Age-Related Changes in Cognitive, Emotional, and Motor Behaviour in Male and Female 3xTg-AD Mice: A Longitudinal Study

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The 3xTg-AD mouse model of Alzheimer's disease has three transgenes (APPswe, PS1M146V, and Tau P301L) that cause the development of amyloid beta plaques, neurofibrillary tangles, and cognitive deficits. The breeding system of the 3xTg-AD mice requires that the transgenic mice be reared by transgenic mothers and the wildtype controls (B6129SF2) reared by wildtype mothers. To assess the effect of maternal genotype we cross-fostered pups to create mixed genotype litters and tested pups in a longitudinal study from 2 to 18 months of age. We found little evidence of a lasting effect of maternal genotype on behaviour or neuropathology. The 3xTg-AD mice had enhanced motor abilities on Rotarod and decreased anxiety-like behaviour from 2 to 18 months of age. We found no deficits in social behaviour at any age tested. The 3xTg-AD mice had a deficit in spatial learning and memory in the MWM from 2 – 18 months of age. To further characterize the motor phenotype we performed an extensive motor test battery at six months of age and found the 3xTg-AD mice had enhanced motor performance on the Rotarod, but worse performance on the grid suspension task. The 3xTg-AD mice had a longer stride length in gait analysis and made more foot slips on the balance beam than wildtype mice. There was no difference in voluntary wheel-running activity between genotypes, but there was a disruption in circadian activity rhythm in 3xTg-AD mice. We then tested mice at 6.5 months of age on a series of cognitive tasks to determine which was the most sensitive to detect cognitive deficits. We found that the Barnes maze was the most sensitive; the 3xTg-AD mice had impaired learning and memory in the Barnes maze but performed better than B6129SF2 wildtype mice in the Y-Maze and in contextual fear conditioning. Neither genotype demonstrated a preference in novel object recognition nor was there a genotype difference in cued fear conditioning. Overall the 3xTg-AD mouse develops some of the deficits that would be expected of a mouse model of Alzheimer's disease but has fairly mild cognitive deficits even at 18 months of age.

LIST OF ABBRIVIATIONS USED

% ..... Percentage

AD..... Alzheimer's Disease

AIC_c ..... Akaike Information Criterion

APOE ..... Apolipoprotein E

APP ..... Amyloid Precursor Protein

Aβ..... Amyloid Beta

CI_{95}..... 95% Confidence Interval

COPT ..... Conditioned Odour Preference Task

DAB ..... Diaminobenzidine Tetrahydrochloride

dH_20 ..... Distilled Water

EPM ..... Elevated Plus Maze

FAD..... Familial Alzheimer's Disease

GSK3..... Glycogen Synthase Kinase 3

HCO ..... Home Cage Observations

H_20_2 ..... Hydrogen Peroxide

MWM..... Morris Water Maze

NFT ..... Neurofibrillary Tangles

NMDA ..... N-methyl-D-aspartate

NORT..... Novel Object Recognition Task

OF ..... Open Field

PB..... Phosphate Buffer

PBS ..... Phosphate Buffered Saline

PPI..... Prepulse Inhibition

PS1 ..... Presenilin 1

PS2 ..... Presenilin 2

SNSP ..... Social Novelty / Social Preference Task

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CHAPTER 1 INTRODUCTION

1.1 ALZHEIMER'S DISEASE

1.1.1 OVERVIEW AND IMPACT

1.1.1.1 BRIEF DESCRIPTION OF HISTORY, SYMPTOMS, AND PROGRESSION OF AD.

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is the most common form of dementia. Alzheimer's disease causes increasing memory loss, can affect many other cognitive functions, and is ultimately fatal. The neuropathology of AD involves the progressive deposition of amyloid beta (Aβ) plaques, neurofibrillary tangles, and other neuropathological hallmarks (Braak and Braak, 1991). The disease and associated neuropathology were described by Alois Alzheimer, its namesake, in 1906, though senile dementia has been known as a disease since antiquity (Goedert and Spillantini, 2006; Cipriani et al., 2011). The prevalence of AD was approximately 1.5% in the Canadian population in 2008 and is expected to rise to 2.8% by 2038, mainly as a result of the ageing population (Alzheimer Society of Canada, 2010).

1.1.1.2 PREVALENCE BY AGE

The prevalence of AD increases dramatically with age. Women generally have a higher prevalence than men at any given age; the prevalence of dementia in those aged 85 or older in Canada in 2008 was 33% in men and 46% in women, and is expected to increase as the population ages (Alzheimer Society of Canada, 2010).

1.1.1.3 ECONOMIC IMPACT OF THE DISEASE IN CANADA AND THE WORLD

Alzheimer's disease causes a significant burden on the Canadian healthcare system and on unpaid caregivers who support those who suffer from AD. The total

economic burden of AD, a measure approximating the direct healthcare costs, indirect costs, and lost potential wages of unpaid caregivers, was approximately $15 billion in 2008 and is expected to rise to $150 billion by 2038 (Alzheimer Society of Canada, 2010; World Health Organization, 2012).

1.1.2 BEHAVIOURAL SYMPTOMS

1.1.2.1 COGNITIVE DEFICITS

The progression of symptoms in AD tends to follow a characteristic pattern, though there is evidence for distinct genetically defined subtypes with differences in symptom progression. The symptoms increase with time, eventually leading to profound cognitive deficits (Becker et al., 1988; Murray et al., 2011). One of the earliest and most prominent symptoms of AD is memory loss, specifically episodic memory, which is the memory for events that have occurred in a person's life. The episodic memory deficit is likely the result of an inability to consolidate memories, as AD patients typically exhibit accelerated forgetting and equally poor recall and recognition. Patients with AD also have deficits in semantic memory, which is general knowledge, concepts, and facts. This has been determined by assessing knowledge for concepts or facts across multiple methods of retrieval. A lack of semantic memory is characterized by a deficit in performance that is consistent across retrieval methods (Weintraub et al., 2012). Working memory and executive function can also be impaired in AD (Baudic et al., 2006; Belleville et al., 2007).

1.1.2.2 NEUROPSYCHOLOGICAL SYMPTOMS

Elevated anxiety is a common symptom in AD, and is present early in the course of the disease. Elevated anxiety is correlated with a higher rate of other behavioural

symptoms and a decrease in the ability to perform activities of daily living (Teri et al., 1999; Ferretti et al., 2001). Apathy and depression are also common symptoms of early AD (Devanand et al., 1996; Landes et al., 2001). A variety of other neuropsychological symptoms can develop in advanced AD, including hallucinations, delusions, and sleep disturbances (Jost and Grossberg, 1996).

1.1.2.5 MOTOR BEHAVIOUR

Motor dysfunction is a fairly common symptom in early AD and typically presents as slowing and a minor deficit in performing complex or fine motor tasks, but without a deficit in gross motor ability (Kluger et al., 1997; Pettersson et al., 2005). Later in the disease more gross deficits in motor functions may be present, including gait disturbances (Braak and Braak, 1991; Allan et al., 2005).

1.1.3 NEUROPATHOLOGY

There are two historical histological hallmarks of AD pathology, Aβ plaques and neurofibrillary tangles, which are thought to be responsible for the synaptic dysfunction and cell death that cause the neural, cognitive, and behavioural symptoms of AD.

1.1.3.1 AB PATHOLOGY

1.1.3.1 What is Aβ, and how does the pathology develop?

In the pathogenesis of AD the amyloid precursor protein (APP) is cleaved by the beta-secretase enzyme then by the gamma-secretase enzyme, producing Aβ peptide. This peptide is normally 40 residues long ($A\beta_{40}$) but a proportion of the peptides are 42 residues long ($A\beta_{42}$). The $A\beta_{42}$ peptide is more likely to aggregate and is the primary form found in Aβ plaques (LaFerla et al., 2007). An autosomal dominant form of AD is caused by mutations in the genes encoding APP or presenilin 1 or 2 (PS1 or PS2), a

component of the gamma-secretase protein, which cause the overproduction of $A\beta_{42}$ relative to $A\beta_{40}$ and the development of amyloid beta pathology (Scheuner et al., 1996). The Aβ peptide can aggregate to form oligomers and fibrils, which can develop into Aβ plaques. The exact mechanism by which any species of Aβ results in neurotoxicity or synaptic dysfunction is a subject of debate, and there is little correlation between the levels of Aβ plaques and cognitive dysfunction in AD patients, however the relationship between autosomal dominant AD and mutations in the genes associated with Aβ production demonstrate that Aβ is an important part of AD neuropathology (Walsh et al., 2002; LaFerla et al., 2007; Benilova et al., 2012).

1.1.3.2 Progression of Aβ pathology

The extracellular Aβ plaques of AD develop in a manner that varies considerably between individuals, but in a generally characteristic pattern, increasing in density in previously affected areas and spreading to additional areas of the brain over time. Braak and Braak (1991, 1996) describe the progression of Aβ plaques in the cortex of the ageing brain in three stages. The first stage of Aβ plaque deposition, A, consists of a low level of Aβ plaques in the basal occipital, temporal, and frontal lobes and in the entorhinal cortex. In stage B there is a medium level of Aβ plaques in most areas of the neocortex, with the exception of the motor and sensory cortex, and few Aβ plaques in the hippocampus. In the final stage, C, almost all of the neocortex has dense Aβ plaques, with some plaques in the hippocampus and many subcortical areas begin to develop Aβ plaques. While most cases of dementia that Braak and Braak (1991, 1996) examined had high levels of Aβ plaques in the brain, many non-demented individuals also had Aβ

plaques throughout the cortex, highlighting the lack of a direct relationship between Aβ plaques and cognitive dysfunction.

1.1.3.2 TAU PATHOLOGY

1.1.3.2.1 What are neurofibrillary tangles and how are they made?

The second hallmark of AD neuropathology, neurofibrillary tangles (NFTs), are aggregations of hyperphosphorylated tau proteins. These NFTs develop in the neurons and cause neuronal dysfunction either through the loss of normal tau function, which stabilizes microtubules, or a pathological function of the aggregated tau protein (for a review see Ballatore et al., 2007). The progression of tau pathology is more closely correlated with cognitive deficits than Aβ plaques and follows a more well defined spatial and temporal pattern in AD (Braak and Braak, 1991, 1996; Arriagada et al., 1992).

1.1.3.2.2 Progression of tau pathology

Generally the NFTs first appear in the entorhinal cortex, then spread to the limbic system and finally to the neocortex. Braak and Braak (1991, 1996) describe six stages (I – VI) of NFT development, which has much less variability between AD patients than the development of Aβ plaques. In stage I there are some NFTs in the area of the cortex where the entorhinal and temporal cortices meet, with the possibility of a few NFTs in the surrounding areas. In stage II the density of NFTs in the stage I areas has increased and spread to the CA1 region of the hippocampus. Stages III and IV involve increasing densities in the affected areas from the preceding stages, a spread of NFTs throughout the hippocampus, and the appearance of low density NFTs in the neocortex. Stages V and VI again involve an increasing density of NFTs in the previously affected areas and the spread of NFTs to the neocortex, and in stage VI to the subcortical nuclei.

1.1.3.3 OTHER NEURODEGENERATION

Synaptic loss and neuronal death are two additional hallmarks of AD that are highly correlated with cognitive deficits. Synaptic loss and neuronal death are thought to result from either Aβ plaques, tau NFTs, or some combination of the two pathologies (Mark et al., 1995; Feinstein and Wilson, 2005; Schindowski et al., 2006; Lacor et al., 2007). Neuronal cell death progresses in a similar pattern as tau, described above, and leads to progressive brain atrophy compared to controls without AD (Braak and Braak, 1991; Chan et al., 2003). The majority of the cell death in AD appears to be the result of apoptosis and, while the exact cause is unknown, there is evidence that both Aβ and tau pathologies can cause apoptosis (Mark et al., 1995; Smale et al., 1995; Feinstein and Wilson, 2005)

1.1.4 GENETIC AND ENVIRONMENTAL RISK FACTORS

There are two main patterns of inheritance for AD, sporadic and familial. Sporadic AD is the primary type, it generally occurs at an advanced age and has genetic risk factors. Familial AD generally has an early onset (before age 65), is inherited, and is typically caused by an autosomal dominant mutation. There are three genes with mutations thought to cause familial AD. One encodes for the amyloid precursor protein which is cleaved to form Aβ, the other two genes encode for presenilin 1 and 2, which forms a portion of the gamma-secretase complex that cleaves the APP. These mutations either cause an increase in the ratio of production of Aβ or in the ratio of $A\beta_{42}$ to $A\beta_{40}$, either of which cause the development of AB plaques. Familial AD makes up a small minority of the cases of AD (<5%), but the study of the genes associated with FAD has

been crucial to understanding the disease process and creating animal models of AD (Campion et al., 1995, 1999; Haass et al., 1995; Hardy, 1997). While the genetic causes of FAD and sporadic AD are different, the underlying neuropathology is very similar, as are the clinical features of the disease, though FAD tends to have a longer disease course, likely because of the earlier age of onset (Duara et al., 1993).

The most common genetic risk factors for sporadic AD are mutations in the gene encoding for apolipoprotein E (APOE), though a number of other genes and loci have been implicated (Lambert et al., 2013). APOE may be involved in the clearance of Aβ in the brain and mutations associated with AD, and mutations in APOE are good predictors for the levels of Aβ, but not tau, pathology in unaffected carriers (Strittmatter et al., 1993; Morris et al., 2010; Castellano et al., 2011). APOE mutations alone are generally not sufficient to cause AD, and so do not generally cause autosomal dominant AD. The most well studied APOE mutation is the ε4 allele. One study found a 2.84 fold increase in the risk of AD in people with one copy of the allele and a 8.07 fold increase with two (Corder et al., 1993). The strongest risk factor for AD is age; After age 65 the risk of AD rises dramatically and by age 85 over 30% of the population has developed AD (Alzheimer Society of Canada, 2010). There are a number of environmental risk factors for AD, including smoking, head injuries, diabetes, and obesity (Mortimer et al., 1991; Anstey et al., 2007; Profenno et al., 2010).

1.1.5 PROPOSED CAUSES OF ALZHEIMER'S DISEASE

1.1.5.1 THE CHOLINERGIC HYPOTHESIS

The cholinergic hypothesis is one of the oldest hypotheses about the development of AD (for a review see Francis and Meaney, 1999; Francis et al., 1999). The cholinergic

hypothesis proposes that the loss of cholinergic neurons in the basal forebrain and other brain areas results in a general decrease in cholinergic neurotransmission, which causes the behavioural symptoms of AD. While many patients with AD exhibit a decrease in cholinergic function early in the disease this is not a universal symptom and there is evidence that neurodegenerative changes not associated with cholinergic transmission also contribute to cognitive decline. The actual cause of AD is likely more complex than the cholinergic hypothesis proposes, however cholinesterase inhibitors, which increase cholinergic neurotransmission, are one of only two classes of drugs currently approved for treatment of AD (Scarpini et al., 2003).

1.1.5.2 THE AMYLOID CASCADE HYPOTHESIS

One of the most influential hypotheses about the development of AD is the amyloid cascade hypothesis (Hardy and Higgins, 1992). The amyloid cascade hypothesis proposes that Aβ is the causative agent of AD. This means that all the other symptoms, including neurofibrillary tangles, neuronal loss, and cognitive deficits are the result of the deposition of Aβ and the formation of plaques, and is likely a result of an imbalance between the production of Aβ and its clearance. There are several facts that support the hypothesis and others that call at least part of it into question. Supporting facts include that the majority of familial AD cases are caused by mutations that directly affect Aβ, that people with Down's syndrome, which involves a duplication of the APP gene and overproduction of Aβ, develop AD at very high rate, that many of the genes implicated in late onset AD are thought to be involved in Aβ production or clearance, and lastly that the majority of mutations associated with tau are not associated with AD. Some limitations include the fact that amyloid pathology does not correlate well with cognitive

developments (Arriagada et al., 1992), that the oligomeric form of Aβ may be the toxic species, not the Aβ plaque, and that few of the therapeutics designed based on the hypothesis have had any success (Lee et al., 2004; Pimplikar, 2009).

1.1.5.3 OTHER HYPOTHESES

Partially as a result of the issues with the amyloid cascade hypothesis, several other hypotheses about the development of AD have been proposed, though none are currently as prevalent as the amyloid cascade hypothesis. The early tau hypothesis proposes that tau, not Aβ, is the causative agent of AD, and is supported by the fact that cognitive functions in AD are much better correlated with tau pathology than Aβ, and proposes that a number of factors, including Aβ, cause tau hyperphosphorylation and neuronal cell death, which releases the hyperphosphorylated tau and begins the cascade (Maccioni et al., 2010). Other proposed causes of AD are the oxidative stress hypothesis, which suggests that an increases in oxidative stress in the brain causes AD (Markesbery, 1997), and the Glycogen synthase kinase 3 (GSK3) hypothesis, which proposes that over activity of GSK3 is responsible for the development of AD, as GSK3's deregulation is hypothesized to cause the hyperphosphorylation of tau and an increase in Aβ production (Hooper et al., 2008).

