recovery was seen in the treated 5xfAD mice. treated 5xfAD mice also showed an 80% reduction in microglia in the hippocampus, cortex and thalamus compared to non-treated TG mice. In these experiments the treatment was conducted after substantial AD pathology had developed in the TG mice, making it hard to establish the role of inflammation in the development of the disease.

5.3 Soluble Aβ and inflammation

Chronic inflammation in AD is usually attributed to the development of amyloid plaques. Recent evidence suggests that soluble Aβ, prior to plaque deposition, is also involved in initiating the inflammatory response. Evidence in support of this includes greater levels of microglia activation in patients with MCI (Bruno et al., 2009; Okello et al., 2009; Parachikova et al., 2007).

A number of experiments using transgenic animals have found increased microglia, astrocytes and pro-inflammatory markers in pre-plaque animals. Microglia levels also correlated significantly with soluble Aβ and cognitive performance in the young pre-plaque mice (Ferretti et al., 2012; Hanzel et al., 2014; Heneka et al., 2005; Ruan et al., 2009; Varvel et al., 2009).

5.4 APPSwe/PS1dE9 mice

The levels of inflammation have been examined in the APPswe/PS1dE mouse during the early stages of AD. Ruan et al. (2009) found clustered microglia (CD11b-positive staining) around amyloid plaques in both the hippocampus and neo-cortex from 4 months of age. GFAP-positive astrocytes were detected in the hippocampus of APPswe/PS1dE9 mice between 2-6 months, prior to plaque development. Zhang et al (2012) also found higher levels of astrocytes and microglia (iba1-positive) in pre-plaque (3.5 month) and older (6 and 12 month) APPswe/PS1dE9 mice compared to controls. Increased levels of Iba1 stained microglia have also been observed in the cortex and hippocampus of 9-month APPswe/PS1dE9 mice relative to those found in wild type controls (Xiong et al., 2015).

5.5 Hippocampus

As mentioned in Chapter 1, the hippocampus is one of the earliest areas affected during the development of AD, involving the deposition of plaques and tangles, as well as hippocampal atrophy (Braak & Braak 1991; Clark et al., 2013; Serrano-Pozo et al. 2011).

5.6 *Striatum*

The striatum is also a key area in the development of AD; however, it is largely overlooked. Striatum pathology occurs slightly after the medial temporal lobe is affected, with plaque deposition occurring during Braak stage 3. Striatal atrophy is present in the sporadic form of AD, but more pronounced in familial forms of the disease (Madsen et al., 2010; De Jong et al., 2008; 2014; Looi et al., 2010). Pievani et al (2013). Micotti et al. (2015) carried out volumetric measurements of striatum size using MRI in an APP/PS1 (TASTPM) mouse between 4 and 26 months. The results showed a global reduction in striatum volume (atrophy) from 8 months onwards in the transgenic mice. The analysis also showed greater volume loss in the ventral striatum compared to the dorsal section. Immunofluorescent analysis of synaptophysin, a protein found in neurons, in six regions throughout the striatum, showed a significant reduction of synapses a 26 months of age compared to wild types.

5.7 Aims & Hypotheses

Given that both the hippocampus and striatum are affected during AD, it seems reasonable that an immune response would be present in both regions. In the APPswe/PS1dE9 mouse model of Alzheimer's disease, increased inflammatory response has consistently been

observed in the hippocampus before and after Aβ plaque development (Pardon et al., 2016; Ruan et al., 2009; Xiong et al., 2015; Zhang et al., 2012). The striatum, however, has not been examined in relation to an inflammatory response in the APPswe/PS1dE9, despite it being an area affected early on in the familial version of AD (De Jong et al., 2008; 2014; Looi et al., 2010; Madsen et al., 2010).

5.8 Experiment 1

Experiment 1 aimed to examine the microglia levels in both the striatum and hippocampus of 4-5 month APPswe/PS1dE9 mice and controls. Microglia levels were measured by staining with the iba1 (ionizing calcium-binding adaptor molecule 1) antibody (Ito et al. 1998). Iba1 is a protein that is specifically expressed in microglia and is up-regulated during microglia activation, therefore a useful tool in measuring the global (resting & active) number of microglia in the brain. Given the hypothesis that an immune response occurs much earlier in the disease's progression than previously thought, we predict that glial activity will be up-regulated in the APPswe/PS1dE9 mice, relative to the wild types, in both the hippocampus and striatum, even though plaque deposition is only just starting to occur.

Experiment 1 also measured the number of microglia clusters in the cortex, hippocampus and striatum. Microglia have been shown to cluster around Aβ plaques, in an attempt to clear away the depositions (Barton et al., 2009). Microglia clusters can therefore be used as an indirect measure of Aβ plaque levels in the young APPswe/PS1dE9 mice. We predict that higher levels of microglia clusters will be observed in the 5-month than in the 4-month APPswe/PS1dE9 mice, whilst the cortex would show the greatest level of clusters relative to the hippocampus and striatum.

5.9 Methods

5.9.1 Tissue processing

Following the end of Experiment 2 (Chapter 3) the mice were sacrificed aged 4 and 5 months via cervical dislocation, the brains were removed and post-fixed in 8% paraformaldehyde, and stored at 4-8^{0}C for at least 48 hours. The brains underwent tissue processing; first they were dehydrated to remove both water and the fixative, by placing the samples into increasing ethanol concentrations. Specifically, into 70% ethanol for 1 hour, a second 70% ethanol for 1 hour, then into 90% ethanol for 1 hour, a second 90% ethanol for 1.5 hours, then the first 100% ethanol for 1.5 hours, second 100%

ethanol for 1.5 hours and the final 100% ethanol for 2 hours. The samples had the ethanol removed via clearing, in which the brains were placed into 100% chloroform for 2 hours, a liquid that is compatible with the embedding medium. Finally the samples were placed into liquid paraffin wax twice, both times for 1 hour. The samples were then embedded in paraffin wax using a tissue embedding station (Leica TP1020).

5.9.2 Slicing

8-µm-thick coronal sections were cut between Bregma 1.18mm to -2.92mm using a microtome (Microtome Slee cut 4060). Each slice contained all neuroanatomical areas associated with the Bregma position. Every 10^{th} slice was retained and mounted on APES (3-Aminopropyltriethoxysilance) coated slides and dried overnight at 40^{o}C. The sections containing the hippocampus were taken from between Bregma 1.18mm and -2.92mm, while sections containing the striatum were taken from between Bregma 1.32mm and -0.82mm via visual examination during sectioning.

5.9.3 Immunohistochemistry

Two slides per brain region, averaging 6 slices per slide, were used for staining. Immunohistochemistry was carried out according to a standardised procedure (Pardon et al., 2016). The sections were

deparaffinized and rehydrated by placing the samples in consecutive rinses of xylene, before being immersed in 100% ethanol, 70% ethanol and finally dH_2O for 5 minutes each. The samples then underwent antigen retrieval by being placed into a tri-sodium citrate buffer (pH = 6.0), heated to 95^0C and maintained at this temperature for 20 minutes. Following heating, the beaker was removed from the water bath, and allowed to cool to 70^0C. The sections were incubated in PBS (phosphate buffered saline) and placed into a humidity chamber before incubation in 1% H_2O_2 (50µl H_2O_2 per 5000µl PBS-Tween) for 20 minutes to prevent endogenous peroxidase activity. Samples were rinsed in PBS before being incubated in a 5% goat serum solution (250µl goat serum to 5000µl PBS-T) for 30 minutes. Sections were then incubated in rabbit anti-Iba1 (Wako, cat. Nr. 019-19741; 1µl iba1 / 6000µl PBS-Tween) for 90 minutes. The sections were once again rinsed in PBS prior to the incubation for 30 minutes with biotinylated secondary anti-body (Vectastain Elite ABC Kit, Rabbit IgG, Vector Labs, Burlingame, CA cat. Nr. PK-6101; 25µl in 5000µl PBS-Tween). Following the second antibody the sections were rinsed in PBS before incubation in ABC-HRP solution (Vectastain Elite ABC Kit R.T.U, Vector Labs cat.nr. PK-7100) according to manufacturer's instructions for 30 minutes. The ABC-

HRP solution is light sensitive, so the humidity chamber was covered. The sections were then rinsed in dH_2O before the final incubation with DAB peroxidase substrate (Vector Labs cat. SK-4100), according to manufacturer's instructions, for around 5 minutes before being rinsed in dH_2O.

The sections then underwent counterstaining using haematoxylin and eosin protocol. The sections were placed into Harris Haematoxylin for 1 minute, followed by acid alcohol (10s), Scotts tap water (15s), followed by alcohol in increasing concentrations (50%, 70%, 90% and 100%) for 10 seconds each before being placed into xylene for 4 minutes. Sections were then mounted with Clearvue mountant (Thermo scientific, cat. Nr. 4212). The slides were cleaned the following day with acetone.

Images of the samples were taken using a Hamamatsu NanoZoomer-XR 2.0-RS C10703 digital scanning system using a TDI NanoZoomer camera (Hamamatsu Photonics K.K. Systems, Japan) using a 20 X magnification and viewed using NDP.view2 software (NanoZoomer Digital Photography).

5.10 Data treatment

5.10.1 Regions of interest

In the sections containing the striatum a .2mm region was defined in both hemispheres (Figure 5.1), whilst in the sections containing the hippocampus a .2mm region was defined in the CA1, CA3 and dentate gyrus (DG), and a .1mm region in CA2 (Figure 5.2 & 5.3) for both hemispheres. 8 striatum slices, and 6 hippocampal slices were used in the analysis.

The number of iba1-stained microglia was then counted in those regions. The counting was done blind (i.e. without knowing the genotype of the sections). The number of iba1-stained microglia was averaged between hemisphere and slice before being analysed using ANOVAs; significant interactions were explored with simple main effects using the pooled error term.