1.1.6 TREATMENT OF ALZHEIMER'S DISEASE

Despite a wealth of research on AD and many clinical trials there are only two classes of drugs currently approved for the treatment of AD, both of which provide mainly symptomatic relief (for a review see Anand et al., 2014 and Scarpini et al., 2003). The first class of drugs are acetylcholinesterase inhibitors, which were developed based on the cholinergic hypothesis and improve cholinergic neurotransmission by decreasing

the rate of the breakdown of acetlycholine. These drugs improve cognitive function in mild and moderate AD, though their effect on disease progression is unclear. The only other drug approved to treat AD is Memantine, which is a NMDA-receptor partial antagonist, may protect neurons from glutamate excitotoxicity. Memantine has been shown to slow the decline of cognitive and other functions in late stage AD, though whether it is of any long term benefit is currently unknown.

1.2 ANIMAL MODELS OF ALZHEIMER'S DISEASE

1.2.1 HISTORY AND TYPES OF MODELS

The first attempts to model AD in animals focused on the cholinergic hypothesis. The first animal models involved treating non-human primates with Scopolamine, an anti-cholinergic drug, which produced cognitive deficits similar those seen in old age. Rodent models of cholinergic dysfunction were then developed using pharmacological and surgical methods (for a review see Bartus, 2000 and LaFerla and Green, 2012). With the rise in popularity of the amyloid cascade hypothesis animal models were created by injecting various forms of Aβ directly into the brain, which resulted in neurotoxicity, causing apoptosis, and cognitive dysfunction. Researchers then developed transgenic animals which harboured human APP and PS1 genes that are associated with familial AD. Those transgenic animals developed Aβ plaques and cognitive dysfunction similar to AD, but did not develop the tau deposits or neurofibrillary tangles (Games et al., 1995; Hsiao et al., 1996; Jankowsky et al., 2004; Oakley et al., 2006). In an attempt to more fully model AD neuropathology the 3xTg-AD transgenic mice were created by inserting transgenic APP, PS1 and a tau gene (tau_{P301L}), which causes the mice to develop neurofibrillary tangles as well as amyloid beta plaques (Oddo et al., 2003).

1.2.2 CRITERIA FOR A "GOOD" MODEL

Willner (1984) proposed three criteria for an animal model of disease; (1) predictive validity, which is that any treatments in the animal will translate into treatments in humans; (2) face validity, whether the symptoms of the model are the same as the human disease; and (3) construct validity, whether the same disease process is being modeled as is present in the human disease. The ideal mouse model of AD would thus replicate the behavioural and neuropathological symptoms of AD, which would mean developing Aβ plaques, neurofibrillary tangles, synaptic dysfunction and cell loss, as well as progressive deficits in memory and finally death. It would also have the same underlying disease process, which for the majority of cases of AD does not involve autosomal dominant mutations in APP or PS1, unlike the majority of mouse models. Treatments which ameliorate symptoms in the ideal model of AD should translate to treatments in humans with AD if the model has good predicative validity, though this has not yet been successful with transgenic models of AD. While the current models of AD do not meet all the criteria, there is value in incomplete models of AD. Webster et al. (2014) provide a thorough review the neuropathology and behaviour of 10 commonly used mouse models of AD.

Modeling only one aspect of the disease can allow for a better understanding of the mechanisms of that aspect and the creation of interventions based on those mechanisms, even if they do not fully address all aspects of the disease (Radde et al., 2008). The cholinergic models of AD are a good example of this, they had little construct validity, as the neuropathology was not similar to AD, but they had similar behavioral symptoms. The cholinergic models also had some predictive validity as the drugs that

ameliorated their symptoms provide some symptomatic relief in AD (Yamada and Nabeshima, 2000). The current transgenic models may lead to a better understanding of the disease and treatments, despite being incomplete.

1.3 THE 3XTG-AD MOUSE MODEL OF ALZHEIMER'S DISEASE

1.3.1 DEVELOPMENT AND GENETICS

The B6;129-Psen1tm1Mpm Tg(APPSwe,tauP301L)1Lfa (3xTg-AD) mouse model of AD was designed to develop both of the pathological hallmarks of AD: Aβ plaques and neurofibrillary tangles. To accomplish this, two transgenes, APP_{swe} and $tau_{P301L,}$ were subcloned onto the same Thy1.2 expression cassette and injected into the single celled embryo of a transgenic mouse with the $PS1_{M146V}$ transgene, to create a homozygous transgenic mouse with all three transgenes. These mice were backcrossed onto the original $PS1_{M146V}$ parental strain. The APP_{swe} gene encodes for a human APP with a the Swedish mutation associated with familial AD, and the $PS1_{M146V}$ transgene encodes for a mouse PS1 with a section of the mouse gene replaced with the homologous section of human PS1 with a mutation associated with familial AD. The tau_{P301L} transgene encodes for human tau and has a mutation associated with the development of frontotemporal dementia. This strain is maintained by breeding homozygous transgenic mice with other homozygous transgenic mice and the approximate wildtype controls (B6129SF2/J) with one another, which means that the strains are bred separately (Oddo et al., 2003).

Early life environment can have a lasting effect on the brain and behaviour (Denenberg et al., 1968; Liu et al., 1997). Several factors have been shown to have long-term effects on behaviour in both humans and rodents, including early stress and differences in

maternal care (Francis and Meaney, 1999). Priebe et al. (2005) cross-fostered pups from two mouse strains with differing levels of maternal care and found that maternal care had a lasting effect on anxiety-like behaviour later in life. In addition to post-natal care, the pre-natal environment can have a lasting effect on brain and behaviour. Francis et al. (2003) cross fostered single cell mouse embryos and compared them to mice cross fostered immediately after birth and found differences in behaviour between the two types of cross fostering, which demonstrates that the pre-natal environment can have a lasting effect on later life development. Because the 3xTg-AD and B6129SF2 strains are bred separately it is possible that the strains have differing levels of maternal care which could have lasting effects on the behaviour throughout the lifespan.

1.3.2 PATHOLOGY

Mastrangelo and Bowers (2008) provide a detailed description of the development of Aβ and tau neuropathology in the 3xTg-AD mouse. The cleaved human Aβ gene product is first detectable in the hippocampus, entorhinal cortex, and motor cortex in 3xTg-AD at two months. Intracellular Aβ deposition begins at two to three months of age in the hippocampus, entorhinal cortex, and motor cortex, and in the amygdala by six months of age. Mastrangelo and Bowers (2008) report that extracellular Aβ deposition does not begin until 15 months of age with dense plaques developing by 18 months of age, however Billings et al., (2005) report that there is extracellular Aβ deposition in the hippocampus and amygdala by six months of age.

Mastrangelo and Bowers, (2008) found that the tau transgene product can be detected in some pyramidal neurons in the hippocampus by two months of age, and in most by six months of age. Phosphorylated tau can be detected in the hippocampus by six

months of age, spreading throughout the hippocampus with age and extending to the motor cortex at nine months of age and the entorhinal cortex by 26 months of age. Paired helical filaments, a component of neurofibrillary tangles, were not detectable until 15 months of age in the CA1 region of the hippocampus and in the subiculum, spreading to other areas of the hippocampus, entorhinal cortex, and amygdala, only at 26 months of age. The original study on 3xTg-AD mice reports that paired helical filaments develop earlier, with extensive staining at 18 months of age (Oddo et al., 2003).

The brains of the 3xTg-AD mice also developed increased inflammation in their brains relative to control mice. The levels of microglial activation increase beginning at 2 months of age in the rostral CA1 region of the hippocampus and remain stable until 18 months of age, when microglial activation spreads to other regions of the hippocampus. A similar pattern of microglial activation was found in the amygdala and motor cortex. Astrocyte activation was first detectable in the hippocampus, with lower levels in the amygdala, entorhinal cortex, and motor cortex, remaining relatively constant until 15 months of age when there was a decrease in the hippocampus that continued until at least 26 months of age (Mastrangelo and Bowers, 2008).

In addition to Aβ and tau neuropathology the 3xTg-AD also develop synaptic dysfunction and deficits in long term potentiation in the hippocampus beginning at six months of age, with the deficit increasing with age (Oddo et al., 2003). The 3xTg-AD develop immunological dysfunction by 2 months of age, with enlarged spleens, which by 12 months of age are an order of magnitude larger than wildtypes, and differences in the levels of immune cells compare to wildtype controls (Marchese et al., 2014).

1.3.3 BEHAVIOURAL DEFICITS

The 3xTg-AD mice develop a number of behavioural deficits which change over time. The behavioural deficits are described in a brief overview here and the following chapters provide a more detailed description.

1.3.3.1 LEARNING AND MEMORY

The 3xTg-AD mice develop deficits in learning and memory which worsen with age, though the nature and age of onset of these deficits is unclear. Spatial memory deficits have been detected using the Morris water maze (MWM) beginning between 2.5 and 4 months of age (Billings et al., 2005; Clinton et al., 2007; Marchese et al., 2014), and these deficits are present at later ages and appear to worsen with age (Billings et al., 2007; McKee et al., 2008; Movsesyan et al., 2008; Corona et al., 2010; García-Mesa et al., 2011; Chen et al., 2013). This spatial memory deficit in 3xTg-AD mice has also been detected using the Barnes maze at six months of age (Clinton et al., 2010). In the radial arm maze, another measure of spatial learning and memory, the 3xTg-AD are impaired in both working and reference memory beginning at two months of age (Stevens and Brown, 2014).

Some studies have reported deficits in contextual fear conditioning in 3xTg-AD mice beginning at six months of age (Billings et al., 2005; España et al., 2010), while others found no difference in contextual fear conditioning at either six or 13 months of age (Pietropaolo et al., 2008; Chu et al., 2012).

In a test of short term memory, the Y-Maze test of spontaneous alternation behaviour there are conflicting reports with some finding a deficit in the 3xTg-AD mice at six to seven months of age and others finding no difference between 3xTg-AD mice

and wildtype controls at six months of age (Rosario et al., 2006; Carroll et al., 2007; Zhang et al., 2010). In the novel object recognition task, some studies have reported deficits beginning between six and nine months of age (Blanchard et al., 2010; Martinez-Coria et al., 2010; Chen et al., 2013), though Clinton et al. (2007) did not find a deficit in the 3xTg-AD until 9 months of age. The cause of these inconsistencies is unclear, they may be related to subtle differences in testing protocols, minor differences in the genetics due to differing sources of the mice, or variability between mice.

1.3.3.2 ANXIETY-LIKE SYMPTOMS

There are conflicting reports about the development of anxiety-like behaviour in the 3xTg-AD mice. There are reports of no differences between 3xTg-AD and controls in anxiety-like behavior on the elevated plus maze (EPM) between 7 and 14 months of age (Pietropaolo et al., 2008; Filali et al., 2012). However there are also reports of an increase in anxiety-like behaviour in the 3xTg-AD mice relative to B6129SF2 mice at 7 months of age (Blanchard et al., 2010; Chen et al., 2013).

1.3.3.3 SOCIAL BEHAVIOUR

There have been few studies of social behaviour in the 3xTg-AD mice. One study using the social recognition test reported that at 18 months of age the 3xTg-AD had impaired social recognition compared to controls (Medeiros et al., 2011).

1.3.3.4 MOTOR BEHAVIOUR

In a neurodevelopmental test battery performed on the mice used for the experiment in chapters 2 – 5 we found that 3xTg-AD pups reached physical milestones earlier than control mice (Blaney et al., 2013). Several studies, including one from our lab, have found that the 3xTg-AD mice have enhanced performance on the Rotarod

compared to control mice beginning at two months and continuing until at least fifteen months of age (Blanchard et al., 2010; Filali et al., 2012; Chen et al., 2013; Oore et al., 2013). On the wire hang test, a measure of forelimb strength, two studies found no deficits in 3xTg-AD mice relative to controls (Sterniczuk et al., 2010a; Arsenault et al., 2011). These results suggest that the 3xTg-AD do no develop motor deficits, which are present in some mouse models with tau mutations, and that they 3xTg-AD may have enhanced motor functioning compared to controls.

1.4 USE OF THE 3XTG-AD MICE TO ASSESS POTENTIAL THERAPIES FOR ALZHEIMER'S DISEASE

The 3xTg-AD mouse model has been used to assess several potential therapies for AD, with varying degrees of success at ameliorating the behavioural and neuropathological deficits. Aβ immunotherapy involves treatment with antibodies against Aβ, which are intended to increase the clearance of Aβ from the brain. In the 3xTg-AD mouse Aβ immunotherapy decreases the levels of Aβ pathology and prevents the development of spatial memory deficits in the MWM (Movsesyan et al., 2008; Giménez-Llort et al., 2013). However there is a report that both Aβ and tau immunotherapy are required to ameliorate spatial memory deficits 3xTg-AD mice in the MWM (Oddo et al., 2006). Aβ immunotherapy also appears to delay, but not prevent, the development of tau pathology (Oddo et al., 2004). Clinical trials in humans with AD had some early success but some trials were halted due to the development of meningoencephalitis, a swelling of the brain and meninges, and others have shown little to no effect on cognitive function, though the trials have not yet tested Aβ immunotherapies' effectiveness in preventing AD (for a review see Lemere, 2013). Other studies have found that ovariectomy or

orchiectomy increases Aβ and tau pathology and causes memory deficits on the Y-maze, and subsequent supplementation with estrogen (in females) or testosterone (in males) reversed those effects, which may have implications for women with AD, and implies that the sex hormones play a role in the development of the neuropathology of AD (Rosario et al., 2006; Carroll et al., 2007; Carroll and Pike, 2008). Though trials in humans have had mixed results, with no large scale clinical trials to date (Mulnard, 2000; Cherrier et al., 2005; Carson, 2006).

1.5 RATIONALE

Several other studies have examined treatments thought to help prevent AD and found that they decrease the levels of neuropathology and cognitive deficits in 3xTg-AD mice, including exercise (Adlard et al., 2005), anti-inflammatory drugs (McKee et al., 2008), previous learning (Billings et al., 2007), and Memantine, a drug approved to treat AD described in section 1.6 (Martinez-Coria et al., 2010). A number of studies have found that novel drugs for AD reduce neuropathology and or cognitive deficits (Caccamo et al., 2006; Green et al., 2007, 2008; Wang et al., 2010; Himeno et al., 2011; Medina et al., 2011), but whether or not these will translate into treatments for AD remains unclear.

As described in the preceding sections, the literature on the behaviour of the 3xTg-AD mouse is inconsistent, and the effect of maternal genotype remains unstudied. Our overall rationale for these experiments was to conduct a detailed behavioural analysis of the 3xTg-AD and B6129SF2 hybrid mice across the lifespan, using multiple tests of each aspect of behaviour, in order to create a more thorough behavioural characterization of these mice. Additionally, we sought to study what, if any, effect maternal genotype has on both the behavioural development of these mice and the development of

neuropathology. After conducting our longitudinal experiment on the behaviour of these mice we were left with two additional experimental questions: 1) as we found enhanced performance in the Rotarod in the longitudinal study, we wanted to determine the nature of the motor phenotype of the 3xTg-AD and B6129SF2 control mice, 2) as we found that the 3xTg-AD had some cognitive deficits in the Morris water maze, but other behavioural studies used different tasks, we wanted to determine which is the most sensitive task to detect cognitive deficits with the 3xTg-AD.

1.6 OUTLINE OF EXPERIMENTS

1.6.1 EXPERIMENT 1. LONGITUDINAL BEHAVIOURAL ASSESSMENT OF THE EFFECTS OF MATERNAL GENOTYPE ON BEHAVIOUR AT 2, 6, 12, AND 18 MONTHS OF AGE.

Experiment 1 and 2 used the same mice in a longitudinal study of the effects of maternal genotype on behaviour throughout the lifespan. After cross fostering, the mice underwent a neurodevelopmental test battery (Blaney et al., 2013). The mice were then tested at 2, 6, 12, and 18 months of age on a behavioural test battery. After the behavioural test battery was complete the levels of amyloid beta plaques and neurofibrillary tangles were assessed and correlated with the behavioural measures. The results of the 2 and 6 month time points were described in my MSc thesis (Stover, 2012). In this dissertation results of the test battery were split into four chapters, chapter 2 details the anxiety and motor behaviour test results, and chapter 3 details the results of the acoustic startle and prepulse inhibition tasks, chapter 4 contains the results of the learning and memory tasks, and chapter 5 has the results of the social behaviour tasks. Chapter 2

has the results of the neuropathology assessment and chapters 2 – 5 all contain correlations of levels of neuropathology to the respective behavioural tasks.

I had assistance with the behavioural testing for this experiment from a number of students. Kaitlyn Gordon assisted with behavioural testing for one cohort of mice for her Honours project during her BSc, Michelle Hicks assisted in the behavioural testing for another cohort of mice for course credit in a third year research project, and Daniel Ikpi assisted with the third cohort during his time as a visiting PhD student in Dr. Richard Brown's lab. Dr. Darvesh provided the lab space for the neuropathology and Andrew Reid and Meghan Cash trained me on how to complete the histology and quantification of neuropathology. I trained the students, and completed the remainder of the behaviour testing, the histology, quantification of neuropathology, statistical analysis, and wrote the chapters.

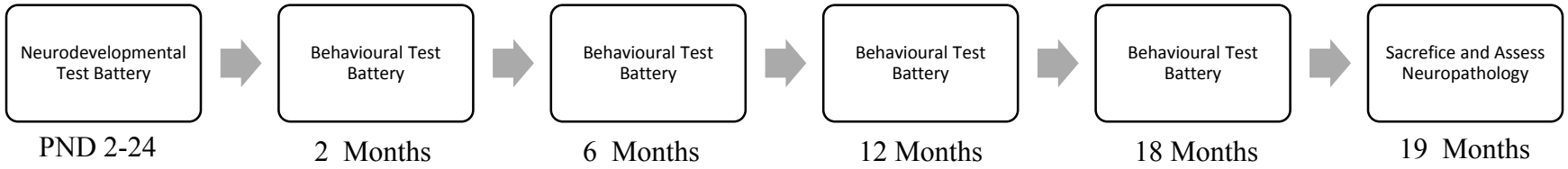

Figure 1. Timeline of experiment 1

1.6.2 EXPERIMENT 2. CHARACTERIZATION OF THE MOTOR PHENOTYPE OF THE 3XTG-AD MOUSE

In the first experiment we found that the 3xTg-AD mice had enhanced performance on the Rotarod beginning at 2 months of age and continuing until 18 months of age and there is additional evidence from the literature that the 3xTg-AD show improved motor functioning compared to control mice (Blanchard et al., 2010; Chen et al., 2013). In order to further characterize the motor phenotype of this strain we ran the 3xTg-AD on a motor test battery. The results of this experiment are presented in chapter 6. This chapter has been published in Behavioural Brain Research (Stover et al., 2015a).

I had assistance in the behavioural testing from Mackenzie A. Campbell, who completed some of the behavioural testing for two of the three cohorts of mice as a part of her honours project for her BSc degree, and Christine M. Van Winssen who also completed some of the behavioural testing for two of the cohorts for course credit in a third year research project. I trained the students, completed the remaining behavioural testing for the two cohorts and completed the behavioural testing for the final cohort. I also completed the statistical analysis and wrote the manuscript, which Dr. Richard Brown edited.

1.6.3 EXPERIMENT 3. EARLY COGNITIVE DEFICIT DETECTION IN THE 3XTG-AD MOUSE MODEL OF ALZHEIMER'S DISEASE AT 6.5 MONTHS OF AGE

For the final experiment in my PhD thesis we sought to determine which cognitive deficits were present at 6.5 months of age in the 3xTg-AD mice, and which test was the most sensitive at detecting those deficits. We used the same mice as in

experiment 2 and ran them on a series of cognitive tasks after they had completed the motor tasks. We compared the effect sizes of the different tasks to determine which was the most sensitive. I had the same assistance as described in experiment 2. The results of this experiment are presented in chapter 7.

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CHAPTER 2 AGE-RELATED CHANGES IN MOTOR BEHAVIOUR AND ANXIETY IN THE 3XTG-AD MOUSE MODEL OF ALZHEIMER'S DISEASE: A LONGITUDINAL STUDY

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2.1 ABSTRACT

The breeding system of the 3xTg-AD mice requires that transgenic mice be reared by transgenic mothers and wildtype controls (B6129SF2) reared by wildtype mothers to create homozygous 3xTg-AD mice. We assessed the effect of maternal genotype by cross fostering pups to create mixed genotype litters and tested pups in a longitudinal study from 2, 6, 12, and 18 months of age. On the Rotarod 3xTg-AD mice performed better than controls, females performed better than males, and all mice reared by wildtype mothers performed better than those reared by 3xTg-AD mothers. In the elevated plus maze and open field, the 3xTg-AD mice had decreased anxiety-like behaviour. Mice reared by 3xTg-AD mothers had a higher density of tau-positive neurons in the amygdala, but no difference in levels of amyloid beta. Overall the 3xTg-AD mice had enhanced motor behaviour, decreased anxiety-like behaviour and maternal genotype had little effect on the phenotype of 3xTg-AD and control mice in adulthood.

2.2 INTRODUCTION

Early life environment and maternal care have a lasting epigenetic effect on neurobehavioral development (Kundakovic and Champagne, 2014). Such factors as early-life stress and differences in level and quality of maternal care (Francis and Meaney, 1999) can have long-term effects on the behaviour of rodents and humans. By cross-fostering litters from two strains of mice with differing levels of maternal care Priebe et al. (2005) found that the level and quality of maternal care affected anxiety-like behaviour later in life.

Studying the interaction of genetics and environment is essential to fully characterize mouse models of AD (Chouliaras et al., 2010). The 3xTg-AD mouse model of Alzheimer's disease (AD) co-expresses three human familial AD genes, presenilin-1 (M146V), amyloid precursor protein (APP swe-K67ON/M671L) and tau (P30IL) (Oddo et al., 2003). This combination of transgenes causes the 3xTg-AD mouse to develop Aß plaques and tau neuropathology. The intracellular Aß is detectable in the hippocampal neurons at around three months of age, and extracellular Aß begins to develop at six and fifteen months of age (Billings et al., 2005; Mastrangelo and Bowers, 2008). Tau pathology is present in the hippocampal neurons at six months of age, and by nine months of age tau pathology spreads to the motor cortex (Mastrangelo and Bowers, 2008).

Previous studies have shown that the 3xTg-AD mice differ from their B6129SF2 control mice in anxiety-like and motor behaviour. We have shown that the 3xTg-AD mice have enhanced motor performance on the Rotarod, a test that measures motor ability, grip strength, and a longer stride than B6129SF2 control mice at six months of

age (Stover et al., 2015a). Enhanced Rotarod performance in the 3xTg-AD mice at six and seven months of age have also been reported previously (Blanchard et al., 2010; Chen et al., 2013; Oore et al., 2013). However, Sterniczuk et al. (2010a) found no differences between 7.5-11 month old female 3xTg-AD and C57BL/6J mice on the Rotarod, which could be the result of having used C57BL/6J rather than B6129SF2 control mice, as there are strain differences in Rotarod performance. The motor enhancement on the Rotarod appears to be stable with age, since Filali et al. (2012) have demonstrated that female 3xTg-AD mice backcrossed to C57BL/6J mice also have enhanced performance on the Rotarod relative to C57BL/6J control mice at 12-14 months of age.

There are conflicting reports about the exploratory behaviour and activity levels of 3xTg-AD mice as measured in the open field. Some reports that the 3xTg-AD mice are less active than B6129SF2 mice from 4-15 months of age (Pietropaolo et al., 2009; García-Mesa et al., 2011; Filali et al., 2012), others report no differences between 3xTg-AD and control mice (Pietropaolo et al., 2008; Blanchard et al., 2010; Sterniczuk et al., 2010a), and still others report increased activity in the open field in female 3xTg-AD relative to B6129SF2 mice at seven (Chen et al., 2013) and 12 months (Pietropaolo et al., 2008) of age. These conflict reports may be the result of a number of factors including different origins of the mice, differing behavioural protocols, and differing housing conditions.

Anxiety is a common symptom of AD in humans (Teri et al., 1999) and 3xTg-AD mice have been tested for anxiety in the elevated plus maze. However, there are conflicting reports about the level of anxiety in these mice. Some studies found no

differences in anxiety-like behavior on the EPM between 3xTg-AD and B6129SF2 control mice at 12-14 months of age (Filali et al., 2012) or 7 to 12 months of age (Pietropaolo et al., 2008). Other studies report increased anxiety-like behaviour in female 3xTg-AD mice relative to B6129SF2 controls at 7 months of age (Blanchard et al., 2010; Chen et al., 2013): One study reported a sex-dependant effect at 15 months of age, where female 3xTg-AD displayed less anxiety-like behaviour than female B6129SF2 controls, but there was no genotype difference in male mice (Pietropaolo et al., 2009). Because measures of anxiety are confounded by differences in motor behaviour (O'Leary et al., 2013), it is important to determine whether anxiety measures in 3xTg-AD mice are not merely reflections of differences in locomotor behavior.

The 3xTg-AD females are bred with 3xTg-AD males to produce homozygous transgenic pups, so 3xTg-AD mice are reared by 3xTg-AD mothers, and wildtype (B6129SF2) mice are reared by B6129SF2 mothers. There are strain differences in maternal care in mice (Brown et al., 1999b; Priebe et al., 2005; Champagne et al., 2007), and since 3xTg-AD and wildtype control pups are reared by mothers of different genotypes, some behavioural deficits in 3xTg-AD pups may be due to differing levels and quality of maternal care. We have shown that maternal genotype affects behaviour in the 3xTg-AD mouse pups between 2 and 24 days by cross fostering pups to mothers of each genotype (Blaney et al., 2013). In order to determine the long term effects of maternal genotype on anxiety-like and motor behaviour, we conducted a longitudinal behavioural study of the cross-fostered pups from Blaney et al., (2013) at 2, 6, 12, and 18 months of age. After behavioural testing was completed we examined whether behaviour differences in early life predicted differences at later ages and we analyzed the levels of

amyloid beta and tau pathology in the brains of these mice at 19 months of age to determine whether the amount of pathology correlates with behaviour.

2.3 METHODS

2.3.1 BREEDING, CROSS-FOSTERING & PRE-WEANING TREATMENT OF MICE

To produce mice for this experiment we bred four pairs of 3xTg-AD mice (JAX # 004807) and four pairs of B6129S/F2 mice (JAX# 101045), which were purchased from Jackson Laboratories (Bar Harbor, Maine). These two strains are bred as separate lines, so 3xTg-AD mice are always reared by 3xTg-AD mothers, and B6129SF2 mice are always reared by B6129SF2 mothers. In order to study the effects of maternal genotype we cross-fostered litters of 3xTg-AD and B6129SF2 mice to create mixed genotype litters; no mother reared her own pups. To study early development in this strain the mice underwent a neurodevelopmental test battery from post natal day 0-24, (see Blaney et al., 2013). After weaning, the mice were housed in same sex mixed genotype groups of two to four mice, in clear plastic cages measuring 18.75 x 28 x 12.5 cm, with wood chip bedding, a PVC tube (4 cm diameter x 7 cm length) for enrichment, and metal wire covers. They were fed Purina rodent chow (#5001, Purina, St. Louis, Missouri) and tap water ad libitum, unless otherwise indicated. The colony room was maintained at 22±2 °C on a reversed 12:12 light:dark cycle, with lights off at 10:00am. This experiment was approved by the Dalhousie University Committee on Animal Care.

2.3.2 PROCEDURE

We tested a total of 78 mice, 40 3xTg-AD (17 male and 24 female) and 38 B6129S/F2 (19 male and 19 female), in a longitudinal study with testing at 2, 6, 12, and

18 months of age. These time points allowed us to evaluate the behaviour of the 3xTg-AD mice before, during, and after development of neuropathology (Oddo et al., 2003; Billings et al., 2005; Mastrangelo and Bowers, 2008). In designing this experiment and in calculating sample size, we reviewed previous studies that detected differences in behaviour between 3xTg-AD and control mice and found that they used between five and fifteen mice per genotype, based on this we chose groups of 38-40 mice per genotype and 8-14 mice per group (pup genotype by sex by foster mother genotype), which should provide sufficient power to detect any differences between the groups; and these numbers remained high until 12 months of age at which point mortality increased dramatically (Billings et al., 2005, 2007; Clinton et al., 2007; Sterniczuk et al., 2010a, 2010b). Because of age and sex differences in mortality rates (Rae and Brown, Unpublished), the sample sizes decreased as mice aged (Table 2.1), by 18 months of age many mice had died which may decrease our power to detect differences at that age. The mice were tested in three cohorts of approximately 27 animals each. The experimenters were blind as to the pup genotype and foster mother genotype of the mice during behavioural testing, however it was not possible to blind the experimenters to the age of the mice due to the longitudinal design. All testing took place during the dark phase of the light:dark cycle.

Table 2.1. Number of mice used at each age.

2 Months of Age			
	Maternal Genotype		
Pup Genotype	B6129SF2	3xTg-AD	Total
B6129SF2	11M, 7F	8M, 12F	38
3xTg-AD	8M, 14F	9M, 9F	40
Total	40	38	78
6 Months of Age			
	Maternal Genotype		
Pup Genotype	B6129SF2	3xTg-AD	Total

B6129SF2	11M, 7F	8M, 12F	38
3xTg-AD	7M, 14F	9M, 8F	38
Total	39	37	76
12 Months of Age			
	Maternal Genotype		
Pup Genotype	B6129SF2	3xTg-AD	Total
B6129SF2	11M, 5F	8M, 12F	36
3xTg-AD	6M, 13F	9M, 8F	36
Total	35	37	72
18 Months of Age			
	Maternal Genotype		
Pup Genotype	B6129SF2	3xTg-AD	Total
B6129SF2	10M, 5F	7M, 7F	29
3xTg-AD	1M, 11F	2M, 5F	19
Total	27	21	48
Mice used for Histology at 19 Months of Age			
	Maternal Genotype		
Pup Genotype	B6129SF2	3xTg-AD	Total
3xTg-AD	1M, 9F	0M, 5F	15
Total	10	5	

2.3.3 TEST BATTERY

The tests were completed in the order described below. This was designed such that the order of administration of the tests was from least to most stressful to minimize the effect of stress on test results. The mice also underwent a test battery to assess prepulse inhibition, cognitive, and social behaviour.

2.3.3.1 ELEVATED PLUS MAZE

The elevated plus maze apparatus and test procedure described by Brown et al. (1999) were used. The apparatus consisted of a plus shaped maze with two open arms (30 x 5 cm) with a 4 mm lip to prevent the mouse from slipping off, and two closed arms (30 x 5 cm) with transparent Plexiglas walls (15 cm high). The arms were connected by a center square (5 x 5 cm). The floor of the maze was grey Plexiglas. Testing was completed in a room (2 x 5 m) illuminated by two 60 W white bulbs. Each mouse was

tested on one five minute trial and between mice the maze was cleaned with a 70% ethanol solution. At the beginning of the trial the mice were placed in the center square facing an open arm. A camera 2.1 m above the maze recorded the movement of the mice throughout the trial. The time in the open and closed arms, and the distance travelled were recorded by a computerized tracking system (Limelight, Actimetrics Inc., Wilmette, IL). The duration of grooming, freezing (remaining completely immobile expect for respiration), the number of bouts of rearing (removing forepaws from the floor of the maze), stretch attend postures (extending its head and then returning to the previous position), and head dips (moving its head over the edge of the maze and pointing downwards) were recorded. In the elevated plus maze distance travelled, rears and head dips are measures of locomotor behaviour and the percentage of time in the closed arms, stretch attend postures, and time spent freezing are measures of anxiety (O'Leary et al., 2013).

2.3.3.2 OPEN FIELD

The open field test was performed using the procedure of Brown et al. (1999). The open field was a square box constructed from wood and painted white (72 x 72 cm with 36 cm high walls). The floor was covered with transparent Plexiglas which had an 18 x 18 cm center square drawn in the middle. Testing occurred in the same room as the elevated plus maze. To begin a trial a mouse was placed in a corner of the open field and their behaviour was recorded for five minutes. The time in the centre square, and total distance travelled were recorded by the Limelight computerized tracking system and the experimenter recorded the number of center square entries, rears against the wall, rears in the center of the maze (forepaws not touching any walls), time spent grooming, number

of stretch attend postures, and time spent freezing. Between mice the maze was cleaned with a 70% ethanol and allowed to dry. In the open field rears and distance travelled are measures of locomotion while stretch attend postures, time in the centre, center rears, and time spent freezing are measures of anxiety.

2.3.3.3 ROTAROD

In order to examine motor coordination and learning, mice were tested on the accelerating Rotarod for six trials a day for five days (Accuscan Instruments Inc. Columbus, Ohio), using the procedure described by Brown and Wong (2007). The Rotarod consisted of a plastic rod (3 cm diameter) which was divided by plastic dividers (14 cm diameter) into four sections (11 cm wide). Holding chambers were located 39 cm below each of the four sections. The mice were weighed each day and then placed on the Rotarod which accelerated from 0 to 48 rpm over a trial lasting a maximum of 360 seconds. The latency to fall for each mouse was recorded. If a mouse did not fall after 360 seconds it was placed in the holding chamber. The mice were given a 60 second inter-trial interval after the last mouse fell from the rod. Mice were tested in the dark phase of the L:D cycle in a room lit by a 60 W red bulb. The average latency to fall each day was analyzed.