5.10.2 Total clusters

Only the APPswe/PS1dE9 mice were used in the analysis of microglia clusters, as they occur around amyloid plaque depositions (Ruan et al., 2009), which are not present in the wild type mice. The sections containing the striatum and hippocampus also contained cortical areas, which were also analysed together.

The total numbers of iba1-stained microglia clusters in the cortex were counted between Bregma 1.32mm / -0.82mm (sections containing the striatum, which included: primary, secondary motor , primary somatosensory, Granular insular, piriform cortex, ventral pallidum)) and 1.18mm and -2.92mm (sections containing the hippocampus, which included: retrosplenial agranular, lateral parietal association, primary, secondary somatosensory, secondary auditory, piriform cortex). The numbers of clusters were also counted inside the hippocampus and striatum sections. Due to the low numbers of microglia clusters observed in the specific regions, the total clusters were analysed in an identical manner to the region of interest data; however, the data were not split between hemispheres.

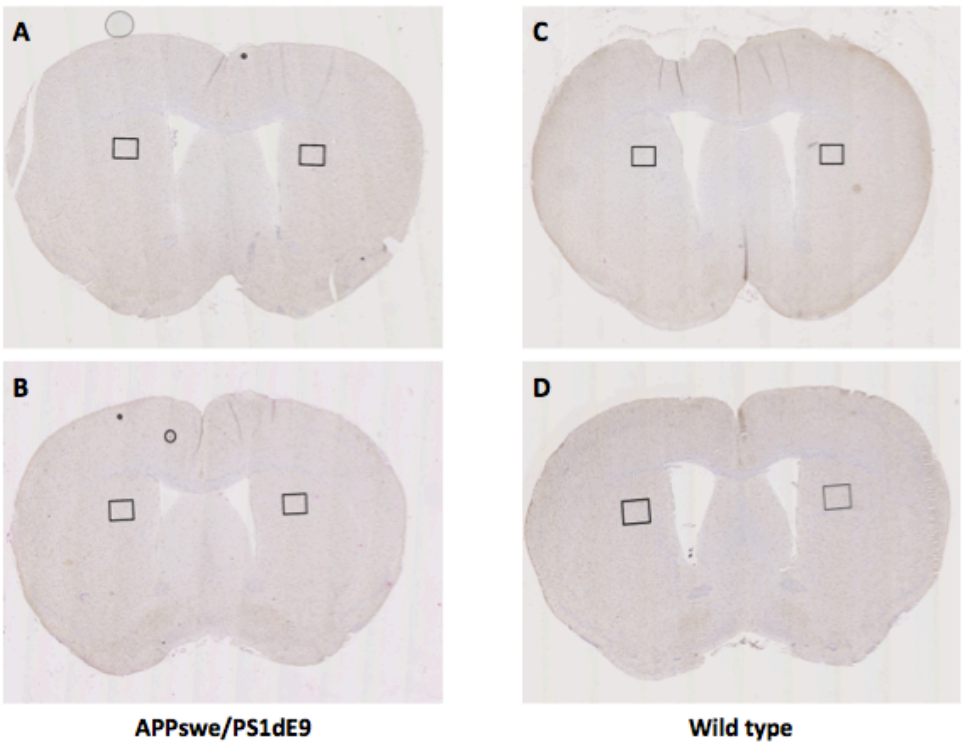


Figure 5.1. Image of iba1-stained microglia striatal sections. Each panel shows the placement (black box) of the .2mm region of interest inside the striatum for a representative sample of APPswe/PS1dE9 (A & B) and wild type (C & D) mice.

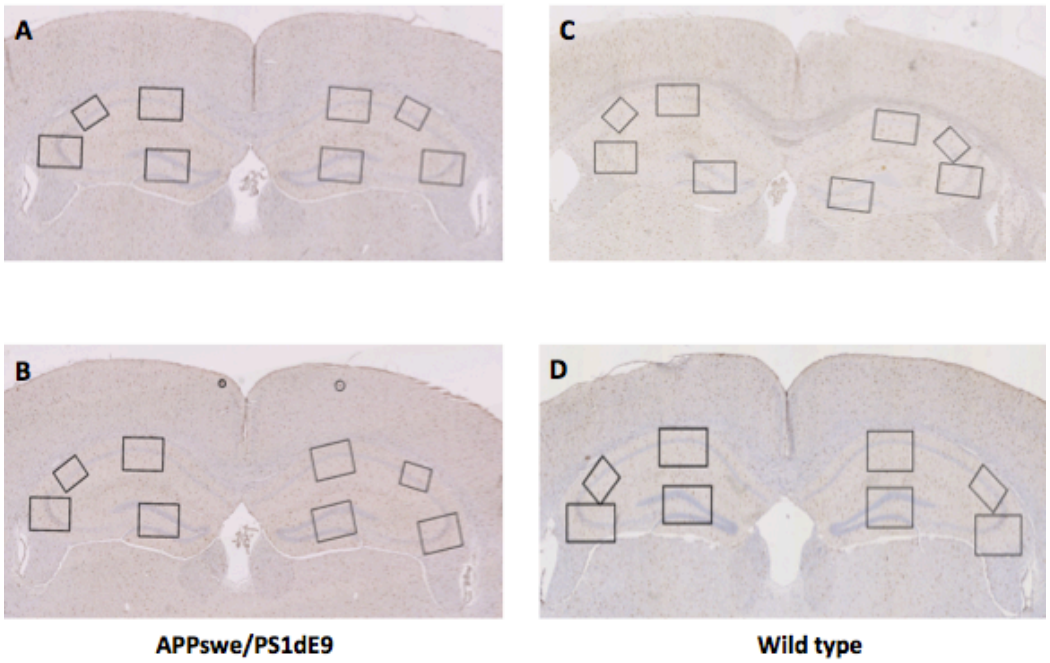


Figure 5.2. Image of iba1-stained microglia in hippocampal sections showing the placement (black box) of the .2mm/.1mm region of interest for a representative sample of APPswe/PS1dE9 (A & B) and wild type (C & D) mice.

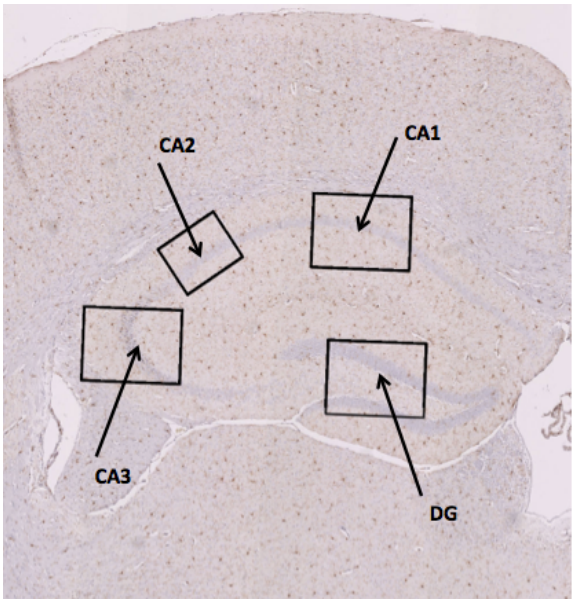


Figure 5.3. A magnified version of Figure 5.2 A, right hemisphere, showing the positions of the regions of interest in the hippocampus (CA1, CA2, CA3 and dentate gyrus - DG).

5.11 Results

5.11.1 Striatum

The number of iba1-stained microglia was greater in the APPswe/PS1dE9 mice (Figures 5.4 & 5.5). An ANOVA with genotype (APPswe/PS1dE9 & wild type) and age (4 months & 5 months) confirmed only a main effect of genotype, $F(1, 20) = 4.93$, $MSE = 429.26$, $p = .038$, $\eta_p^2 = .198$ and of age, $F(1, 20) = 16.53$, MSE = 429.26, $p = .001$, $\eta_p^2 = .453$. No other main effects or interactions were observed (smallest $p = .521$). The analysis confirmed more iba1-stained microglia in the striatum in the APPswe/PS1dE9 mice than the controls, and in the 5-month-old mice than the 4-month-old mice.

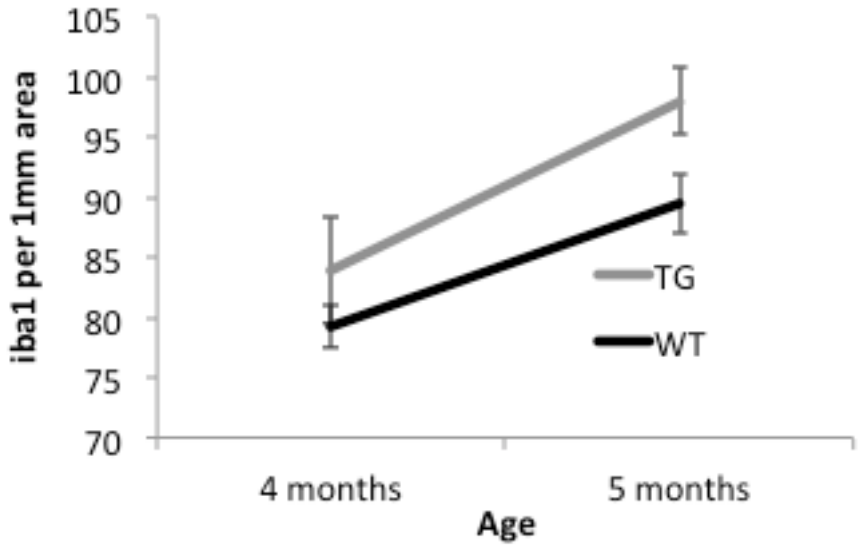
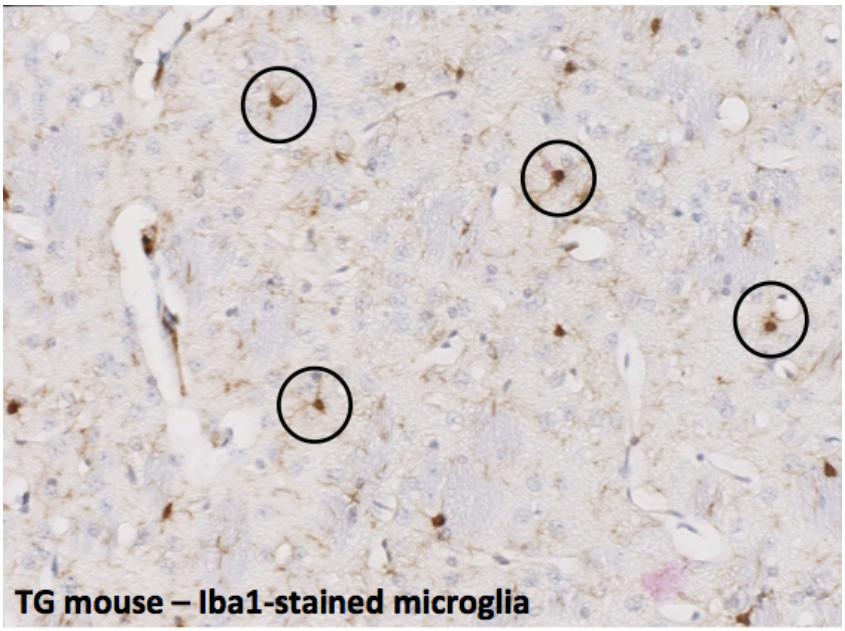


Figure 5.4. Group mean number of striatal iba1-stained microglia in TG and WT mice, split by age (4 & 5 months). Error bars show standard error of the mean.



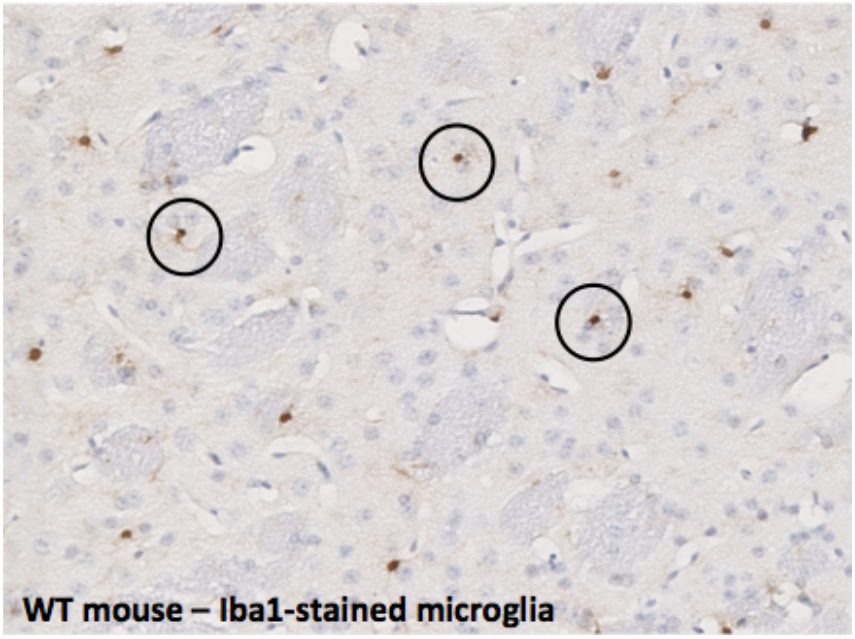


Figure 5.5. A representative sample of iba1-stained microglia in one striatal region of interest (.2mm), in one TG and one WT mouse. Circle shows an individual iba1-stained microglia.

5.11.2 Cortex Clusters

An ANOVA with age (4 month & 5 months) as a factor revealed a main effect of age, $F(1, 11) = 17.10$, $MSE = 357.88$, $p = .002$, $\eta_p^2 = .631$ reflecting a greater number of clusters at 5 months than at 4 months. This is to be expected (Figure 5.6 & 5.7), as microglia cluster around amyloid plaques, and the number of plaques significantly increases with age.

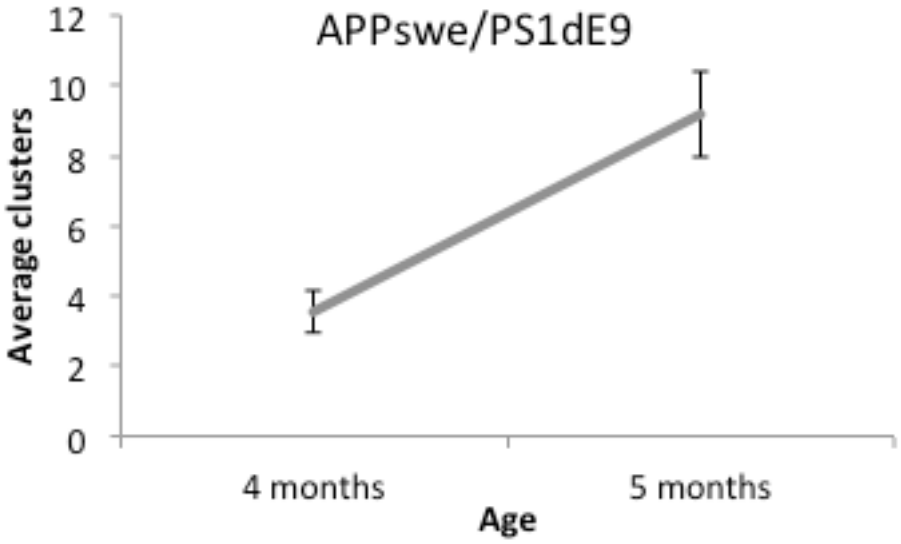


Figure 5.6. Mean number of iba1-stained microglia clusters in the cortex between Bregma 1.32mm to -0.82mm (Striatum sections) in APPswe/PS1dE9 mice split by age. Error bars show standard error of the mean.

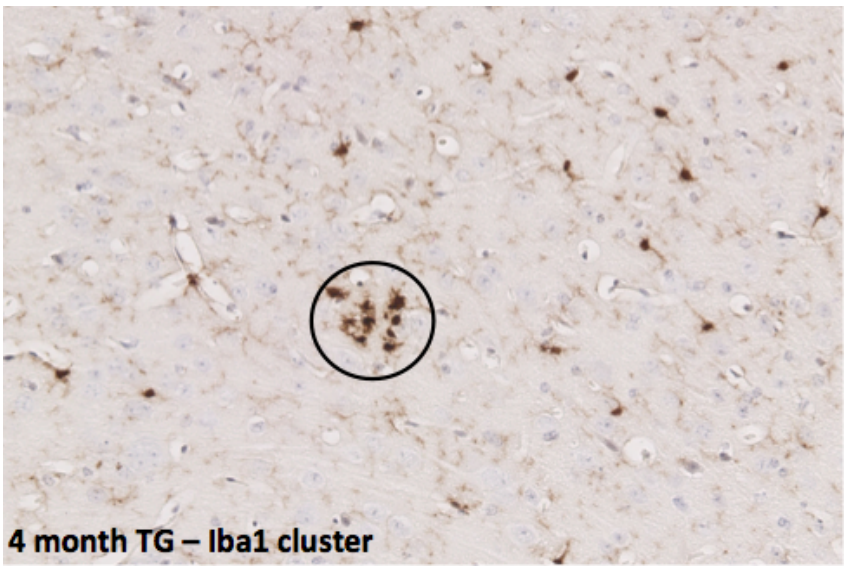


Figure 5.7. A representative iba1-stained microglia cluster, in a 4-month-old APPswe/PS1dE9 mouse.

5.11.3 Hippocampus – region of interest

APPswe/PS1dE9 mice appeared to show a greater number of iba1-stained microglia than the wild type mice (Figure 5.8 & 5.9). An ANOVA with genotype, age and area (CA1, CA2, CA3, DG) found a main effect of genotype, $F(1, 20) = 6.92$, $MSE = 1087.75$, $p = .016$, $\eta_p^2 = .257$, reflecting more iba1-stained microglia in the APPswe/PS1dE9 mice than the controls. The analysis also showed a main effect of age, $F(1, 20) = 24.80$, $MSE = 1087.75$ $p < .001$, $\eta_p^2 = .554$, which confirmed more iba1-stained microglia in the 5 month mice than in the younger (4-month) animals. Finally, there was a main effect of area $F(3, 60) = 9.16$, $MSE = 322.53$, $p < .001$, $\eta_p^2 = .314$, showing different levels of iba1-stained microglia in different regions of the hippocampus. Pairwise comparisons (Bonferroni correction) tests showed greater iba1 activation in CA1 than in CA3 ($p < .001$), and more in the DG than CA3 ($p = .002$). No other main effect or interaction was found to be significant (smallest $p = .063$ for area * genotype interaction).