2.3.4 HISTOLOGY

After behavioural testing was completed at 19 months of age the levels of amyloid beta and tau pathology in the brains of the mice were evaluated. We used 15 mice for this analysis (see Table 2.1).

2.3.4.1 TISSUE PREPARATION

Mice were deeply anaesthetized with an intraperitoneal injection of sodium pentobarbital (200mg/kg) and perfused with 0.1M phosphate buffered saline (PBS), followed by 4% paraformaldehyde in 0.1M phosphate buffer (PB). The brains were removed and post-fixed in 4% paraformaldehyde in PB for one hour. They were then cryo-protected in PB with 30% sucrose for a minimum of 72 hours. Entire brains were cut in 40µm thick equal sections in coronal plane using a Leica SM2000R microtome with a freezing stage (Leica Microsystems Inc., Nussloch, Germany) and stored in PBS with 0.1% sodium azide. The brains were separated into six series, and adjacent sections were used for Aß and tau immunohistochemistry.

2.3.4.2 AB IMMUNOHISTOCHEMISTRY

Aβ immunohistochemistry was performed using the procedure described by Darvesh et al. (2012). Briefly, sections were rinsed for 5 minutes in 0.05 M PB (pH 7.4), followed by distilled water (dH_2O), and then treated with 90% formic acid for 2 minutes to improve antigen retrieval (Kitamoto et al., 1987). Sections were then rinsed 5 times in dH_2O for 1 minute each and twice in PB for 15 minutes. Sections were placed in 0.3% H_2O_2 in PB for 30 minutes and rinsed for 30 minutes in PB. Sections were then incubated in PB containing 0.1% Triton X-100, normal goat serum (1:100), and a polyclonal rabbit anti-amyloid antibody (1:400; 71-5800, Invitrogen, Camarillo, CA), which is specific for the 4- to 5-kDa amyloid peptide derived by cleavage from the amyloid precursor protein (Jankowsky et al., 2007), for approximately 16 hours at room temperature. After rinsing, sections were incubated in PB with 0.1% Triton X-100, biotinylated goat anti-rabbit secondary antibody (1:500), and normal goat serum (1:1000) for 1 hour. After another

rinse, sections were placed in PB with 0.1% Triton X-100 and Vectastain Elite ABC kit (1:182; PK-6100, Vector Laboratories, Burlingame, CA), according to the manufacturer's instructions for 1 hour. Sections were rinsed and developed in a solution of PB containing 1.39 M 3,3 diaminobenzidine tetrahydrochloride (DAB). After 5 minutes, 50 μL of 0.3%H_2O_2 in dH_2O was added per milliliter of DAB solution, and the sections incubated for 10 minutes. The reaction was stopped by rinsing the sections in 0.01 M acetate buffer (pH 3.3). In control experiments, no staining was observed when the primary antibody was omitted. The total area of Aβ plaque load was assessed in the hippocampus, amygdala, and the cerebral cortex using the National Institute of Health ImageJ software. The areas of interest were parcellated according to the Paxinos and Franklin mouse atlas (Paxinos and Franklin, 2001). An intensity threshold was chosen to distinguish between plaque immunoreactivity and background staining and was kept constant throughout quantification.

2.3.4.3 TAU IMMUNOHISTOCHEMISTRY

The tau Immunohistochemistry was performed as above except the primary antibodies were polyclonal rabbit anti-human tau (Dako #A0024) and the secondary antibodies were biotinylated goat anti-rabbit secondary antibody (1:500). The levels of tau pathology in the amygdala and the pyramidal cell layer of the hippocampus were quantified using unbiased stereology, as described by Tran et al. (2011). Breifly, the StereoInvestigator software was used to selecte the area of interest optical fractionator stereological method was used to estimate the number of tau positive neurons in that area. There was little tau straining in the cerebral cortex or other layers of the hippocampus, so they were not analyzed.

2.3.5 STATISTICAL ANALYSES

Statistical analyses were performed with R (www.R-project.org) using linear mixed effects models, with genotype, foster mother genotype, sex and age as possible predictors. For behaviours without repeated measures all of the possible models were constructed, and the second order Akaike information criterion (AIC_c), Akaike weight, and evidence ratio were calculated for each model. The model with the lowest AICc was chosen as the 'best' model. For behaviours with a repeated measures component the model was chosen using backwards elimination. Models with three-way or greater interactions involving age were excluded due to the low numbers of mice in some groups at 18 months of age. The chosen model was compared to the null model with a χ^2 test (Akaike, 1974; Burnham and Anderson, 2002). We calculated confidence intervals (95%) for all effects in the model. For measures with significant effects of genotype, sex, or foster mother genotype, the effect size was calculated using Cohen's d with a Hedge's (d_{unb}) for an unbiased measure (Hedges, 1981; Cumming, 2014).

In order to examine the relationship between locomotor behaviour before weaning and at later ages we included activity in the open field from the study conducted by Blaney et al. (2013) as a possible predictor of locomotor behaviours in the OF, EPM, and Rotarod.

The relationship between neuropathology and behaviour was analyzed by correlating levels of neuropathology with behavioural measures that had genotype differences using Pearson's r for all mice with neuropathology data. If there was more than one measure that evaluated a similar behaviour (e.g. Time in the closed arms and distance in the closed arms in the elevated plus maze), the measure with the larger effect

size was included in the correlation to decrease the number of similar measures included in the correlation and to prevent issues with multiple comparisons.

2.4 RESULTS

The number of animals tested at each age group, their genotypes and sex are summarized in Table 2.1.

2.4.1 ELEVATED PLUS MAZE

2.4.1.1 LOCOMOTOR BEHAVIOURS

The best model for distance travelled had genotype, foster mother genotype, sex, age, a genotype by foster mother genotype interaction, a genotype by sex interaction, a foster mother genotype by sex interaction and a genotype by age interaction (AICc=4064.248, Figure 2.1A; See also Supplemental Table 2.1.1 and Supplemental Figure 2.1A), which differed significantly from the null model ($\chi^2(12, N=272)= 159.39$, $p <0.0001$). Confidence intervals (CIs) indicated that B6129SF2 mice travelled a greater distance than 3xTg-AD mice (CI_{95}= 275.49 – 546.24cm) and mice reared by B6129SF2 mothers travelled a greater distance than those reared by 3xTg-AD mothers (CI_{95}= -429.91 – -62.08cm). Although there was no overall sex effect (CI_{95}= -90.53 – 283.16cm), the pup genotype by sex interaction showed that 3xTg-AD females travelled a greater distance than 3xTg-AD males (CI_{95}= -11.159 – 378.292 cm) while B6129SF2 females travelled a shorter distance than B6129SF2 males (CI_{95}= -285.729 – 90.072 cm). The maternal genotype by sex interaction showed that females reared by B6129SF2 mothers travelled a greater distance than males (-32.838 – 344.130 cm) while there was no sex difference in mice reared by 3xTg-AD mothers (CI_{95}= -116.455 – 257.676 cm). The total distance travelled was highest at 2 months of age and was lower at 6, 12, and 18

months of age (2m vs 6m: CI_{95}= -931.03 – -594.91, 12m: CI_{95}= -981.14 – -652.04, 18m: CI_{95}= -1045.25 – -685.13). The pup genotype by foster mother genotype interaction occurred because there was no difference between maternal genotypes in 3xTg-AD mice (CI_{95}= -243.726 – 132.136 cm), but B6129SF2 mice reared by B6129SF2 mothers travelled a greater distance than B6129SF2 mice reared by 3xTg-AD mothers (CI_{95}= 58.214 – 430.646 cm). The age by genotype interaction showed a large difference between genotypes at two months of age (CI_{95}= 527.876 – 903. 998cm) but this difference was smaller later ages (18m: CI_{95}= 111.660 – 588.456cm).

For the number of rears the best model had only age (AICc=1451.552, Figure 2.1B; See also Supplemental Table 2.1.2 and Supplemental Figure 2.1B), which differed significantly from the null model ($\chi^2(3, N=272)= 12.548$, $p =0.0057$). The number of rears decreased from 2 to 6 months of age (CI_{95}= -2.057 – 0.043 rears), and from 2 to 12 months of age (CI_{95}= 2.235 – -0.060 rears), and returned to near the 2 month levels at 18 months of age (CI_{95}= -0.531 – 1.917 rears).

The best model for the number of head dips had age, genotype, and a genotype by age interaction (AICc=1985.685, Figure 2.1C; See also Supplemental Table 2.1.3 and Supplemental Figure 2.1C), which differed significantly from the null model ($\chi^2(4, N=272)= 22.008$, $p =0.0025$). The number of head dips decreased from 2 to 18 months of age (CI_{95}= -7.202 – -0.960 dips). And although there was no difference between genotypes (CI_{95}= -8.171 – 2.407 dips), the genotype by age interaction showed that the 3xTg-AD mice made more head dips than B6129SF2 mice at 18 months of age (CI_{95}= 1.647 – 9.784 dips), but there was no genotype difference at earlier ages.

2.4.1.2 ANXIETY-LIKE BEHAVIOURS

The best model for the percentage of total distance travelled in the closed arms had genotype, sex, and a genotype by sex interaction (AICc=2417.806, Figure 2.1D; See also Supplemental Table 2.1.4 and Supplemental Figure 2.1C), which was significantly different from the null model ($\chi^2(3, N=272)= 35.188$, $p <0.0001$). The CIs indicated that B6129SF2 mice travelled a greater percentage of their distance in the closed arms than 3xTg-AD mice (CI_{95}= 12.282 – 24.083%) and females had a higher distance in the closed arms than males (CI_{95}= 1.742 – 13.600%). The genotype by sex interaction showed that 3xTg-AD females travelled a greater distance than males (CI_{95}= 5.271% – 22.201%), but there was no difference between sexes in B6129SF2 mice (CI_{95}= -6.470 – 10.265%).

For the percentage of time spent in the closed arms the best model had genotype and sex (AICc=2440.189, Figure 2.1E; See also Supplemental Table 2.1.5 and Supplemental Figure 2.1E), which was significantly different from the null model ($\chi^2(2, N=272)= 44.419$, $p <0.0001$). The B6129SF2 mice spent a greater percentage of time in the closed arms (CI_{95}= 17.289 – 29.387%) and females spent more time in the closed arms than males (CI_{95}= 0.090 – 12.340%).

The best model for time spent freezing in the EPM had age, age, and a sex by age interaction (AICc=2245.262, Figure 2.1F; See also Supplemental Table 2.1.6 and Supplemental Figure 2.1F) which differed significantly from the null model ($\chi^2(7, N=272)= 26.037$, $p <0.0005$). The amount of time spent freezing was higher at 6 and 18 than at 2 and 12 months of age (2 vs 6: CI_{95}= 1.392 – 9.756s, 6 vs 12: -11.734 – -3.091s, 12 vs 18: 1.125 – 10.914s). There was no overall effect of sex (CI_{95}= -4.102 – -5.618s), but the sex by age interaction showed that at 6 months of age males spent more time

freezing than females (CI_{95}= 1.805 - 15.644s), at 18 months of age females spent more time freezing than males (CI_{95}= -1.041 – 15.887s), and there was no difference between males and females at other ages.

For the number of stretch attend postures the best model had only age (AICc=1138.767, Figure 2.1G; See also Supplemental Table 2.1.7 and Supplemental Figure 2.1G), which differed significantly from the null model ($\chi^2(3, N=272)=23.481$, $p <0.0001$). The number of SAPs increased from 2 to 12 (CI_{95}= 0.478 –1.703 SAPs) and 2 to 18 (CI_{95}= 0.676 – 2.080 SAPs) months of age.

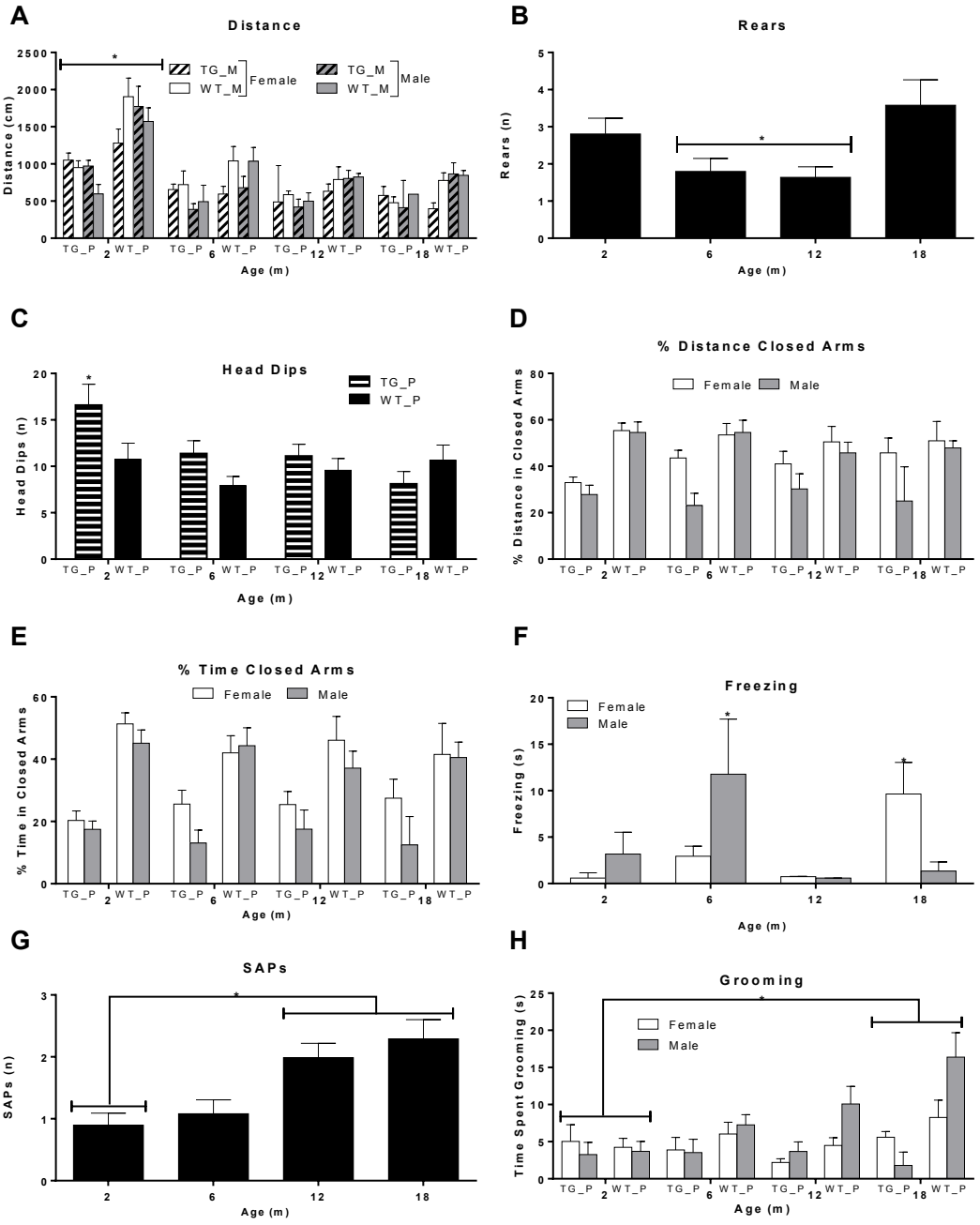

Figure 2.1 The Elevated Plus Maze. The mean (± S.E.M) total distance travelled (A), number of rears (B) and head dips (C), percentage of distance (D) and time (E) spent in the closed arms, the time spent freezing (F), number of stretch attend postures (G) and time spent grooming (H) of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers in the elevated plus maze**.**

2.4.1.3 GROOMING

For the amount of time spent grooming the best model had genotype, sex, and age, a genotype by sex interaction, and a genotype by age interaction (AICc=1878.803, Figure 2.4.1H; See also Supplemental Table 2.1.8 and Supplemental Figure 2.1H), which differed significantly from the null model ($\chi^2(9, N=272)= 44.089$, $p <0.0001$). The B6129SF2 mice spent more time grooming than 3xTg-AD mice (CI_{95}= 3.197 – 11.893s), males spent more time grooming than females (CI_{95}= -0.849 – 3.362s), and the amount of time spent grooming increased from 2 to 18 months of age (CI_{95}= 2.422 – 7.615s). The genotype by sex interaction occurred because male B6129SF2 mice spent more time grooming than females (CI_{95}= 0.207 – 6.152s), but there was no difference between the sexes in 3xTg-AD mice (CI_{95}= -3.591 – 2.285s). The genotype by age interaction was caused by B6129SF2 mice spending more time grooming than 3xTg-AD mice at 12 months of age (CI_{95}= 1.094 – 8.007s) and 18 months of age (CI_{95}= 3.234 – 11.951s) but not earlier ages.

2.4.2 OPEN FIELD

2.4.2.1 LOCOMOTOR BEHAVIOURS

In the open field the best model for the distance travelled had only age (AICc=4354.535, Figure 2.2A, See also Supplemental Table 2.2.1 Supplemental Figure 2.2A), which was significantly different from the null model ($\chi^2(3, N=273)=49.604$, $p <0.0001$). The distance travelled decreased with age (2-18m: CI_{95}= -908.243 – -460.112cm), though it did not change from 12-18 months of age (CI_{95}=-254.140 – 198.252cm).

For the number of rears the best model had genotype, foster mother genotype, sex, age, a genotype by age interaction, and a sex by age interaction (AICc=1886.184, Figure 2.2B; See also Supplemental Table 2.2.7 and Supplemental Figure 2.2G), which was significantly different from the null model ($\chi^2(12, N=273)=69.217$, $p <0.0001$). The B6129SF2 mice tended to rear more than 3xTg-AD mice (CI_{95}= -0.137 – 4.145 rears), mice reared by 3xTg-AD mothers reared more than mice reared by B6129SF2 mothers (CI_{95}= 0.635 – 4.804 rears), and females reared more than males (CI_{95}= -0.948 – 3.409 rears). The number of rears decreased from 2 to 6 months of age (CI_{95}= -5.260 – -0.731 rears), and from 2 to 12 months of age (CI_{95}= -9.021 – -4.471 rears), and then increased to 2 months levels at 18 months of age (CI_{95}= -4.398 – 0.952 rears). The genotype by age interaction occurred because at only 6 months of age B6129SF2 mice reared more than 3xTg-AD mice (CI_{95}= 0.780 – 7.554 rears). The sex by age interaction occurred because only at 2 months of age females reared more than males (CI_{95}= 2.475 – 9.188 rears).

2.4.2.2 ANXIETY-LIKE BEHAVIOURS

For the number of entries into the center square, the best model had pup genotype, foster mother genotype, age, a pup genotype by age interaction, and a foster mother genotype by age interaction (AICc=914.622, Figure 2.2C, See also Supplemental Table 2.2.2 and Supplemental Figure 2.2B), which differed significantly from the null model ($\chi^2(11, N=273)=51.550$, $p <0.0001$). The 3xTg-AD mice entered the centre square more often than B6129SF2 mice (CI_{95}= 0.129 – 0.739 entries), but there was no difference between foster mother genotypes (CI_{95}= -0.577 – 0.036 entries). The number of entries into the centre square decreased with age (2-18m: CI_{95}= -1.220 – -0.294 entries) and the genotype by age interaction showed that 3xTg-AD mice entered the center square more

often than wildtype mice at 2 months of age (CI_{95}= 0.684 – 1.831 entries) but by 18 months of age there was no longer a difference between genotypes (CI_{95}= -0.547 – 0.920 entries). Similarly the foster mother genotype by age interaction occurred because at 2 months of age mice reared by 3xTg-AD mothers entered the center square more often than mice reared by B6129SF2 mothers (CI_{95}= 0.447 – 1.560 entries), but by 18 months of age there was no difference (CI_{95}= -0.557 – 0.885 entries).

For the amount of time spent in the center the best model had genotype, age, and a genotype by age interaction (AICc= 1210.696, Figure 2.2D; See also Supplemental Table 2.2.3 and Supplemental Figure 2.2C), which differed significantly from the null model ($\chi^2(7, N=273)=29.165$, p =0.0001). The 3xTg-AD mice spent more time in the center square than B6129SF2 mice (CI_{95}= 0.408 – 1.500 s), and the amount of time in the center square decreased from 2 to 6 (CI_{95}= -1.455 – -0.137 s) and 2 to 12 months of age (CI_{95}= -1.772 – -0.392 s), but increased at 18 months of age (CI_{95}= -1.219 – 0.340 s). The genotype by age interaction occurred because at 2 and 18 months of age 3xTg-AD mice spent more time in the center square than B6129SF2 mice (CI_{95}= 0.943 – 2.847s, and CI_{95}= 0.042 – 2.569s, respectively), but there was no difference at 6 or 12 months of age (CI_{95}= -0.526 – 1.415s, and CI_{95}= -0.911 – 1.123s, respectively).

For the number of center rears the best model had pup genotype, foster mother genotype, sex, age, a foster mother genotype by age interaction, and a sex by age interaction (AICc=1102.712, , Figure 2.2E; See also Supplemental Table 2.2.4 and Supplemental Figure 2.2D), which differed significantly from the null model ($\chi^2(12, N=273)=36.768$, p =0.0002). The 3xTg-AD mice had more center rears than B6129SF2 mice (CI_{95}= 0.092 – 1.05 rears), there was no difference between foster mother genotypes

(CI_{95}= -0.317 – 0.665 rears), and there was some evidence that males had more center rears than females (CI_{95}= -0.0004 – 0.975 rears). The number of rears increased from 2 to 18 months of age (CI_{95}= 0.188 – 1.442 rears), but there no difference between 2 and 6 months of age (CI_{95}= -0.801 – 0.287 rears), or 2 and 12 (CI_{95}= -0.990 – 0.134 rears). The foster mother genotype by age interaction occurred because mice reared by 3xTg-AD mothers had more center rears than mice reared by B6129SF2 mothers only at 18 months of age (CI_{95}= 0.454 – 2.56 rears), and the sex by age interaction occurred because only at 18 months of age males reared in the center more than females (CI_{95}= 0.766 – 2.786 rears).

For the time spent freezing the best model had only foster mother genotype (AICc=2159.739, Figure 2.2F; See also Supplemental Table 2.2.6 and Supplemental Figure 2.2F), which was significantly different from the null model ($\chi^2(8, N=273)=6.766$, $p =0.0093$). Mice reared by B6129SF2 mothers spent more time freezing than mice reared by 3xTg-AD mothers (CI_{95}= 0.986 – 7.033 s).

For the number of stretch attend postures the best model had only age (AICc=1139.625, Figure 2.2H; See also Supplemental Table 2.2.8 and Supplemental Figure 2.2G), which differed significantly from the null model ($\chi^2(3, N=273)=16.649$, $p <0.0002$). The number of SAPs increased from 2 to 6 (CI_{95}= 0.528 – 1.620 SAPs), and 2 to 12 (CI_{95}= 0.395 – 1.527 SAPs) months of age, but there was no difference between 2 and 18 months of age (CI_{95}= -0.460 – 0.809 SAPs).

2.4.2.3 GROOMING

For the amount of time spent grooming the best model had genotype, foster mother genotype, sex, age, a genotype by sex interaction, and a foster mother genotype

by sex interaction (AICc=1912.996, Supplemental Table 2.2.5, Figure 2.2H, and Supplemental Figure 2.2E), which differed significantly from the null model ($\chi^2(8, N=273)=39.429$, $p <0.0001$). B6129SF2 mice spent more time grooming than 3xTg-AD mice (CI_{95}= 1.132 – 5.141 s), there was some evidence that mice reared by B6129SF2 mothers spent more time grooming than mice reared by 3xTg-AD mothers (CI_{95}= -0.759 – 3.027 s), and the amount of time spent grooming decreased from 2 to 12 months of age (CI_{95}= -0.109 – -5.09s). There was no difference between females and males (CI_{95}= -1.031 – 2.738 s). The genotype by sex interaction occurred because in 3xTg-AD mice there was no difference between females and males (CI_{95}= -4.624 – 1.088 s), while in B6129SF2 mice males spent more time grooming than females (CI_{95}= 0.952 – 6.175 s). Similarly the foster mother genotype by sex interaction occurred because in mice reared by 3xTg-AD mothers there was no difference between males and females (CI_{95}= -3.775 – 1.653 s), while in mice reared by B6129SF2 mothers males spent more time grooming than females (CI_{95}= 0.059 – 5.437 s).

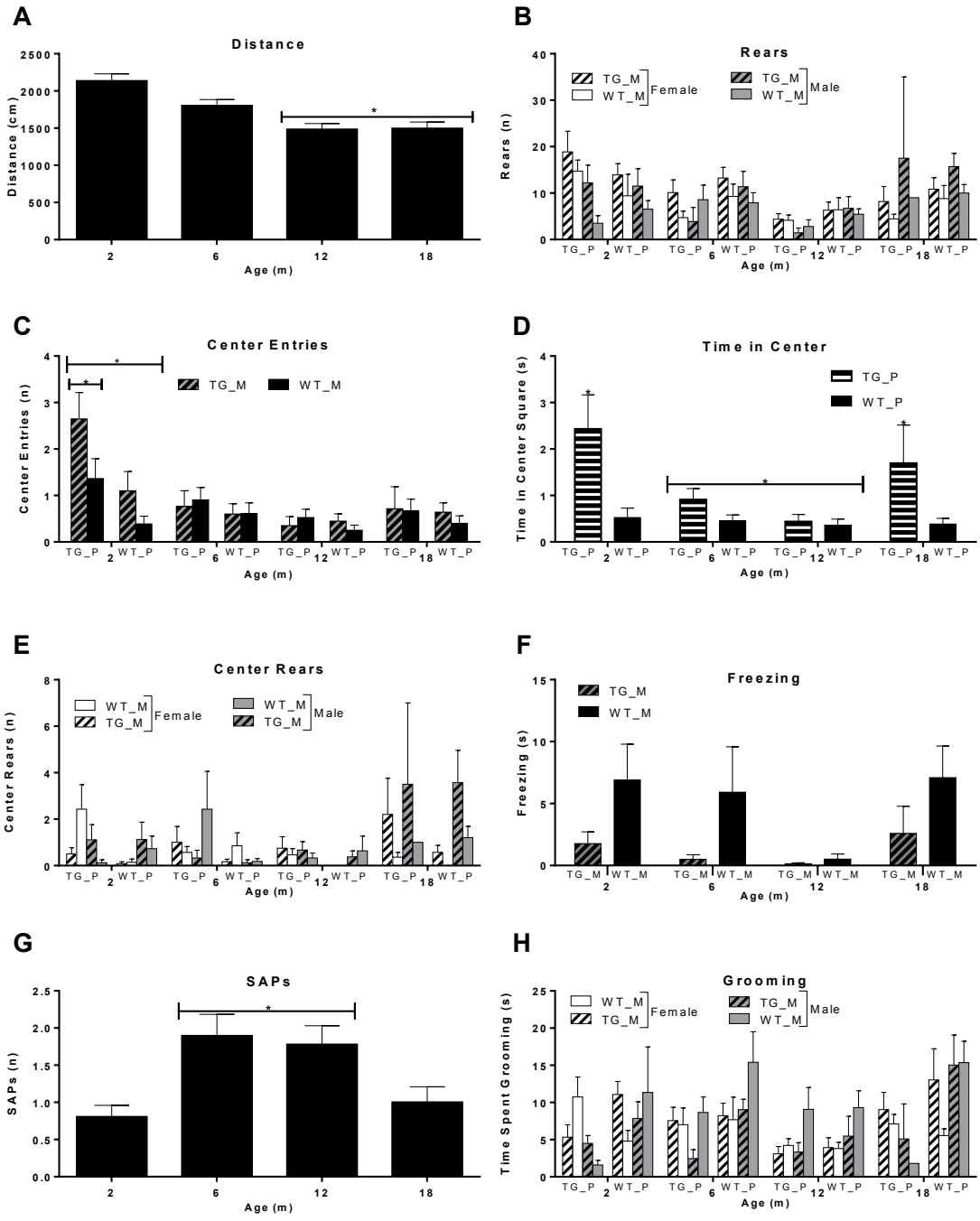

Figure 2.2 The Open Field. The mean (± S.E.M) total distance travelled (A), number of rears (B), center entries (C), time spent in the center (D), number of center rears (E), time spent freezing (F), number of stretch attend postures (G), and time spent grooming (H) of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers in the open field.

2.4.3 ROTAROD

The best model for body weight had pup genotype, foster mother genotype, sex, age, a genotype by sex interaction, a genotype by age interaction, and a foster mother genotype by sex by age interaction (AICc=1539.390, Figure 2.3A; See also Supplemental Table 2.3), which differed significantly from the null model ($\chi^2(20,N= 273)= 318.88$, $p < 0.001$). Male mice were heavier than female mice ($CI_{95}= 0.761 – 6.665$g) and the mice increased in weight with age (2-18m: $CI_{95}= 9.150 – 14.377$ g). Although there was no main effect of genotype ($CI_{95}= -2.485 – 0.960$ g), in female mice 3xTg-AD mice weighed more than B6129SF2 mice ($CI_{95}= 0.0251 – 4.889$ g) and at 12 months of age 3xTg-AD mice weighed more than B6129SF2 mice ($CI_{95}= 0.353 –4.712$ g). At 12 months of age in female mice only the mice reared by 3xTg-AD mothers weighed more than the mice reared by B6129SF2 mothers ($CI_{95}= 1.827 – 9.359$ g).

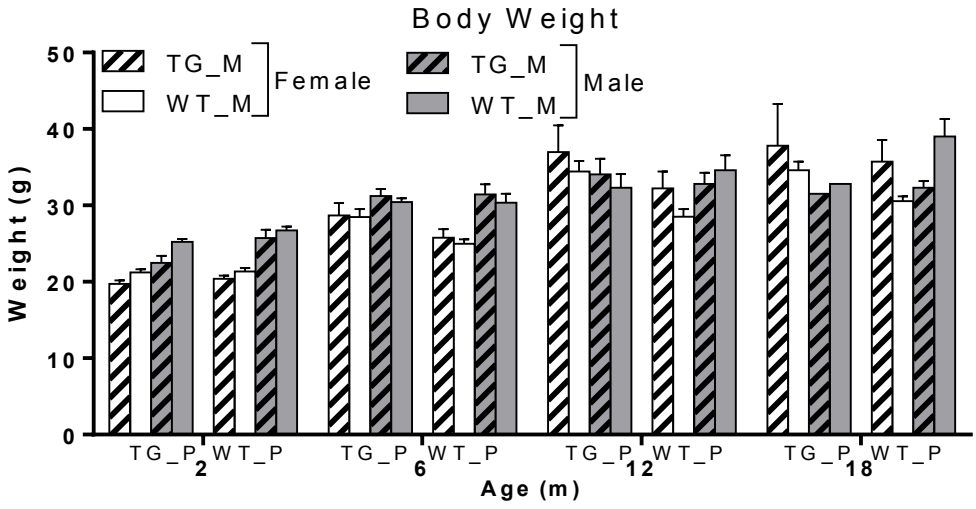

Figure 2.3 Body Weight. The mean (± S.E.M) total body weight of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers.

For latency to fall from the Rotarod the best model had day, pup genotype, foster mother genotype, sex, age, a day by pup genotype interaction, a day by age interaction, a pup genotype by sex by age interaction, and a foster mother genotype by sex by age interaction (AICc=13771.900, Figure 2.4A; See also Supplemental Table 2.4), which differed significantly from the null model ($\chi^2(43,N= 1355)= 808.53$, $p < 0.001$). The latency to fall increased from day 1 to 5 (CI_{95}= 65.882 – 78.280s), 3xTg-AD mice had a longer latency to fall than B6129SF2 mice (CI_{95}= 24.678 – 52.691s), mice reared by B6129SF2 mothers had a longer latency to fall than mice reared by 3xTg-AD mothers (CI_{95}= 0.495 - 27.643s), and females had a longer latency to fall than males (CI_{95}= 2.913 – 29.998s). The latency to fall increased from 2 to 6 (CI_{95}= 3.820 – 14.093s) and 2 to 12 months of age (CI_{95}= 11.462 – 21.880s), but decreased from 2 to 18 months of age (CI_{95}= -20.324 – -4.062s). There was a day by genotype interaction as in 3xTg-AD mice the latency to fall increased each day, while in B6129SF2 mice the latency to fall increased only until day 4 (CI_{95}= -0.282 – 16.518s). The day by age interaction occurred because at every age the latency to fall increased from day 1 to day 5, except at 18 months of age when there no difference between day 1 and 2 (CI_{95}= -12.951 – 5.708s). The genotype by sex by age interaction occurred because the female 3xTg-AD mice had a longer latency to fall than the males only at 18 months of age (CI_{95}= 28.158 – 91.967s), while there was no sex difference in B6129SF2 mice (CI_{95}= -19.785 – 23.130s). The foster mother genotype by sex by age interaction occurred because only at 18 months of age female mice reared by B6129SF2 mothers had a longer latency to fall than male mice (CI_{95}= 20.064 – 69.423s), while there was no sex difference in mice reared by 3xTg-AD (CI_{95}= 8.180 – 44.111s). In order to determine if body weight was a confounding factor for the

sex effects we included body weigh as a possible variable, however it was not included in the best model indicating that it was not better at explaining the differences than sex.