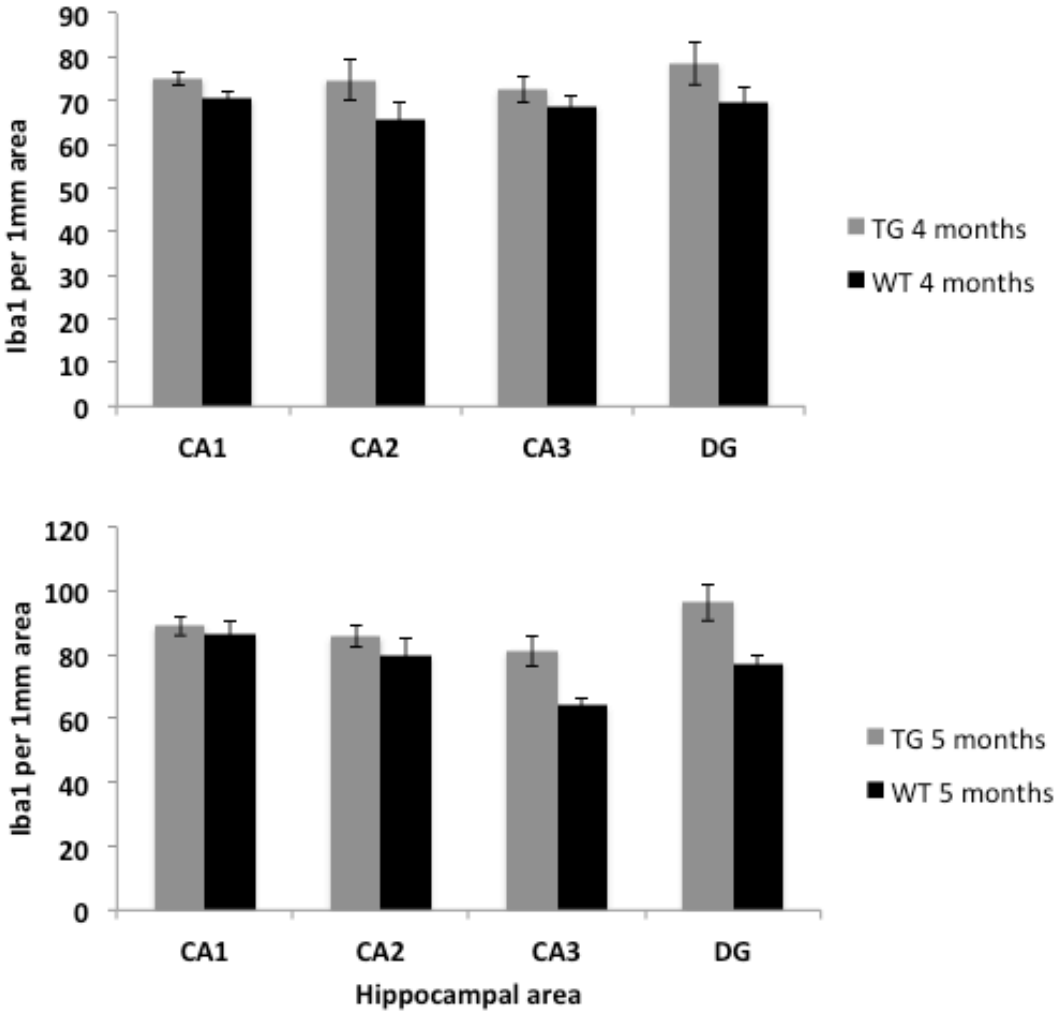


Figure 5.8. Group mean number of iba1-stained microglia in the CA1, CA2, CA3 and the dentate gyrus (DG) regions of the hippocampus for 4 month (top panel) and 5 month (bottom panel) TG and WT mice. Error bars show standard error of the mean.

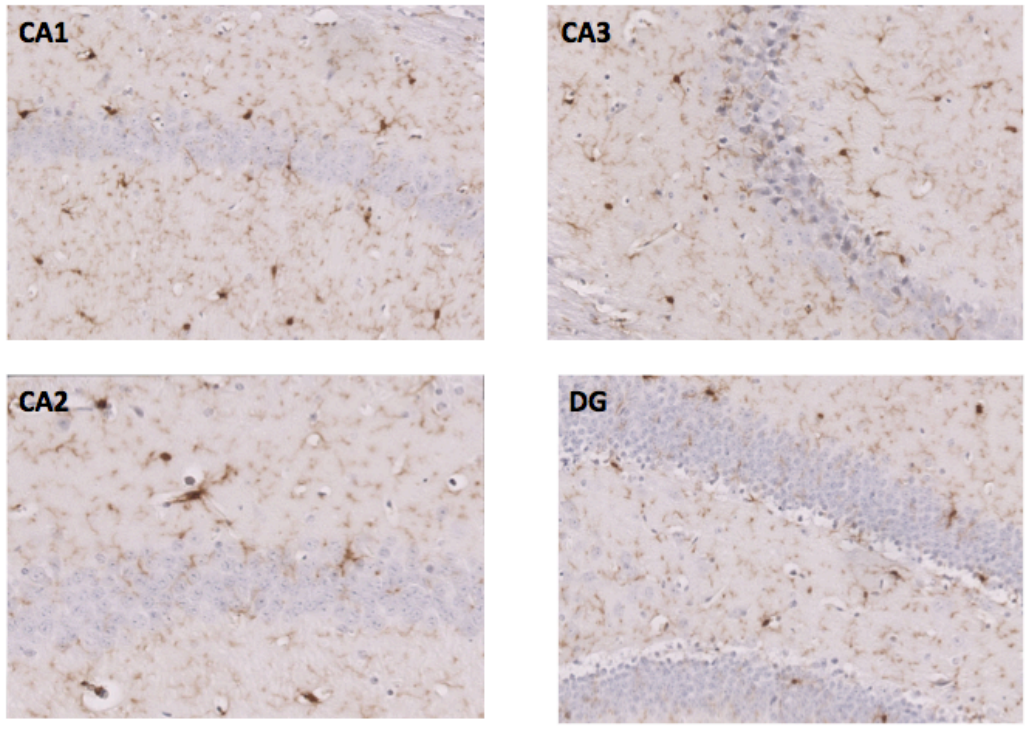


Figure 5.9. A representative sample of Iba1-stained microglia in the CA1, CA2, CA3 and dentate gyrus (DG) in one 4-month APPswe/PS1dE9 (top panel) and one wild type (bottom panel) mouse.

5.11.4 Cortex Clusters

The number of iba1-stained microglia clusters in the cortex and hippocampus were analyzed between Bregma -1.28mm to -2.92mm (Figure 5.10, 5.11 & 5.12). The 5-month mice appeared to show more clusters in both the hippocampus and cortex in comparison to the younger (4 month) mice. ANOVA with age and area (cortex & hippocampus) as factors revealed a main effect of age, $F(1, 10) = 20.18$, $MSE = 80.59$, $p = .001$, $\eta_p^2 = .669$, and a main effect of area, $F(1, 10) = 51.12$, $MSE = 33.68$, $p = .000$, $\eta_p^2 = .836$. An area * age interaction was also found, $F(1,10) = 22.99$, $MSE = 33.68$, $p = .001$, $\eta_p^2 = .697$, and post hoc tests showed more iba1-stained microglia in the cortex of the 5-month mice than the 4-month-old animals, $F(1, 10) = 40.65$, $MSE = 57.14$, $p < .001$. A difference in iba1 levels was also seen between the cortex and hippocampus, but only in the 5-month-old mice, $F(1, 10) = 71.34$, $MSE = 33.68$, $p < .001$. No other main effect or interaction was found to be significant (smallest $p = .110$).

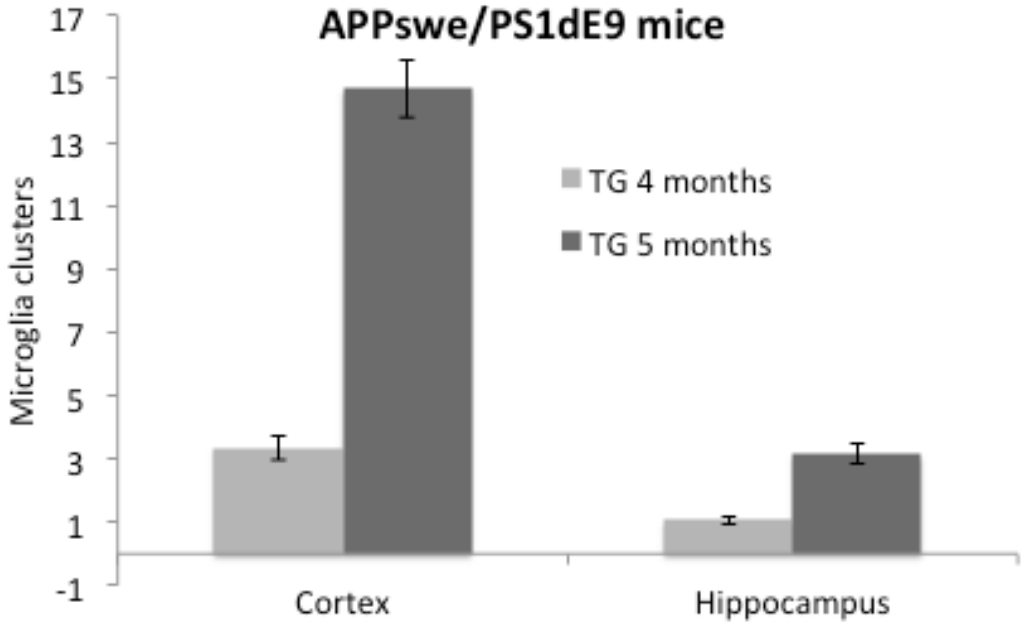


Figure 5.10. Mean number of iba1-stained microglia clusters in the APPswe/PS1dE9 mice in both the cortex and hippocampus at two different ages (4 and 5 months). Error bars show standard error of the mean.