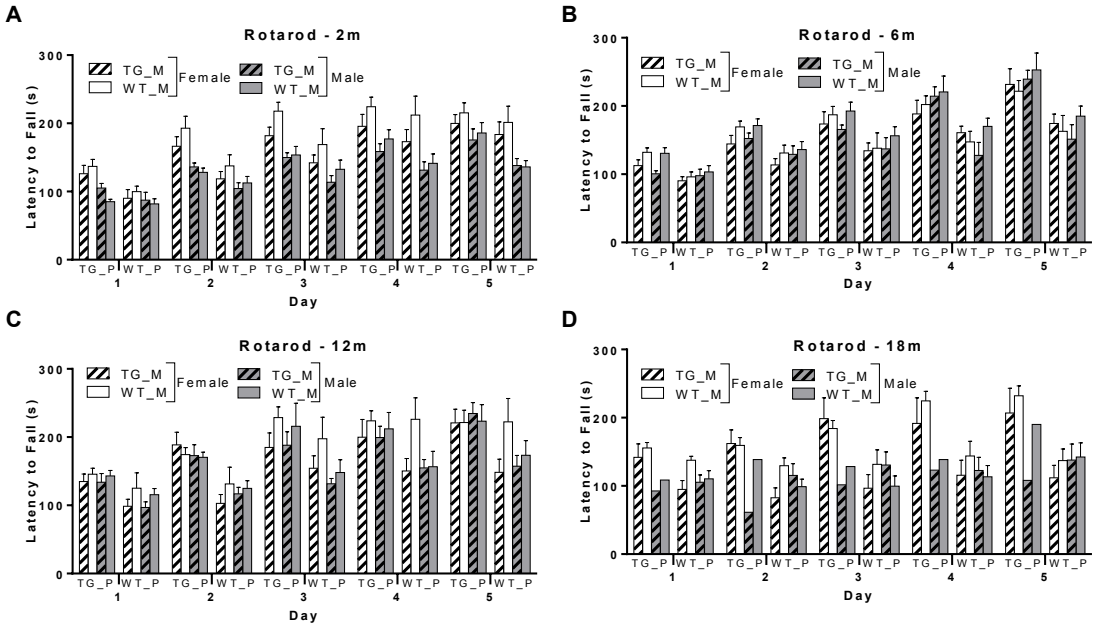

Figure 2.4 Rotarod. The mean (± S.E.M) latency to fall from the Rotarod at 2 (A), 6 (B), 12 (C), and 18 (D) months of age in 3xTg-AD and B6129SF2 mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers.

2.4.4 NEUROPATHOLOGY

Due to the low number of surviving males (Table 2.1) we were only able to analyze differences in neuropathology in 3xTg-AD mice between foster mother genotypes. A summary of the mice used for this analysis is presented in Table 2.1. Some mice died during or immediately after testing at 18 months of age which further reduced the number of animals available for neuropathology analysis. We measured densities of tau positive neurons in the amygdala and pyramidal layers of the hippocampus of the 3xTg-AD mice since there was little tau staining in the other layers of the hippocampus or other regions of the brain. We also stained B6129SF2 control mice for tau and found

no tau positive neurons. For the density of tau positive neurons in the hippocampus the model with foster mother genotype was not significantly different from the null model ($F(1,14)= 3.420$, $p > 0.05$, Figure 2.5A), and the confidence interval indicated there was no difference between foster mother genotypes (CI_{95}= -4.592e-05 – 3.560e-06 neurons/μm^3). The model for the density of tau positive neurons in the amygdala was significantly different from the null model ($F(1,14)= 6.538$, $p < 0.05$. Figure 2.5B), as mice reared by 3xTg-AD mothers had a higher density of tau positive neurons in the amygdala (CI_{95}= 2.33e-06 – 2.772e-05 neurons/μm^3).

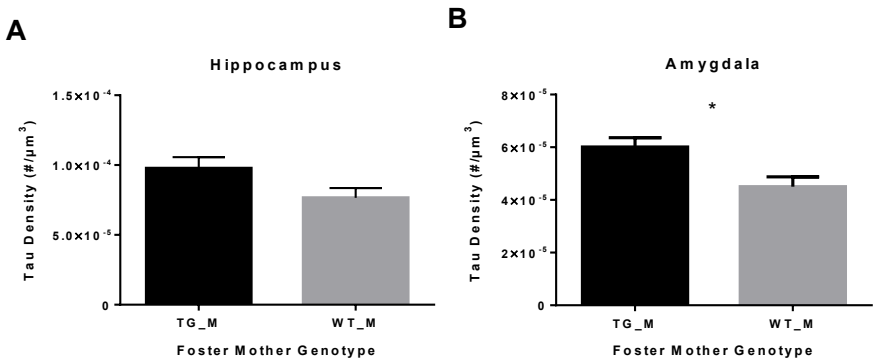

Figure 2.5 Tau Pathology. The mean (± S.E.M) density of tau positive neurons in the hippocampus (A) and amygdala (B) of 3xTG-AD mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers.

For the level of Aβ staining in the hippocampus, amygdala, and cerebral cortex of the 3xTg-AD mice, the models were not significantly different from the null models and the confidence intervals indicated there was no difference between foster mother genotypes (all $F(1,12) <1$, $p > 0.05$, CI_{95}= -8.550 – 8.998 % coverage, CI_{95}= -8.084 – 7.244 , CI_{95}= -0.866 –4.406, respectively, Figure 2.6). We found no Aβ staining in B6129SF2 brains. Example sections of 3xTg-AD and B6129SF2 brain stained for Aβ and tau are presented in Supplemental Figures 2.3 and 2.4.

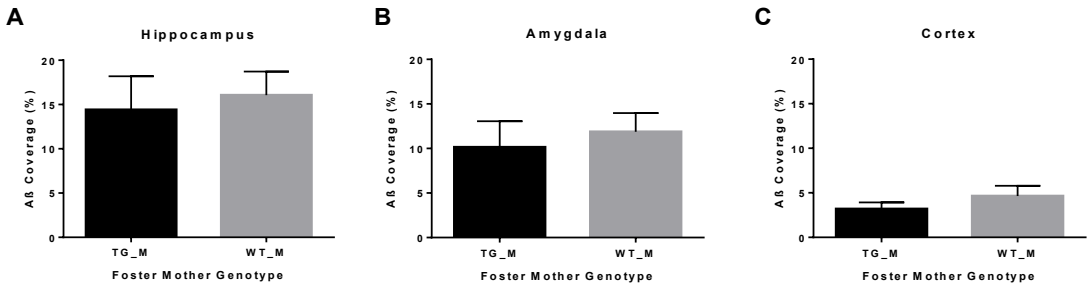

Figure 2.6 Aβ Pathology. The mean (± S.E.M) percentage of the hippocampus (A) amygdala (B), and cortex (C) covered by amyloid beta plaques of 3xTG-AD mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers.

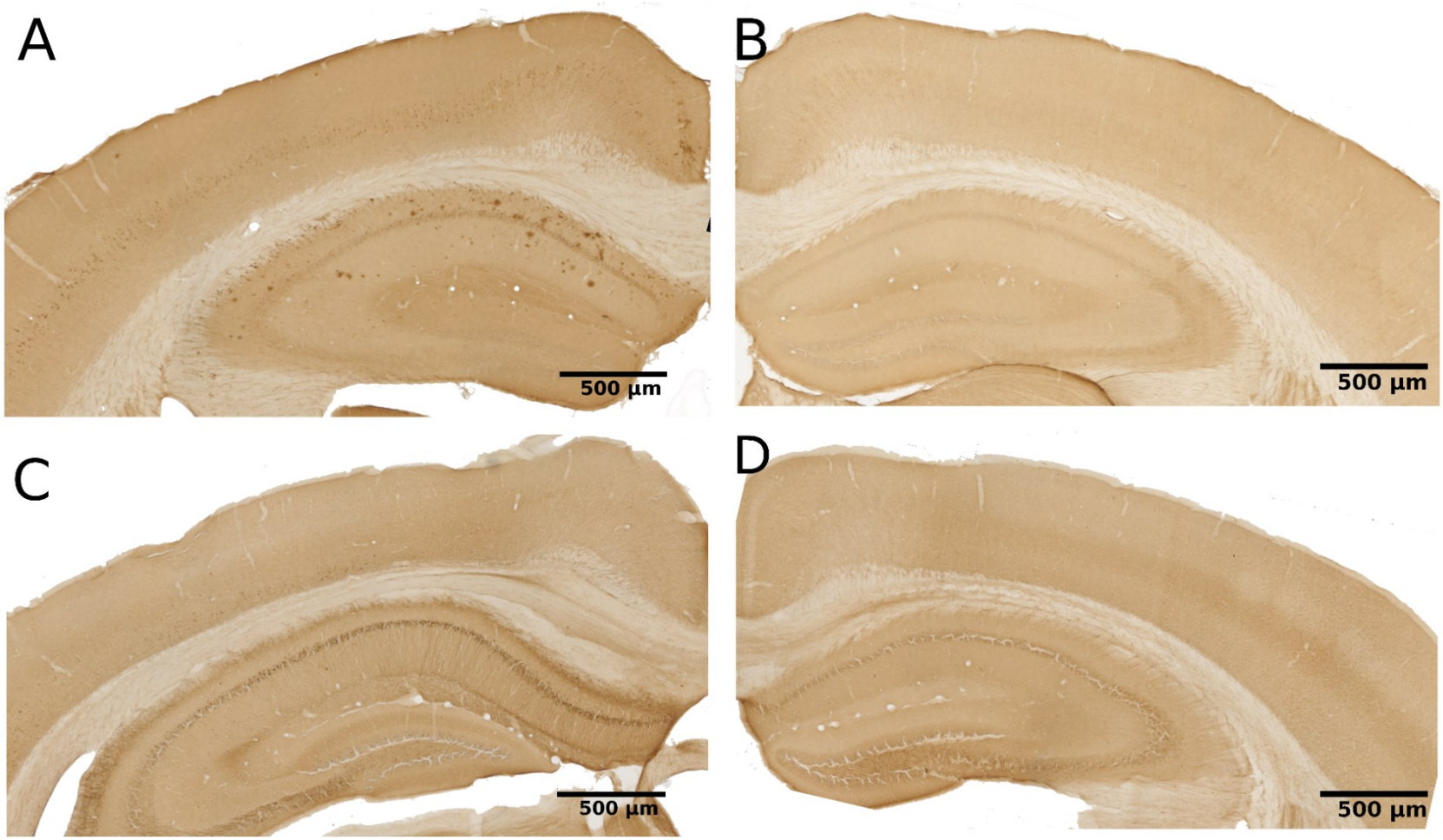

Figure 2.7 Hippocampus and Cortex Immunohistochemistry. Example sections of 19 month old 3xTg-AD mice stained for amyloid beta (A) or tau (C) and B6129SF2 control mice strained for amyloid beta (B) or tau (D)

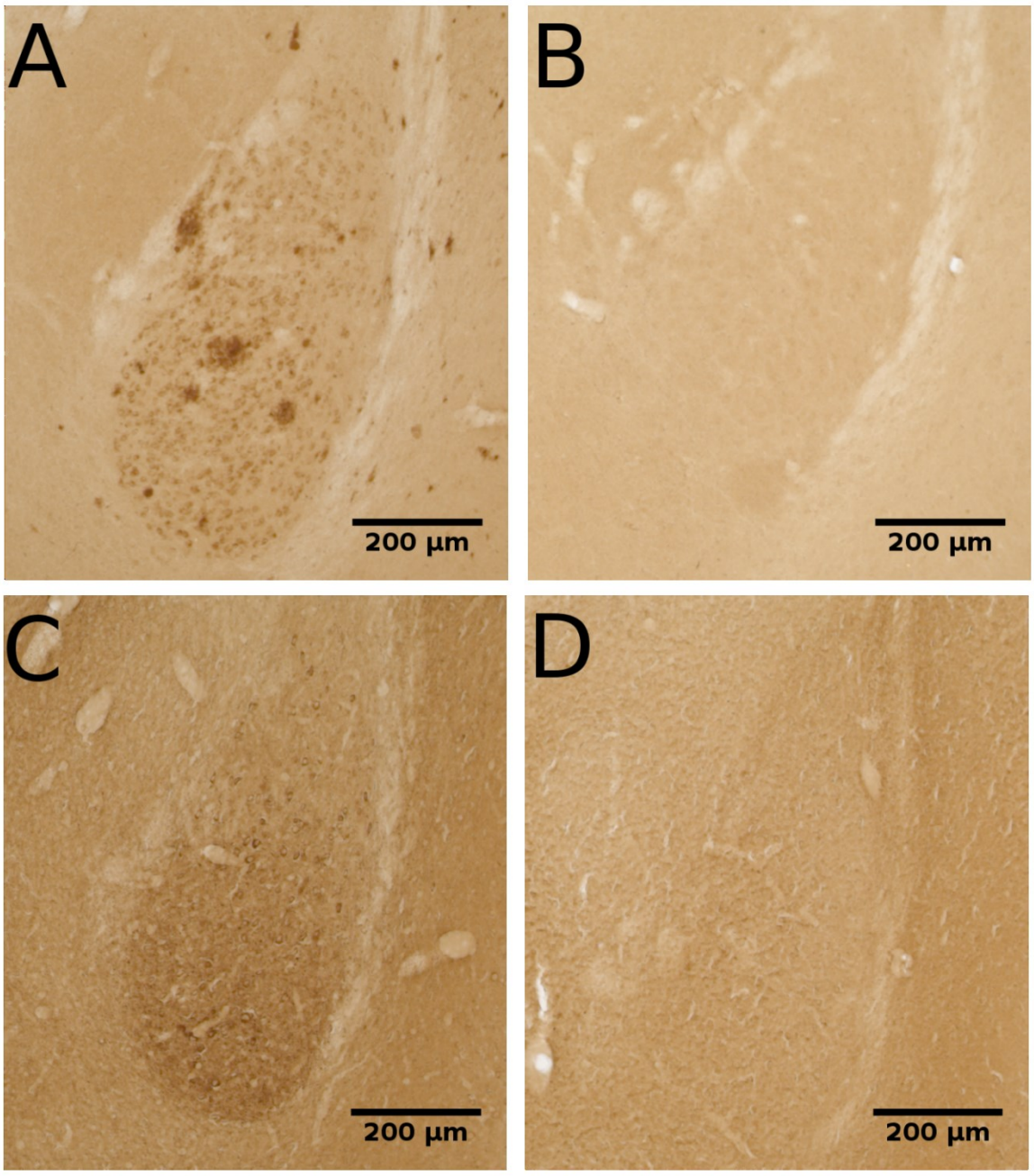

Figure 2.8 Amygdala Immunohistochemistry. Example sections of 19 month old 3xTg-AD mice strained for amyloid beta (A) or tau (C) and B6129SF2 control mice strained for amyloid beta (B) or tau (D).

2.4.5 RELATIONSHIP OF NEUROPATHOLOGY AND BEHAVIOUR

We sought to determine if there was a relationship between neuropathology and behaviour using Pearson's r correlations between levels of neuropathology and behavioural measures with a genotype difference. There were no significant correlations between any of the behavioural measures and levels of neuropathology (all $p > 0.05$, Supplemental Table 2.5). This may be due to our relativity low sample size at 19 months of age, or there may not be enough verifiability in either neuropathology or behaviour among the mice at 18 months of age for there to be a correlation between the two measures.

2.4.6 EFFECT SIZE COMPARISON

To compare the effects across behavioural measures we calculated Cohen's d with a Hedge's correction for measures with differences in pup genotype, sex, or foster mother genotype. For pup genotype effects the largest difference in behaviour was the decreased percentage of time that the 3xTg-AD mice spent in the closed arms of the EPM relative to B6129SF2 control mice (d_{unb}= -1.035, CI_{95}= -0.870 – -1.291, Table 2.2), followed by the increased latency to fall from the Rotarod of the 3xTg-AD relative to B6129SF2 control mice (d_{unb}= 0.846, CI_{95}= 0.734 – 0.957, Table 2.2).

Table 2.2 Genotype Effect Size Estimates. Calculated with a pooled SD and Hedges correction for models including genotype with CI's that provided evidence for an effect. Positive values indicate 3xTg-AD mice had higher scores than B6129SF2 wildtype mice and negative scores indicate B6129SF2 wildtype mice had higher scores than 3xTg-AD mice. A '#' indicates that the confidence interval includes zero.