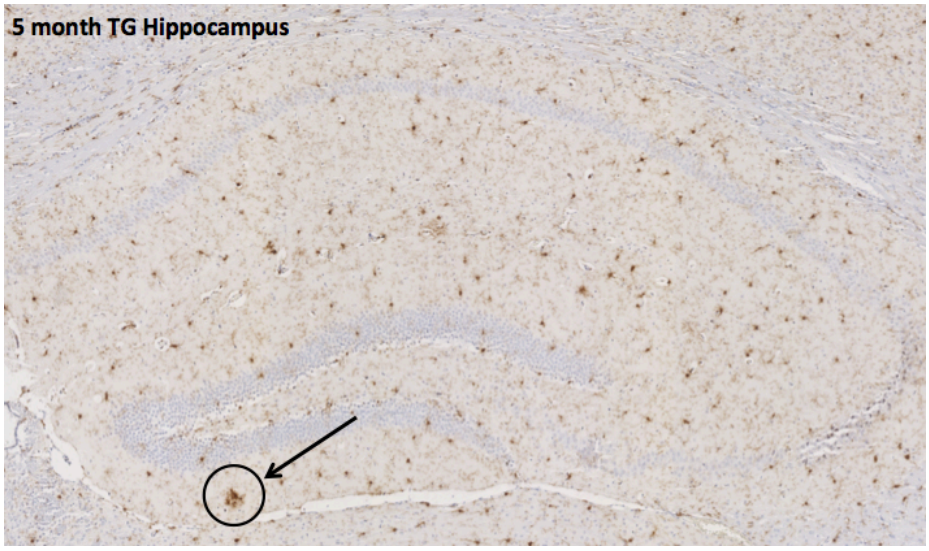


Figure 5.11. Shows a representative iba1-stained microglia cluster in the hippocampus of a 5-month-old APPswe/PS1dE9 mouse

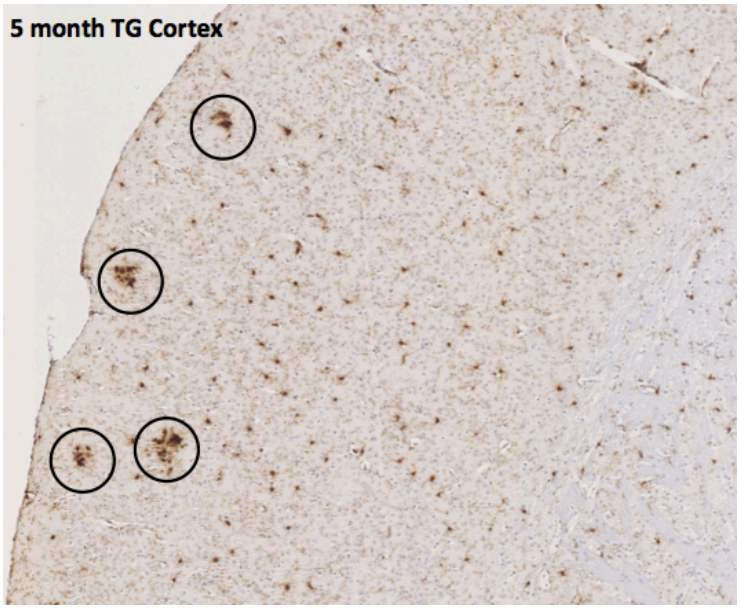


Figure 5.12. Representative Iba1-stained microglia clusters in the cortex of a 5-month-old APPswe/PS1dE9 mouse.

5.12 Discussion

Experiment 1 examined the microglia levels in the striatum and hippocampus of 4 and 5-month APPswe/PS1dE9 mice. The results show a greater number of iba1-stained microglia in the APPswe/PS1dE9 mice than in the wild types in both the striatum and the hippocampus at both age points. The results also showed a greater number of iba1-stained microglia in the 5-month-old mice, regardless of genotype, compared to the 4-month-old animals, which has previously been observed in aging (Safaiyan et al, 2016) and AD studies (Caruso et al., 2013). Previous studies have identified greater numbers of microglia (via iba1-staining) in the hippocampus and

cortex of APPswe/PS1dE9 mice from a variety of ages (Garcia et al., 2014; Zhang et al., 2012). No studies to date have examined microglia in the striatum during AD development, and although it is not conventionally associated with AD, it is an area damaged by the disease (Braak & Braak, 1990; De Jong et al., 2008; 2014; Looi et al., 2010; Madsen et al., 2010). The results present a novel finding of more iba1-stained microglia in 4- and 5-month APPswe/PS1dE9 mice than wild types.

Microglia clusters have been shown to congregate around amyloid plaques at as early as 4-months of age in the APPswe/PS1dE9 mouse (Ruan et al., 2009). Whilst the current experiment has not confirmed the existence of plaques via specific Aβ staining, there presence can be inferred from the number of microglia clusters in both the cortex and hippocampus. The results showed greater number of clusters of iba1-stained microglia in the older 5-month-old mice than in the 4-month-old animals in both the cortex and hippocamus, suggesting increased plaque deposition with age - an effect that has been consistently observed (Garcia-Alloza et al., 2006; Jankowsky et al., 2004). The greater level of clustered iba1-stained microglia in the cortex than in the hippocampus is also indicative of AD pathology, the cortex showing plaque deposition

prior to the hippocampus (Braak & Braak, 1991). The microglia clusters in the cortex were specifically observed in the associative cortices, primary and secondary motor cortex and to some in the somatosensory cortices, which is consistent with previous research (Braak & Braak 1991; Thal et al., 2002). No iba1-stained microglia clusters were found in the striatum of APPswe/PS1dE9 mice at 4 or 5 months. This lack of microglia clusters in the striatum may well reflect a lack of Aβ plaque deposition at those age points, which is consistent with the literature.

5.12.1 Neuro-pathological Findings and Behavioural Findings

The early inflammation in the striatum may well be linked to the subtle impairments in appetitive Pavlovian conditioning seen in Chapters 2 and 3. A body of work has linked the involvement of the striatum, particularly the ventral portion, to Pavlovian learning. Day et al. (2007) measured dopamine release within the rat nucleus accumbens during an appetitive CS-US Pavlovian conditioning procedure. Early during conditioning the arrival of the US signaled the transient release of dopamine, however; with greater conditioning, dopamine release became predicted by CS onset. Presentation of the US in isolation, after conditioning, no longer

produced dopamine release. Darvas et al. (2014) found that mice with striatal dopamine depletion showed impaired conditioned responding during an appetitive Pavlovian CS-US association, however, this improved with pharmacological injections of L-dopa. Blaiss et al. (2009) tested rats on an appetitive CS-US association. Rats with post-training lesions of the NAc core, prior to a test session, resulted in a disruption in the level of conditioned responding. Finally, for example Ferreira et al. (2003) gave rats either electrolytic or neurotoxic lesions of the dorsal striatum prior to undergoing tone fear conditioning. Both types of lesions resulted in reduced conditioned freezing to the tone compared to shams.

The experiments point towards the involvement of the ventral striatum, and the neurotransmitter dopamine in Pavlovian conditioning. The ventral striatum also has anatomical connections to other important regions affected by AD, including the hippocampus, amygdala and medial orbitofrontal cortex (Likieholm & O'Doherty 2012). Future research could look to correlate the level of inflammation in the ventral striatum with both appetitive and aversive cue conditioning in the APPswe/PS1dE9 mice during early and late stages of AD pathology.

Almost no research has shown that the striatum is critically involved in sNOR performance. As mentioned in Chapter 4, both the hippocampus and perirhinal cortex play important roles in the different variations of recognition memory.

5.12.2 Neuro-pathological Findings and Timing

The increased inflammation, and presumable AD pathology, in the striatum at 4 / 5 months of age could well be involved in the loss of precision in timing a target duration seen in Experiments 1 and 2 from Chapter 3. The striatum has been implicated in normal interval timing performance. Matell et al (2003) trained rats to level press at two distinction durations whilst monitoring the firing pattern of striatal neurons. The study found that 22% of striatal neurons showed a specific temporal firing pattern for the two timed durations. Neurons showed a gradual ramped firing pattern over each CS duration, whilst, genetic knock out of striatal NMDA R1 receptors in mice also resulted in an inability to time specific intervals (Jin et al., 2010). Interestingly disruption of the 5-hydroxytryptaminergic pathway, which possess functional connections between the hippocampus, amygdala and striatum produces similar increased variability in timing target duration using

genetically modified mice (Drew et al., 2007; Morrissey et al., 1994; Ward et al., 2009).

The hippocampus is also implicated in alter timing behaviour; however, shows a specific pattern of underestimation, or responding earlier than the specific target duration (Balci et al., 2009; Tam & Bonardi, 2012; 2012; Yin & Meck et al., 2012), which was not observed in the APPswe/PS1dE9 mice.

As the hippocampus and stratum are anatomically linked, subtle AD pathology, resulting in increased inflammation may well be enough to cause the observed loss of precision in timing a specific target duration.

5.13 General discussion

Chapter five has shown higher levels of iba1-stained microglia in the striatum of 4- and 5-month old APPswe/PS1dE9, than wild type mice. No microglia clusters were found in the striatum at either age. These results support the idea that inflammation precedes plaque deposition during the development of AD pathology, which is consistent with some of animal literature (Rodriguez et al., 2009; Ruan et al., 2009; Zhang et al., 2012).

Regarding the hippocampus, the results presented support research showing increased inflammatory markers (microglia &

astrocytes) in pre-plaque APPswe/PS1dE9 mice (Ruan et al., 2009; Zhang et al., 2012). The results showed greater iba1-stained microglia in the hippocampus of APPswe/PS1dE9 mice at 4 and 5 months compared to wild types. This increase was also age dependent. Microglia clusters were observed in the hippocampus from 4 months of age in the APPswe/PS1dE9 mice, and increased by 5 months. 4 month APPswe/PS1dE9 mice showed an up regulation of iba1-stained microglia in the hippocampus and the very early stages of Aβ plaque deposition, shown by the average of 1 microglia cluster.

The cortex showed an age dependent increase in the number of microglia clusters from 4 to 5 months in the APPswe/PS1dE9 mice. Greater numbers of clusters were found in the cortex than in the hippocampus, consistent with the Braak stages of AD development. Cortex clusters were low (average of 3) at 4 months of age but increased by 5 months (average of 11) suggesting that 4/5 months is a key time point in the acceleration of AD pathology in the APPswe/PS1dE9 mouse.

6.1 Chapter 6. General Discussion

This thesis aimed to assess cognitive function in a young APPswe/PS1dE9 mouse model of Alzheimer's disease, using experimental tasks based on associative learning. Chapters 2 to 4

addressed different associative learning tasks in relation to early stage AD while Chapter 5 examined the early neuroinflammatory changes that may underpin the cognitive impairments we observed.