Measure	d_{unb}	95% Confidence Interval	
3xTg-AD higher than B6129SF2		Lower	Upper
Rotarod - Latency	0.846	0.734	0.957
Open Field - Center Time	0.408	0.166	0.649
Open Field - Center Entries	0.347	0.106	0.589
Open Field - Center Rears	0.214	-0.026	0.454 #
B6129SF2 higher than 3xTg-AD			
Elevated Plus Maze - Time Closed Arms	-1.035	-1.291	-0.780
Elevated Plus Maze - % Distance Closed Arms	-0.784	-1.032	-0.535
Elevated Plus Maze - Distance	-0.666	-0.913	-0.420
Elevated Plus Maze - Grooming	-0.462	-0.705	-0.219
Open Field - Grooming	-0.433	-0.675	-0.191
Open Field - Rears	-0.253	-0.493	-0.012

The effect sizes for sex differences were generally lower than for genotype: the largest effect size was the increased latency to fall from the Rotarod in female mice relative to male mice (d_{unb}= 0.329 CI_{95}= 0.221 – 0.437, Table 2.3).

Table 2.3 Sex Effect Size Estimates. Calculated with a pooled SD and Hedges correction for models including sex with CI's that provided evidence for an effect. Positive values indicate female mice had higher scores than male mice and negative scores indicate male mice had higher scores than female mice. A '#' indicates that the confidence interval includes zero.

Measure	d_{unb}	95% Confidence Interval	
Female higher than Male		Lower	Upper
Rotarod - Latency	0.329	0.221	0.437
Elevated Plus Maze - Distance Closed Arms	0.220	-0.021	0.461 #
Open Field - Rears	0.176	-0.064	0.417 #
Elevated Plus Maze - Time Closed Arms	0.114	-0.127	0.355 #
Male higher than Female			
Elevated Plus Maze - Grooming	-0.259	-0.501	-0.018
Open Field - Center Rears	-0.146	-0.386	0.095 #

The effect sizes for foster mother genotype were generally lower than both pup genotype and sex, the largest with a confidence interval that did not include 0 was for the number of rears in the OF, which was higher in mice reared by 3xTg-AD mothers (d_{unb}= 0.348, CI_{95}= 0.107 – 0.589, Table 2.4). Figure 2.9 gives a visual image of the effect sizes for pup genotype, sex, and maternal genotype.

Table 2.4 Foster Mother Genotype Effect Size Estimates. Calculated with a pooled SD and Hedges correction for models including sex with CI's that provided evidence for an effect. Positive values indicate mice reared by 3xTg-AD mothers had higher scores than mice reared by B6129SF2 mothers and negative scores indicate mice reared by B6129SF2 had higher scores than mice reared by 3xTg-AD mothers. A '#' indicates that the confidence interval includes zero

Measure	d_{unb}	95% Confidence Interval	
3xTg-AD Mothers higher than B6129SF2 Mothers		Lower	Upper
Neuropathology - Amygdala Tau	1.318	-0.087	2.724 #
Open Field - Rearing	0.348	0.107	0.589
B6129SF2 Mothers higher than 3xTg-AD Mothers			
Open Field - Freezing	-0.318	-0.559	-0.078
Rotarod Latency	-0.303	-0.411	-0.196
Open Field - Grooming	-0.173	-0.413	0.067 #
Elevated Plus Maze - Distance	-0.167	-0.407	0.073 #

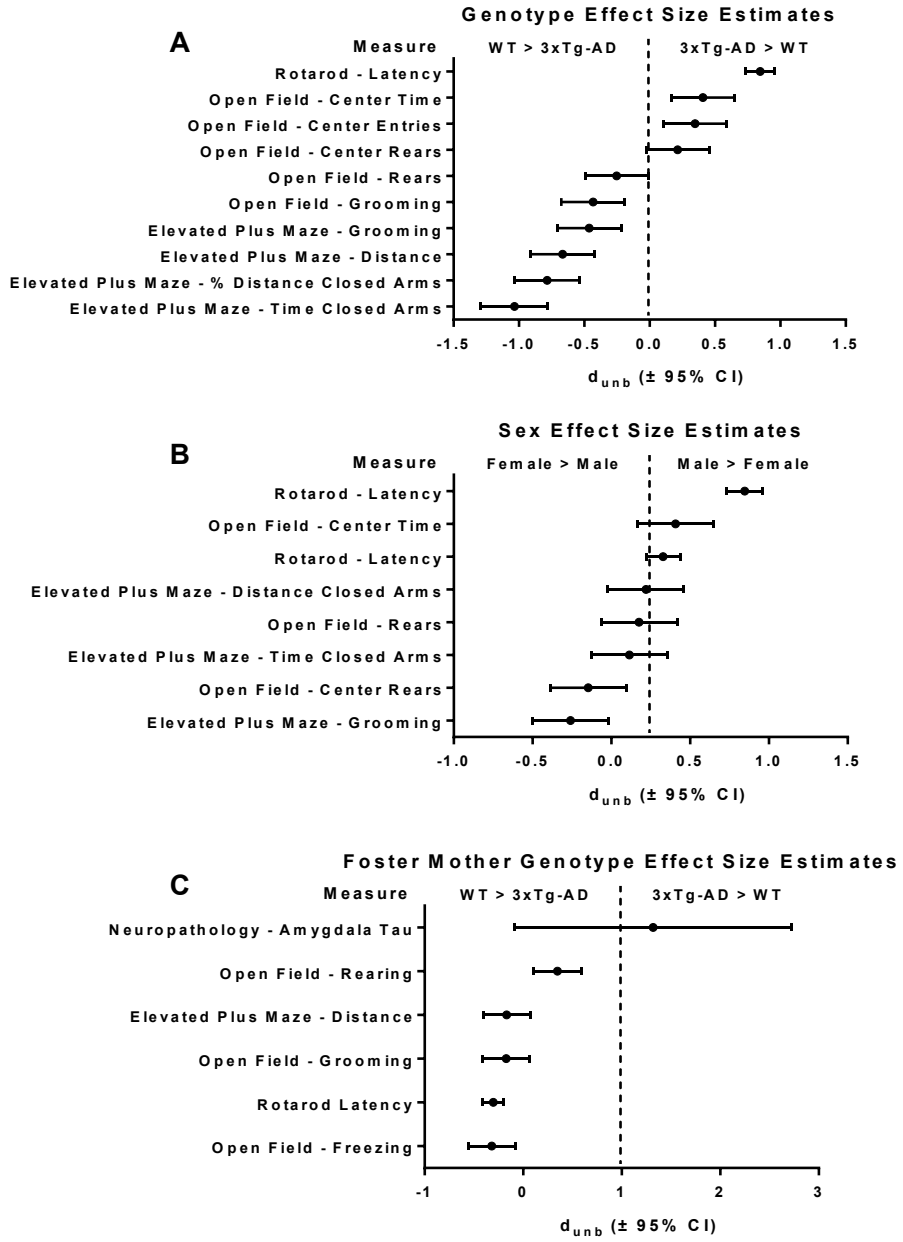

Figure 2.9 Effect Size Estimates. The Cohen's d effect size estimates with a Hedge's correction and 95% confidence intervals. The vertical dotted line represents an effect size of 0 (no difference between groups). For genotype (A), positive values indicate that 3xTg-AD mice had higher scores than B6129SF2 (WT) wildtype mice. For sex (B) positive values indicate that female mice had higher scores than male mice, and for foster mother genotype (C) positive values indicate mice reared by 3xTg-AD mothers had higher scores than mice reared by B6129SF2 (WT) mothers.

2.5 DISCUSSION

2.5.1 DO 3XTG-AD AND B6129SF2 MICE DIFFER IN ANXIETY-LIKE BEHAVIOUR ACROSS AGES?

The 3xTg-AD exhibited less anxiety-like behaviour than B6129SF2 control mice. In the EPM the 3xTg-AD spent less time and travelled less of their total distance in the closed arms than B619SF2 mice. There was a high level of variability in freezing behaviour and so it is difficult to draw any conclusions based on the results. In the open field the 3xTg-AD mice exhibited less anxiety-like behaviour than the B6129SF2 mice as they entered the center of the OF more often and reared more in the center of the maze.

There is a great deal of variability in the measurement of anxiety-like behaviours both within and between laboratories (Crabbe et al., 1999; Wahlsten et al., 2003, 2006), so we chose to conduct two tests of anxiety-like and locomotor behaviour to ensure our results were consistent at least within our laboratory. Different tests of anxiety measure different aspects of anxiety-like behaviour in mice, and so the measures interpreted as anxiety-like behaviour should be carefully chosen (O'Leary et al., 2013). Taking into consideration the consistent results between the OF and EPM it appears that the 3xTg-AD mice exhibit less anxiety-like behaviour in both the EPM and OF and this difference appears to persist with age, which is supported by some previous findings (Blanchard et al., 2010; Chen et al., 2013). The decreased anxiety-like behaviour of the 3xTg-AD in the EPM has a large effect size, and in the OF there is a moderate effect size (Table 2.2), which further supports the idea that the 3xTg-AD have decreased anxiety-like behaviour. The anxiety-like behaviour could be explained by behavioural disinhibition, another symptom of AD, and several other mouse models of AD have reduced anxiety-like

behaviour (Lalonde et al., 2004; Ognibene et al., 2005; Jawhar et al., 2012), so reduced anxiety-like behaviour may still be the manifestation of AD like symptoms in a mouse model.

2.5.2 DO 3XTG-AD AND B6129SF2 MICE DIFFER IN LOCOMOTOR BEHAVIOUR ACROSS AGES?

In the elevated plus maze the B6129SF2 mice exhibited more locomotor behaviour, travelling a greater distance than the 3xTg-AD mice, though this difference decreased with age. There was no genotype difference in locomotor activity in the open field, so it is difficult to draw an overall conclusion about genotype differences in locomotor behaviour. The lack of a consistent difference in locomotor behaviour between these two tests may be related to the nature of the tests, given that they have completely different apparatus, or the amount of variability in the repeated testing of the mice in these tasks.

2.5.3 DO 3XTG-AD AND B6129SF2 MICE DIFFER IN MOTOR COORDINATION AND MOTOR LEARNING ACROSS AGES?

The 3xTg-AD mice had enhanced performance on the Rotarod compared to the B6129SF2 mice. The latency to fall increased from 2 to 12 months of age, then began to decrease at 18 months of age. At 18 months of age there were a number of interactions; the sex effect disappeared in B6129SF2 mice and in mice reared by 3xTg-AD mothers. Our finding of enhanced motor performance in the 3xTg-AD had a large effect size (Table 2.2). The enhanced motor performance of the 3xTg-AD on the Rotarod is a robust difference, as it has been reported by several researchers, even using 3xTg-AD mice that have been backcrossed to a C57BL/6J strain, which indicates the effect is likely a result

of the transgenes (Blanchard et al., 2010; Filali et al., 2012; Chen et al., 2013; Oore et al., 2013). The only dissenting report is from Sterniczuk et al. (2010a), who used the C57BL/6J control strain, which may explain their finding, as there are strain differences in motor behaviour. In a previous study we examined the motor behaviour of 6 month old 3xTg-AD mice in detail (Stover et al., 2015a), and found enhanced performance on the Rotarod but a deficit in the grid suspension task in 3xTg-AD mice relative to B6129SF2 mice, which indicated the motor phenotype of these mice is more complex than a simple enhancement of motor behaviour.

2.5.4 ARE THERE SEX DIFFERENCES IN BEHAVIOUR IN 3XTG-AD AND B6129SF2 MICE?

Males exhibited less anxiety-like behaviour as they spent less time in the closed arms in the EPM, though for the distance in the closed arms the effect was only present in the 3xTg-AD mice. Female mice performed better than male mice on the Rotarod, which indicates they have enhanced motor behaviour, and although female mice weighed less than male mice this did not account for their enhanced performance.

2.5.5 IS ADULT LOCOMOTOR BEHAVIOUR RELATED TO LOCOMOTOR BEHAVIOUR DURING DEVELOPMENT?

None of the best models for any locomotor activity contained the locomotor activity from the automated OF during development, which indicates there is no relationship between locomotor activity before weaning and later in life.

2.5.6 DOES MATERNAL GENOTYPE EFFECT BEHAVIOUR IN ADULT MICE?

There was some evidence that mice reared by 3xTg-AD mothers exhibited less anxiety-like behaviour than mice reared by B6129SF2 mothers, as they entered the center of the OF more often, and spent less time freezing. Mice reared by B6129SF2 mothers travelled a greater distance than those reared by B6129SF2 mice in the EPM, but not the OF, which may indicate they have a higher level of anxiety, but without consistent results this is difficult to confirm. In the Rotarod the mice reared by B6129SF2 mothers had a better motor performance than mice reared by 3xTg-AD mothers.

2.5.7 IS THERE A RELATIONSHIP BETWEEN NEUROPATHOLOGY AND BEHAVIOUR?

The only difference in neuropathology we observed was an increase in the density of tau positive neurons in the amygdala of mice reared by 3xTg-AD mothers compared to mice reared by B6129SF2 mothers, which had a large effect size, though the range of the 95% confidence interval included 0, so caution should be used when interpreting the effect size. We found no correlations between neuropathology levels and behaviour. It does not appear that maternal genotype had a large effect on neuropathology at 19 months of age, possibly because the effect was small enough that it was masked by age or genotype effects.

2.5.8 GENERAL DISCUSSION AND CONCLUSIONS

The mice seem to habituate to the mazes over time as distance travelled and other locomotor behaviours decreased with age, however the effect of habituation is impossible to separate from the effect of age due to the longitudinal design. Interestingly there seems

to be a shift in behaviour at 18 months of age with a greater number of interactions occurring, though with no overall pattern of differences. This shift could be the result of a general increase in variability at 18 months of age, or the result of a survivor effect, as the number of surviving mice decreased by a third from 12 to 18 months of age, and these mice may be differnet in some way, possibly with lower levels of neuropathology or other differences that contributed to their longer lives.

An issue that must be considered in longitudinal behaviour studies is the effect of previous experience on later test scores. For example, the tests of anxiety-like behaviour often rely on the aversive nature of the open aspects of a maze (the open arms of the EPM or the center of the OF), but animals may become habituated with repeated exposure, so habitation could confound the results. In the current study we have attributed general changes over time to habituation in the case of the tests of anxiety-like behaviour, and with motor learning in the Rotarod. Another issue is with the statistical analysis: In our long-term longitudinal study almost 40% of the animals died before the final time point. In a traditional repeated measures ANOVA with age as a factor those animals would have to be excluded due to missing data, which is why we chose to use linear mixed effects modeling, which does not require each mouse to have a value at each time point. Despite these issues, longitudinal studies are the only way to measures changes in behaviour in the same animals over time, which is important for tracking the changes caused by both the transgenes and early-life environment.

In summary, we found that the 3xTg-AD mouse model of AD has decreased anxiety-like behaviour on the EPM and OF, enhanced motor performance on the Rotarod. These findings appear to be relatively stable across the lifespan, with some evidence of

change beginning at 18 months of age. Future studies on mice older than 18 months would be useful but the relatively high mortality in the transgenic mice poses a problem. The only difference that we found in neuropathology was an increased density of tau positive neurons in the amygdala of mice reared by 3xTg-AD, but no differences in the hippocampus or in amyloid beta staining. It is unclear why there would be a difference in the amygdala and not the hippocampus, it is possible that we were unable to detect the effect in the hippocampus due to our low sample size. We found that females had better performance on the Rotarod than males, both of which had moderate effect sizes, but little evidence of a sex difference in anxiety-like behaviour. While there were several individual effects of foster mother genotype on measures in the four tasks that we conducted there were few consistent or stable findings, though the mice reared by B6129SF2 mothers had a better motor performance on the Rotarod than mice reared by 3xTg-AD mothers, which contradicts our previous finding of decayed development of motor reflexes in mice reared by B6129SF2 mothers, and the effect size was fairly small. Overall it appears that maternal genotype had little lasting effect on pup behaviour, but that the 3xTg-AD decreased anxiety-like behaviour and enhanced performance on the Rotarod compared to B6129SF2 mice.

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2.8 SUPPLEMENTAL TABLES

Description of supplemental tables 2.1 – 2.4.

These supplemental tables provide the statistics used to determine the best model for each measure. The best five models for each measure are included. The model column describes the factors analyzed, terms separated by a '*' indicate both individual main effects and interactions between those two terms, terms separated by a '+' are both simple main effects, and terms separated by a ':' indicate an interaction alone. The 'AICc' column is the second order Akaike information criterion, which is a measure used to evaluate the models based on the complexity and how well the model fits the data; lower values are better. The "Δ AICc" column provides the difference between the given model's AICc and the model with the lowest AICc. The 'Wt' column is the Akaike weight, a measure of relative likelihood that the fit is the best, ranging from 0 (unlikely) – 1(likely). The 'ER' column is the evidence ratio which provides the likelihood that the model with the lowest AICc is better than the model in question.