6.2 Chapter 2: Conditioning and extinction

6.2.1 Summary of findings

Chapter 2 examined the performance of 4-5 month APPswe/PS1dE9 mice on Pavlovian conditioning and extinction to both contextual and discrete cues. Experiment 1 simultaneously tested contextual and cue conditioning. The results showed normal appetitive conditioning to both discrete and contextual cues, whilst also showing discrimination between the reinforced and non-reinforced context & cue. Chapter 2 went on to test both cue and context conditioning separately. Experiment 2 tested appetitive conditioning and discrimination to a discrete cue (A+ vs B-), before reversing the reward contingencies (A- vs B+), which previous research has shown is impaired in the APPswe/PS1dE9 mouse (Filali & Lalonde., 2009; Montgomery et al., 2009). No genotype differences were observed during conditioning to the discrete cues or during the reversal-learning phase, despite APPswe/PS1dE9 mice appearing to show numerically faster extinction of A+ during reversal. The final experiment in Chapter 2 examined appetitive conditioning and extinction of contextual cues.

The results showed some evidence of impaired context discrimination between the reinforced and non-reinforced contexts; however, only during the penultimate conditioning session. No differences were found in extinction. Thus Chapter 2 only found limited evidence of impaired contextual conditioning during Experiment 3.

6.2.2 Implications

Because the hippocampus is one of the first areas affected by the pathology of AD, it is conceivable that hippocampal mediated tasks might be more susceptible to disruption during the development of the disease. Previous research in APPswe/PS1dE9 mouse has shown a disruption in contextual fear conditioning (CFC), which is thought to be a hippocampal-dependent task, between 3 and 8 months (Bonardi et al., 2011; Jacobsen et al., 2006; Kilgore et al., 2010; Lin et al., 2015; Maroof et al., 2016; Vegh et al., 2014)

The hippocampal-dependent nature of CFC, is based on the processing of contextual information, not the US modality. The results of Experiment 3 showed a slight impairment in discriminating between a reinforced and a non-reinforced context in 4-month-old APPswe/PS1dE9 mice, which has not previously been observed, supporting the idea that contextual processing is impaired in AD.

This tentative result is supported by literature that shows that lesions of the hippocampus disrupt conditioned place preference, where one context is paired with an appetitive reward (A+) and another is unreinforced (B-). Lesioned animals spent less time in the reinforced arm compared to shams (Ferbinteanu & McDonald 2001; Meyers et al., 2006).

Experiments 1, 2 and 3 (Chapter 3) and previous research (Bonardi et al., 2011; Stover & Brown., 2012) in the APPswe/PS1dE9 mouse have failed to show any differences in cue conditioning using an appetitive US in mice between 4 and 8 months of age. No impaired appetitive cue conditioning was observed at 8 months (Chapter 3, Experiment 3) when there is extensive deposition of amyloid plaques in the hippocampus. These results are inconsistent with the literature that shows ventral hippocampal lesions impaired cued conditioning (Bast et al., 2001; Chen et al., 2016; Maren et al., 1999; Maren & Holt; 2004; Richmond et al., 1999; Zhang et al., 2001; 2014).

Interestingly Lin et al. (2015) showed evidence of impaired cue conditioning using an aversive US in 2, 3 and 4-month APPswe/PS1dE9 mice, consistent with the hippocampal lesions data. The authors; however, argued that the very early impairment in cue

conditioning was a result of early stage changes in the amygdala at 2 months prior to the hippocampus. The changes in the amygdala include increased amyloid beta levels, a reduction in dendrite sizes in BLA neurons, reduced brain-derived neurotrophic factor (BDNF) and serotonin rececptors (5-HT).

The results from all the contextual and cue conditioning tasks mentioned above in general are consistent with the argument that processing of aversive information as opposed to contextual conditioning per se is impaired.

This hypothesis highlights the amygdala, which is involved in aversive processing, as an area affected during the early development of AD. Evidence comes from Lesions of the amygdala and specific nuclei results in disruption of conditioned fear to both discrete cues (Goosens & Maren 2001; Maren et al. 1996; 1999; Vazdarjanova & McGaugh 1998, for a review see LeDoux 2003) and contexts (Flavell & Lee 2012; Maren et al., 1996; 1999, for a review see LeDoux 2003). In relation to AD, the amygdala has been investigated but not to the same degree as the hippocampus. A number of imaging studies in patients with early stage AD have shown evidence of amygdala atrophy compared to controls, and is significantly related to clinical measures of AD (Mini Mental State

Examination) (Basso et al., 2006; Farrow et al., 2007; Poulin et al. 2011). Therefore tasks that measure amygdala function may also be a useful measure of early AD.

6.2.3 Future research

Future research should firstly consider carrying out a replication of Experiment 3 using an older cohort of APPswe/PS1dE9 mice. Given the subtle impairments in context discrimination in 4 month mice, it may well be possible to see both a slower rate of context acquisition as well as impaired context discrimination in 8-10 and 12-15 month mice. This would be a useful way to track how the progression of AD affects the performance on this particular task.

Experiments 1 and 3 looked at associations between contexts and rewards; however, contexts can also serve another function, to help retrieve information about discrete CS-US associations that occurred within the context; occasion setting (Bouton & Swartzentruber 1986; Hall & Honey 1989). During an occasion setting task animals are trained on a CS (X) - US association in a context (A), during test rodents will show greater conditioned responding to X in context A than in a novel context B. Performance on contextual occasion setting experiments has been impaired in

animals with hippocampus lesions (Good & Honey 1991; Good et al., 1998; Holt & Maren 1999; Yoon et al., 2001. But see Hall et al., 1996 for no effect of lesion). Honey & Good (1993) trained hippocampal rats and controls on a context-specific association task. Rats were appetitively reinforced during the presentation of cue X in context A, and cue Y in context B. After acquisition, rats received conditioning trials with X and Y in the conditioned context (same), but also received probe trials of the other cue (different). Controls showed greater responding to the same cue compared to the different cue, whilst hippocampal rats showed greater responding to the different cue, reflecting a lack of contextual control over CS responding.

Given the impaired performance of APPswe/PS1dE9 mice in contextual fear conditioning task, the evidence of impaired contextual conditioning from Chapter 2 and that the hippocampus is one of the first areas to develop Alzheimer's pathology this type of contextual occasion setting may well also be impaired.

Currently no experiments have examined reversal learning to contextual cues in mouse models of AD. Reversal learning to both contextual and discrete cues has been examined in a group of patients with MCI which often precedes the diagnosis of AD. Levy-Gigi et al. (2011) presented participants with stimulus compounds

consisting of two dimensions: a central shape (cue: 1, 2 etc.) and a background colour (context: A, B etc.) on cards. During training four cards are presented (A1, B2, C3, D4) some of which are reinforced with a positive outcome (+) and some with a negative outcome (-). Participants can flip over the cards for the desired outcome or skip each card. During reversal they were presented with cards from training or new compounds with either a change in cue (A3) or context (C1). If the original compound had a positive outcome (A1+) then its reversals will be negative (A2- & B1-), and vice versa. No difference in performance was observed between groups during the training phase; however, during the reversal phase MCI patients made significantly more incorrect choices of contextual cues. The level of right hippocampal volume was also correlated with performance on contextual reversal in the MCI group, implicating the hippocampus in reversal learning to contexts.

This type of procedure could easily be adapted and carried out in the APPswe/PS1dE9 mouse. Using either aversive or appetitive rewards, animals could be reinforced in one context (A+) and not in another (B-), the same as Experiment 3 before having the reward contingencies reversed.

6.3 Chapter 3: Trace and delay conditioning

6.3.1 Summary of findings

The experiments in Chapter 3 looked delay and trace conditioning in 4 and 5-month APPswe/PS1dE9 mice, whilst also assessing timing the arrival of the conditioned food reward. Experiment 1 found evidence of intact delay and trace conditioning. The analysis of timing revealed that both genotypes could time the arrival of the reward (peak time) to the delay CS, whilst APPswe/PS1dE9 mice were impaired in their timing precision (larger peak spread) compared to wild type mice. No differences in timing were observed in the trace-conditioning task. Experiment 2 focused on the delay conditioning impairment in Experiment 1. Experiment 2 conditioned mice to a 10s and 20s delay CS. The results showed intact conditioning to both CS's in both genotypes. During the peak sessions, APPswe/PS1dE9 mice once again showed evidence of impaired timing accuracy (larger peak spreads) compared to wild type mice, whilst maintaining normal timing of the arrival of the reward. Given the results found from Experiments 1 and 2, it was hypothesized that timing performance would become even worse in older, more diseased, APPswe/PS1dE9 mice. Thus Experiment 3 carried out a replication of Experiment 2 in 8-month-old APPswe/PS1dE9 and wild type mice. The conditioning results were

the same as Experiment 2, no difference between genotypes. However, the peak test results failed to show any impairments in timing by the APPswe/PS1dE9 mice, which is counterintuitive, as performance would be expected to worsen with a progressive neuro-degenerative disease. The APPswe/PS1dE9 mice did show numerically greater spreads than the wild types only for the longer 20s CS, which does support the general findings to some degree.

6.3.2 Implications

Previous research into the estimation of time durations in human Alzheimer's disease populations has found a lack of precision (Carrasco et al. 2000; Hellstrom & Almkvist. 1997; Nichelli et al. 1993; Papagno et al. 2004; Rueda & Schmitter-Edgecombe. 2009). Experiments 1 and 2 have shown for the first time a loss of precision in timing the arrival of a US in, early stage pathology, APPswe/PS1dE9 mice, which is reasonably analogous task to the measure used in humans. Chapter 3 gives further support to the observed disruption of timing in AD, which is a research area that has been largely ignored.

Neuro-anatomically a number of different brain regions have been linked to normal interval timing behaviour. A leftward shift or an underestimation of the target duration has been consistently

observed in animals with either hippocampal lesions (Balci et al., 2009; Tam & Bonardi, 2012; 2012; Yin & Meck et al., 2012) or fimbra-fornix lesions (Meck et al. 1984, 1987, 1988; Olton et al., 1988) or frontal cortex (Dietrich et al., 1997; Dietrich & Allen 1998).

However, these impairments are not consistent with the increased spread or variability seen in Experiment 1 and 2. Morrissey et al (1994) has shown that lesions of the 5-hydroxytryptaminergic pathway which has functional connections between the parietal cortex, hippocampus, amygdala and striatum results only in increased variability or spread in timing a target duration. Similarly mice overexpressing the D2 dopamine receptor within the striatum also showed increased variability and spread in timing rewards (Drew et al., 2007; Ward et al., 2009) which is much more consistent with the results seen in Chapter 3.

The increased spreads seen in the young APPswe/PS1dE9 mice could well be the result of early alterations in both the hippocampus and stratum that are functionally connected. The same mice have also shown an inflammatory response in both the hippocampus and striatum, prior to plaque development, suggesting that some pathological development is occurring in those areas, and possibly having an effect upon timing behaviour.

6.3.3 Future research

Future research should initially aim to replicate Experiment 3 with the 8-month-old cohort to confirm the finding presented in Experiment 3.

A further avenue to explore is trace conditioning. Making the trace interval between the end of the CS and the presentation of food much longer can increase the task difficulty. Hippocampal lesions only appear to disrupt trace fear conditioning with longer trace intervals in mice (intact 1s and 3s trace conditioning, but impaired 20s trace interval, Chowdhury et al., 2005; 30s in Raybuck et al., 2011) and in rats (28s in, Quinn et al., 2002). The intact trace conditioning performance in the young APPswe/PS1dE9 mice in Experiment 1 may well be due to the trace interval only being 5 seconds long, which may not be long enough to produce a deficit, especially given the early stage of AD pathology.

Altering the peak procedure during trace conditioning trials is another area for future research. During Experiment 1 the trace interval was lengthened during the peak test sessions; however, the trace interval may well be a less accurate predictor of the reward. During trace conditioning, the CS elicits greater levels of conditioned responding than the interval and may be more strongly associated

with the US. Future research may look to replicate trace conditioning from Experiment 1, lengthening the trace CS during the unreinforced test trials, instead of the interval.

6.4 Chapter 4: Recognition memory

6.4.1 Summary of findings

Chapter 4 examined sNOR memory in 4-month APPswe/PS1dE9 mice and considered the performance in terms of Wagner's (1981) SOP model. Experiments 1a & d examined sNOR with a 5-minute delay between sample and test. The results presented contradictory evidence, 1a showing a lack of novelty preference in the APPswe/PS1dE9 mice, whilst 1d showed a novelty preference in both genotypes. Experiments by Bonardi (2016) replicated Experiments 1a using both a 5 min and 24 hour sample-test interval and showed normal novelty preference in the APPswe/PS1dE9 mice. The combined data supports intact sNOR in young APPswe/PS1dE9 mice.

In terms of Wagner's SOP model, both self and retrieval-generated priming could support normal sNOR performance. Experiments 1b and 1c attempted to examine performance on a RR memory task, which is a relatively clean measure of SGP. The results failed to show normal RR performance in either genotype, making it

impossible to see differences in the APPswe/PS1dE9 mouse. Bonardi et al. (2016) carried out replications of Experiment 1b, using a 5-min and 24 hour interval between the first and second sample phase. The results showed normal RR performance in both genotypes, which supports intact SGP in young APPswe/PS1dE9 mice.

Experiment 2 attempted to further assess RGP performance of APPswe/PS1dE9 mice using a context-priming procedure. Bonardi et al. (2016) found that 5-month APPswe/PS1dE9 mice showed impaired recognition memory, using an OIC procedure, which is a pure measure of RGP. Generalization decrement has been suggested as an alternative explanation for the OIC genotype difference making it hard to truly assess RGP performance. Experiment 2 cannot be explained using generalization decrement and also measures RGP. Experiment 2 showed numerically impaired performance in the APPswe/PS1dE9 mice compared to controls; however, this did not reach significance.

6.4.2 Implications

Chapter 4 examined recognition memory in the APPswe/PS1dE9 mouse model of AD. The first implication is that the OIC task (Bonardi et al. 2016) is a more sensitive measure of early AD pathology than the classic sNOR memory task, which is consistently

used in laboratories across the world. Early changes in hippocampal function during AD development could well account for the lack of observed deficits in sNOR performance until much later in the disease development. Lesions of the hippocampus in general do not disrupt sNOR performance (Barker & Warburton, 2011; Good et al., 2007; Mumby et al., 2002; Langston & Wood, 2010, Lee et al., 2005, but see Clark et al., 2000 for impaired performance). Whilst neuroanatomical studies have identified a number of specific brain regions in OIC performance; particularly lesions of the hippocampus (Barker & Warburton, 2011; DeVito & Eichenbaum, 2010; Good et al., 2007; Tam et al., 2014, but see Langston & Wood for normal performance using the same start position during sample and test), perirhinal cortex (Barker et al., 2007; Barker & Warburton, 2011, Hannesson et al., 2004; Lee et al., 2013) and medial prefrontal cortex (Barker et al., 2007; Barker & Warburton, 2011) produce impaired OIC memory. These three regions are also anatomically connected and therefore functionally involved with each other. The early accumulation of soluble Aβ (Garcia-Alloza et al., 2006; Volianskis et al., 2010), inflammation (Ruan et al., 2009; Chapter 4) and neural degeneration (Vegh et al., 2014; Volianskis et al., 2010) found in the hippocampus of young 2-4 month APPswe/PS1dE9 may well be

resulting in the impaired object-in-context performance seen in Chapter 3.

The second implication, according to the SOP model, is that OIC is impaired in young APPswe/PS1dE9 mice, reflecting impaired RGP. Currently an alternative explanation involving generalization decrement (GD) can possibly explain the OIC results (Bonardi et al. 2016). Experiment 2 failed to show significant context priming performance in the wild types or impaired performance in the APPswe/PS1dE9 mice, currently making it impossible to rule out the involvement of GD in OIC performance.

Third, the evidence points towards intact SGP in the young APPswe/PS1dE9 mice, and this SGP is involved in regulating sNOR performance in the mice, and is not impaired until 8 months of age onwards (Cheng et al., 2014; Donkin et al., 2010; Jardanhazi-Kurutz et al., 2010; Li et al., 2014).

6.4.3 Future research

The first avenue would be to attempt to replicate Experiment 2, the results showed a trend towards impaired performance in the APPswe/PS1dE9 mice, whilst wild types showed intact recognition memory. This experiment would be useful in ruling out a generalization decrement account of the impaired OIC performance

by the APPswe/PS1dE9 in mice in Bonardi et al. (2016) Currently the context priming recognition memory procedure has only been carried out successfully in rats (Whitt et al., 2012), and possible alterations in the procedure may be able to improve the performance in mice.

Another possible explanation for the impaired performance in the OIC task (Bonardi et al., 2016) could be due to task difficulty. Both the sNOR and RR tasks only involve exploring two objects during any phase of the tasks, whilst the OIC task involves exploring four objects during acquisition and test. The APPswe/PS1dE9 recognition memory deficit could be due to the increased number of objects the animals had to remember between sample and test. A simpler version of the OIC task also been demonstrated in a mouse model of AD. This version of the OIC task involved pre exposing the animals too two different object (A & B) before testing the animals with two identical object (A & A) in the same locations. It would be useful to test the performance of young APPswe/PS1dE9 mice on this simpler version of the OIC task, to rule out the possible effect of task demand on RGP performance.

An interesting line of research would attempt to assess Langaton & Wood (2010) hypothesis that the hippocampus is

specifically involved in the association of objects, local contextual information and the allocentric location of the animal. When testing animals on the two-object version of the OIC task, if the hippocampal lesioned animals were place into the arena in the same starting position during sample and test (egocentric), no impaired performance compared to shams was observed. However, if you varied the starting location between sample and test (allocentric) a robust deficit in discrimination was observed in the hippocampal animals. The authors argued that animals could solve the egocentric version by encoding the object locations in relation to their own bodily axes, and did not need to encode the objects and context in relation to each other (allocentric version). Whilst the animals in both Chapter 3 Experiment 2 and the OIC task by Bonardi et al (2015) were all place into the arena using the same starting location, it would be interesting to specifically examine the difference and whether this is effected by the early hippocampal changes seen in the 4 month old APPswe/PS1dE9 mouse.

Another future line of research would be to examine object recognition performance in humans using Wagner's (1981) SOP model of memory as a theoretical framework. The visual paired comparisons (VPC) task involves using visual eye tracking to

measure attention to a novel stimulus. Currently only the sNOR version of the VPC task has been assessed in both healthy and clinical populations. Healthy controls show significantly more eye fixation on novel stimuli than familiar compared to both MCI and AD patients (Crutchner et al. 2009, Zola et al. 2012). It would be interesting to assess healthy subjects performance on the RR and OIC variations, using the VPC method. It would then be interesting to assess the performance of preclinical AD and MCI patients' performance on all three tasks, to assess whether or not differentiating between self and retrieval-generated priming would be a useful measure in the early diagnosis of AD. It would also be interesting to see if early preclinical AD patients show a similar pattern of results as the young APPswe/PS1dE9 mice (Bonardi et al., 2016).

6.5 Chapter 5: Immunohistochemistry

6.5.1 Summary of findings

The experiments in Chapter 5 examined the early neuropathological development of Alzheimer's disease. A growing body of evidence has implicated neuro-inflammation in the brain during the early stages of the AD development (Bruno et al., 2009; Gasic-Milenkovic et al., 2003; Kummer et al., 2011; Lue et al., 2001; McGeer & McGeer 1995; Okello et al., 2009; Parachikova et al., 2007). Experiment 1 looked at

the levels of iba1 (calcium-binding adaptor molecule 1) microglia and microglia clusters in the striatum, hippocampus and cortex of 4 and 5-month-old APPswe/PS1dE9 mice and wild types. The results found an increased level of iba1-stained microglia in both the striatum and hippocampus of APPswe/PS1dE9 mice compared to wild types. Larger numbers of microglia clusters in the 5-month APPswe/PS1dE9 mice compared to the younger 4-month mice was also found. Increased microglia in the hippocampus is consistent with previous research (Ruan et al., 2009; Zhang et al., 2012), whilst the increased microglia in the striatum in APPswe/PS1dE9 mice compared to controls is a novel finding, which has previously not been reported.

6.5.2 Implications

The results from Chapter 5 support a growing body of evidence, which highlights neuroinflammation as an important factor in the development of AD. The amyloid cascade-inflammatory (ACI) hypothesis (McGreer & McGreer 2013) of AD argues that increased inflammation in the brain is a response to the deposition of Aβ plaques, and later neurofibrillary tangles. Chapter 5 showed increased microglia in the striatum, without any evidence of Aβ plaque deposition, which supports a growing evidence base that

suggests neuroinflammation occurs prior to the development of Aβ plaques, against the ACI hypothesis. Greater microglia levels were observed in the hippocampus, whilst the levels of microglia clusters, an indirect measure of Aβ plaques, were very low in 4-month APPswe/PS1dE9 mice, this result is in agreement with the (ACI) hypothesis, and gives support for the hippocampus being an early target of AD pathology.

6.5.3 Future research

Future research examining the pathology during the early development of AD could well focus on more specific areas associated with particular task impairments. Experiments should measure the levels of both soluble and insoluble amyloid and microglia activation as markers of the level of AD pathology.

For example, experiments focusing on contextual fear conditioning should examine these parameters specifically in both the hippocampus and amygdala of transgenic mice. This is due to the hippocampus playing an important role in contextual processing (Kim & Fanselow 1992, 1997; Good & Honey 1997; Maren et al., 1997; Phillips & LeDoux 1992, 1994) and the amygdala is involved in aversive processing (Flavell & Lee 2012; Maren 1999). Conventional neuroscience research into contextual fear conditioning in AD, has

focused on the levels of hippocampal pathology, but largely ignored other brain regions associated with the task demands.

6.6 General conclusions

In this thesis, I have examined the performance of APPswe/PS1dE9 mice on associative learning and memory tasks. I have attempted to use novel experimental designs that are not predominantly employed to measure the development of AD pathology in conventional neuroscience research. The experiments carried out in this thesis have uncovered differences in learning and memory in the APPswe/PS1dE9 mice during the early development of Alzheimer's disease. The differences include impaired appetitive conditioning of contextual cues (Chapter 2), impaired precision in the timing the arrival of an appetitive reward (Chapter 3), tentative evidence for impaired retrieval-generated priming (Chapter 4) and early increases in the level of neuro-inflammation in both the hippocampus and striatum (Chapter 5).

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Appendix

Experimental design

Appendix 1. Experimental designs for Chapter 2

	Acquisition	*Test*	
		Context	*Cue*
Experiment 1	*AX+*	*A*	*CX*
	BY-	*B*	*CY*
Experiment 2	*X+*	*X-*	
	Y-	*Y+*	
Experiment3	*A+*	*A-*	
	B-	*B-*	

Note: A & B = Contexts, X & Y = auditory stimuli, + = reinforced, - = non-reinforced

Appendix 2. Experimental designs of Chapter 3

	Acquisition	*Test*
Experiment 1	*X+*	*X+*
	Y+	*X*----------
		Y+
		Y----------
Experiment 2 & 3	X+	X+
	Y+	X----------
	Z-	Y+
		Y----------
		Z-

Note. X, Y & Z = auditory stimuli, + = reinforced, - = non-reinforced, ---------- = non-reinforced & extended CS

Appendix 3. Experimental designs for Chapter 4

	Sample Phase1	*Sample phase 2*	*Test Phase*
Experiment 1a	*X X*		*X Y*
Experiment 1d *5 minute interval*	*X X*		*X Y*
Experiment 1b	X X	Y Y	X Y
Experiment 1c 3 hour interval	X X	Y Y	X Y
Experiment 2	*PW QX*	*PP*	*RW RX*
5 minute interval	*PY QZ*		*RY RZ*

Note: P, Q, R = Stimuli, contexts, W, X, Y, Z = stimuli, objects,

Appendix 4. Object counterbalancing between animals in Experiment 1a, 1b, 1c & 1d: Novel object recognition and relative recency.

Animals	*Object X*	*Object Y*
Experiment 1a		
2tg & 2wt	*Egg Cup*	*Candleholder*
2tg & 2wt	*Candleholder*	*Egg cup*
2tg & 2wt	*Multi adaptor plug*	*Paperweight*
1tg & 1wt	*Paperweight*	*Multi adaptor plug*
Experiment 1b		
2tg & 2wt	*Multi adaptor plug*	*Paperweight*

2tg & 2wt	*Paperweight*	*Multi adaptor plug*
2tg & 2wt	*Egg Cup*	*Candleholder*
1tg & 1wt	*Candleholder*	*Egg cup*
Experiment 1c		
2tg & 2wt	*Ball*	*Lego*
2tg & 2wt	*Lego*	*Ball*
2tg & 2wt	*Shaker*	*Can*
1tg & 1wt	*Can*	*Shaker*
Experiment 1d		
2tg & 2wt	*Shaker*	*Can*
2tg & 2wt	*Can*	*Shaker*
2tg & 2wt	*Ball*	*Lego*
1tg & 1wt	*Lego*	*Ball*

Tg = APPswe/PS1dE9 mice, wt = Wild type mice

Appendix 5: Dimensions of the junk objects in Chapter 4

	Object Size	
	Diameter (cm)	*Height (cm)*
Can with stripe	*7*	*6*
Black Pingpong	*5*	*5.5*
Football	*6*	*6*
White Pingpong	*4*	*5*
Shampoo	*4*	*10*
Silver shaker	*4.5*	*10*
Shot glass	*4*	*7*
Marbles tube	*3*	*9*
Egg cup	*4.5*	*8.5*
Tabasco	*3.5*	*14*
Battery	*3*	*5.5*
Bulb	*5.5*	*10*
Paperweight	*6*	*6.5*
Tealight	*7.5*	*2.5*

Appendix 6: Dimensions of the junk objects in Chapter 4

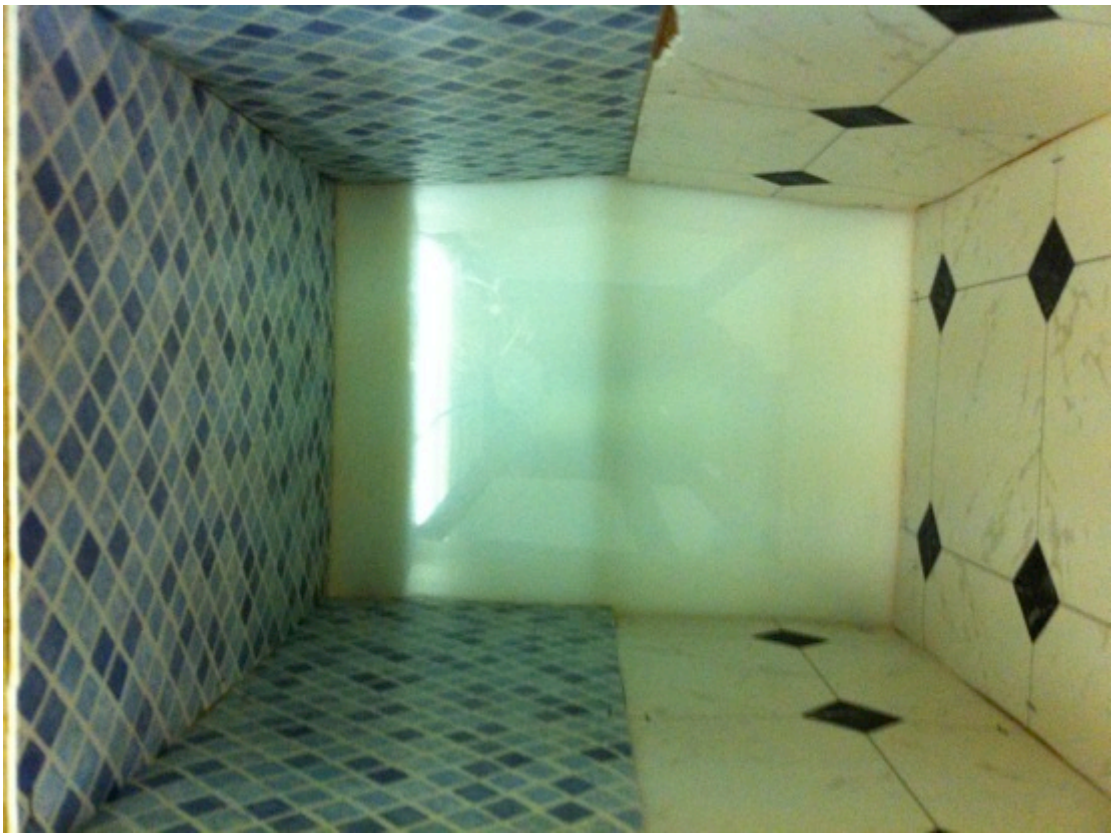
	Object Size		
	Length (cm)	*Width (cm)*	*Height (cm)*
Star	*7*	*8*	*6*
Sand shaker	*4*	*4*	*7.5*
Lego	*7*	*4*	*5*
Plug	*5*	*4.5*	*6.5*
Marmite	*6.5*	*3.4*	*7*
Deodorant	*5*	*2*	*10*

Appendix Photos

A) Photo of arena used during Chapter 4



B) Photo of the arena + context inserts used during Chapter 4



C) Objects used during Chapter 4





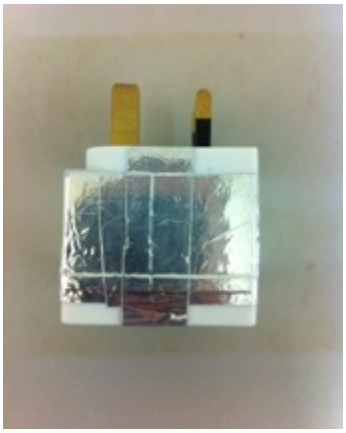

















D) Context inserts used during Chapter 4