Supplemental Table 2.1.1 Elevated Plus Maze – Distance

Model	AICc	Δ AICc	Wt	ER
(Genotype+FMGenotype+Sex+Age)+(Genotype:FMGenotype)+(Genotype:Sex) +(FMGenotype:Sex)+(Genotype:Age)	4064.248	0	0.063	1
(Genotype+FMGenotype+Sex+Age)+(Genotype:FMGenotype:Sex)+(Genotype:Age)	4064.964	0.715	0.044	1.430
(Genotype+FMGenotype+Sex+Age)+(Genotype:Sex)+(FMGenotype:Sex) +(Genotype:FMGenotype:Sex)+(Genotype:Age)	4064.964	0.715	0.044	1.430
(Genotype+FMGenotype+Sex+Age)+(Genotype:FMGenotype)+(Genotype:Sex) +(FMGenotype:Sex)+(Genotype:FMGenotype:Sex)+(Genotype:Age)	4064.964	0.715	0.044	1.430
(Genotype+FMGenotype+Sex+Age)+(Genotype:FMGenotype)+(FMGenotype:Sex) +(Genotype:FMGenotype:Sex)+(Genotype:Age)	4064.964	0.715	0.044	1.430

Supplemental Table 2.1.2 Elevated Plus Maze – Number of Rears

Model	AICc	Δ AICc	Wt	ER
Age	1451.552	0	0.330	1
Sex+Age	1453.446	1.894	0.128	2.578
FMGenotype+Age	1453.452	1.900	0.128	2.585
Genotype+Age	1453.645	2.093	0.116	2.848
FMGenotype+Sex+Age	1455.367	3.815	0.049	6.737

Supplemental Table 2.1.3 Elevated Plus Maze – Number of Head Dips

Model	AICc	Δ AICc	Wt	ER
(Genotype+Age)+(Genotype:Age)	1985.685	0	0.198	1
(Genotype+Sex+Age)+(Genotype:Age)	1986.346	0.661	0.143	1.392
(Genotype+Sex+Age)	1987.013	1.328	0.102	1.943
(Genotype+Age)	1987.148	1.463	0.096	2.078
(Genotype+FMGenotype+Age)+(Genotype:FMGenotype)+(Genotype:Age)	1987.621	1.936	0.075	2.633

Supplemental Table 2.1.4 Elevated Plus Maze – Distance in Closed Arms

Model	AICc	Δ AICc	Wt	ER
Genotype+Sex+Genotype:Sex	2417.806	0	0.328	1
Genotype+Sex	2419.597	1.791	0.134	2.449
(Genotype+FMGenotype+Sex)+(Genotype:Sex)	2419.772	1.966	0.123	2.673
(Genotype+FMGenotype+Sex)+(Genotype:FMGenotype)+(Genotype:Sex)	2421.298	3.492	0.057	5.732
Genotype+FMGenotype+Sex	2421.687	3.882	0.047	6.965

Supplemental Table 2.1.5 Elevated Plus Maze – Time in Closed Arms

Model	AICc	Δ AICc	Wt	ER
Genotype+Sex	2440.189	0	0.247	1
(Genotype+Sex)+(Genotype:Sex)	2441.610	1.421	0.121	2.035
Genotype	2442.016	1.827	0.099	2.493
(Genotype+FMGenotype+Sex)	2442.145	1.956	0.093	2.659
(Genotype+FMGenotype+Sex)+(Genotype:FMGenotype)	2443.579	3.390	0.045	5.446

Supplemental Table 2.1.6 Elevated Plus Maze – Time Spent Freezing

Model	AICc	Δ AICc	Wt	ER
(Sex+Age)+(Sex:Age)	2245.262	0	0.128	1
(FMGenotype+Sex+Age)+(Sex:Age)	2246.168	0.906	0.081	1.573
(Genotype+Sex+Age)+(Sex:Age)	2246.319	1.058	0.076	1.697
(FMGenotype+Sex+Age)+(FMGenotype:Sex)+(Sex:Age)	2247.057	1.796	0.052	2.454
(Genotype+FMGenotype+Sex+Age)+(Genotype:FMGenotype)+(FMGenotype:Sex) +(Sex:Age)	2247.071	1.809	0.052	2.471

Supplemental Table 2.1.7 Elevated Plus Maze – Number of SAPs

Model	AICc	Δ AICc	Wt	ER
Age	1138.767	0	0.083	1
FMGenotype+Age	1139.566	0.799	0.056	1.491
(Genotype+Age)+(Genotype:Age)	1139.942	1.175	0.046	1.799
Sex+Age	1140.604	1.837	0.033	2.505
Genotype+Age	1140.749	1.982	0.031	2.694

Supplemental Table 2.1.8 Elevated Plus Maze – Time Spent Grooming

Model	AICc	Δ AICc	Wt	ER
(Genotype+Sex+Age)+(Genotype:Sex)+(Genotype:Age)	1878.803	0	0.085	1
(Genotype+Age)+(Genotype:Age)	1879.328	0.525	0.065	1.300
(Genotype+Sex+Age)+(Sex:Age)	1879.690	0.887	0.054	1.558
(Genotype+Sex+Age)+(Genotype:Sex)+(Sex:Age)	1879.783	0.980	0.052	1.633
(Genotype+Sex+Age)+(Genotype:Sex)+(Genotype:Age)+(Sex:Age)	1879.867	1.064	0.050	1.702

Supplemental Table 2.2.1 Open Field – Distance Travelled

Model	AICc	Δ AICc	Wt	ER
Age	4354.535	0	0.134	1
(Sex+Age)	4355.159	0.624	0.098	1.366
(FMGenotype+Age)	4355.699	1.164	0.075	1.790
(Genotype+Age)	4356.295	1.760	0.056	2.411
(FMGenotype+Sex+Age)	4356.350	1.815	0.054	2.478

Supplemental Table 2.2.2 Open Field – Number of Rears

Model	AICc	Δ AICc	Wt	ER
(Genotype+FMGenotype+Sex+Age)+(Genotype:Age)+(Sex:Age)	1886.184	0	0.090	1
(Genotype+FMGenotype+Sex+Age)+(Genotype:Age)+(FMGenotype:Age)+(Sex:Age)	1887.151	0.967	0.056	1.622
(Genotype+FMGenotype+Sex+Age)+(Genotype:Sex)+(Genotype:Age)+(Sex:Age)	1887.532	1.349	0.046	1.963
(Genotype+FMGenotype+Sex+Age)+(Genotype:FMGenotype)+(Genotype:Age)+(Sex:Age)	1888.373	2.189	0.030	2.988
(Genotype+FMGenotype+Sex+Age)+(FMGenotype:Sex)+(Genotype:Age)+(Sex:Age)	1888.441	2.257	0.029	3.091

Supplemental Table 2.2.3 Open Field – Center Entries

Model	AICc	Δ AICc	Wt	ER
(Genotype+FMGenotype+Age)+(Genotype:Age)+(FMGenotype:Age)	914.622	0	0.159	1
(Genotype+FMGenotype+Sex+Age)+(Genotype:Age)+(FMGenotype:Age)	916.704	2.082	0.056	2.832
(Genotype+FMGenotype+Sex+Age)+(FMGenotype:Sex)+(Genotype:Age)+ (FMGenotype:Age)	916.798	2.177	0.054	2.969
(Genotype+FMGenotype+Age)+(Genotype:FMGenotype)+(Genotype:Age)+ (FMGenotype:Age)	916.861	2.240	0.052	3.064
(Genotype+FMGenotype+Age)+(Genotype:Age)	916.969	2.348	0.049	3.234

Supplemental Table 2.2.4 Open Field – Time in Center

Model	AICc	Δ AICc	Wt	ER
(Genotype*Age)	1210.696	0	0.230	1
(Genotype+Age)	1212.412	1.716	0.097	2.359
(Genotype+FMGenotype+Age)+(Genotype:Age)	1212.427	1.731	0.097	2.377
(Genotype+Sex+Age)+(Genotype:Age)	1212.732	2.036	0.083	2.768
(Genotype+FMGenotype+Age)+(Genotype:FMGenotype)+(Genotype:Age)	1213.184	2.489	0.066	3.471

Supplemental Table 2.2.5 Open Field – Center Rears

Model	AICc	Δ AICc	Wt	ER
(Genotype+FMGenotype+Sex+Age)+(FMGenotype:Age)+(Sex:Age)	1102.712	0	0.038	1
(Genotype+FMGenotype*Sex*Age)	1102.964	0.253	0.033	1.135
(Genotype+FMGenotype+Sex+Age)+(Genotype:Sex)+(FMGenotype:Age)+(Sex:Age)	1103.658	0.947	0.024	1.605
(Genotype+FMGenotype+Sex+Age)+(FMGenotype:Sex)+(FMGenotype:Age)+(Sex:Age)	1104.097	1.386	0.019	1.999
(Genotype+FMGenotype*Sex*Age)+(Genotype:Sex)	1104.131	1.420	0.019	2.033

Supplemental Table 2.2.6 Open Field – Time Spent Freezing

Model	AICc	Δ AICc	Wt	ER
FMGenotype	2159.739	0	0.175	1
FMGenotype+Age	2160.732	0.992	0.107	1.642
FMGenotype+Sex	2161.711	1.971	0.065	2.680
Genotype+FMGenotype	2161.732	1.992	0.065	2.708
Genotype+FMGenotype+Age	2162.708	2.969	0.040	4.412

Supplemental Table 2.2.7 Open Field – Number of stretch Attend Postures

Model	AICc	Δ AICc	Wt	ER
Age	1139.625	0	0.239	1
Genotype+Age	1140.864	1.240	0.128	1.859
Sex+Age	1141.296	1.672	0.103	2.307
FMGenotype+Age	1141.728	2.103	0.083	2.862
Genotype+Sex+Age	1142.382	2.757	0.060	3.969

Supplemental Table 2.2.8 Open Field – Time Spent Grooming

Model	AICc	Δ AICc	Wt	ER
(Genotype+FMGenotype+Sex+Age)+(Genotype:Sex)+(FMGenotype:Sex)	1912.996	0	0.064	1
(Genotype+Sex+Age)+(Genotype:Sex)	1913.866	0.870	0.041	1.545
(Genotype*FMGenotype*Sex+Age)	1914.260	1.264	0.034	1.881
(Genotype+FMGenotype+Sex+Age)+(Genotype:FMGenotype)+(Genotype:Sex) +(FMGenotype:Sex)	1914.324	1.328	0.033	1.943
(Genotype+FMGenotype+Sex+Age)+(Genotype:Sex)+(FMGenotype:Sex)+(Sex:Age)	1914.618	1.622	0.028	2.250

Supplemental Table 2.3 Body Weight

Model	AICc	Δ AICc	Wt	ER
(Genotype+FMGenotype+Sex+Age)+(Genotype:Sex)+(Genotype:Age)+ (FMGenotype:Sex:Age)	1539.390	0.000	0.039	1.000
(Genotype+FMGenotype+Sex+Age)+(Genotype:FMGenotype)+(Genotype:Sex)+ (Genotype:Age+(FMGenotype:Sex:Age)	1541.768	2.378	0.012	3.284
(Genotype+FMGenotype+Sex+Age)+(Genotype:Sex)+(Sex:Age)+ (Genotype:Sex:Age)+(FMGenotype:Sex:Age)	1541.778	2.388	0.012	3.301
(Genotype+FMGenotype+Sex+Age)+(Genotype:Age)+(FMGenotype:Sex:Age)	1542.193	2.803	0.010	4.061
(Genotype+FMGenotype+Sex+Age)+(Genotype:FMGenotype:Sex)+ (Genotype:Age)+(FMGenotype:Sex:Age)	1544.002	4.611	0.004	10.032

Supplemental Table 2.4 Rotarod - Latency to Fall

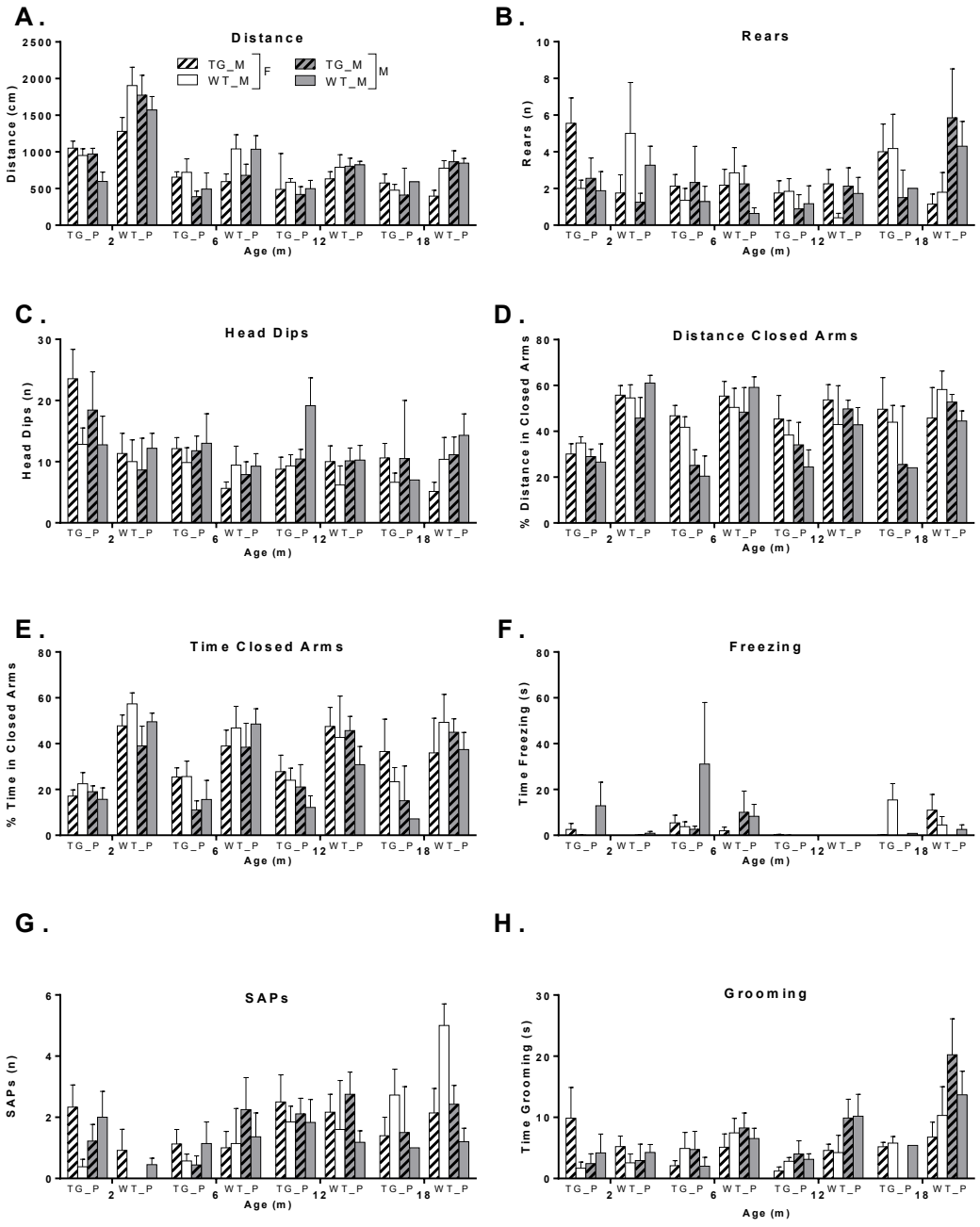
Model	AICc	Δ AICc	Wt	ER
(Day+Genotype+FMGenotype+Sex+Age)+(Day:Genotype)+(Day:Age)+ (Genotype:Sex:Age)+(FMGenotype:Sex:Age)	13771.900	0	0.004	1
(Day+Genotype+FMGenotype+Sex+Age)+(Day:Genotype:Sex)+(Day:Age)+ (Genotype:Sex:Age)+(FMGenotype:Sex:Age)	13773.239	1.339	0.002	1.953
(Day+Genotype+FMGenotype+Sex+Age)+(Day:Genotype)+(Day:Sex)+(Day:Age)+ (Genotype:Sex:Age)+(FMGenotype:Sex:Age)	13773.321	1.421	0.002	2.035
(Day+Genotype+FMGenotype+Sex+Age)+(Day:Genotype)+ (Genotype:FMGenotype)+(Day:Age)+(Genotype:Sex:Age)+(FMGenotype:Sex:Age)	13774.009	2.110	0.001	2.872
(Day+Genotype+FMGenotype+Sex+Age)+(Day:Genotype)+(Day:Age)+ (Genotype:FMGenotype:Age)+(Genotype:Sex:Age)+(FMGenotype:Sex:Age)	13775.141	3.242	0.001	5.058

Supplemental Table 2.5 Correlations of Neuropathology and Behavior. Pearson's-r scores for correlations between measures levels of amyloid beta and tau in the brain of 3xTg-AD mice and behavioral measures with a genotype difference at 18 months of age. A '*' indicates a significant correlation ($p < 0.05$).

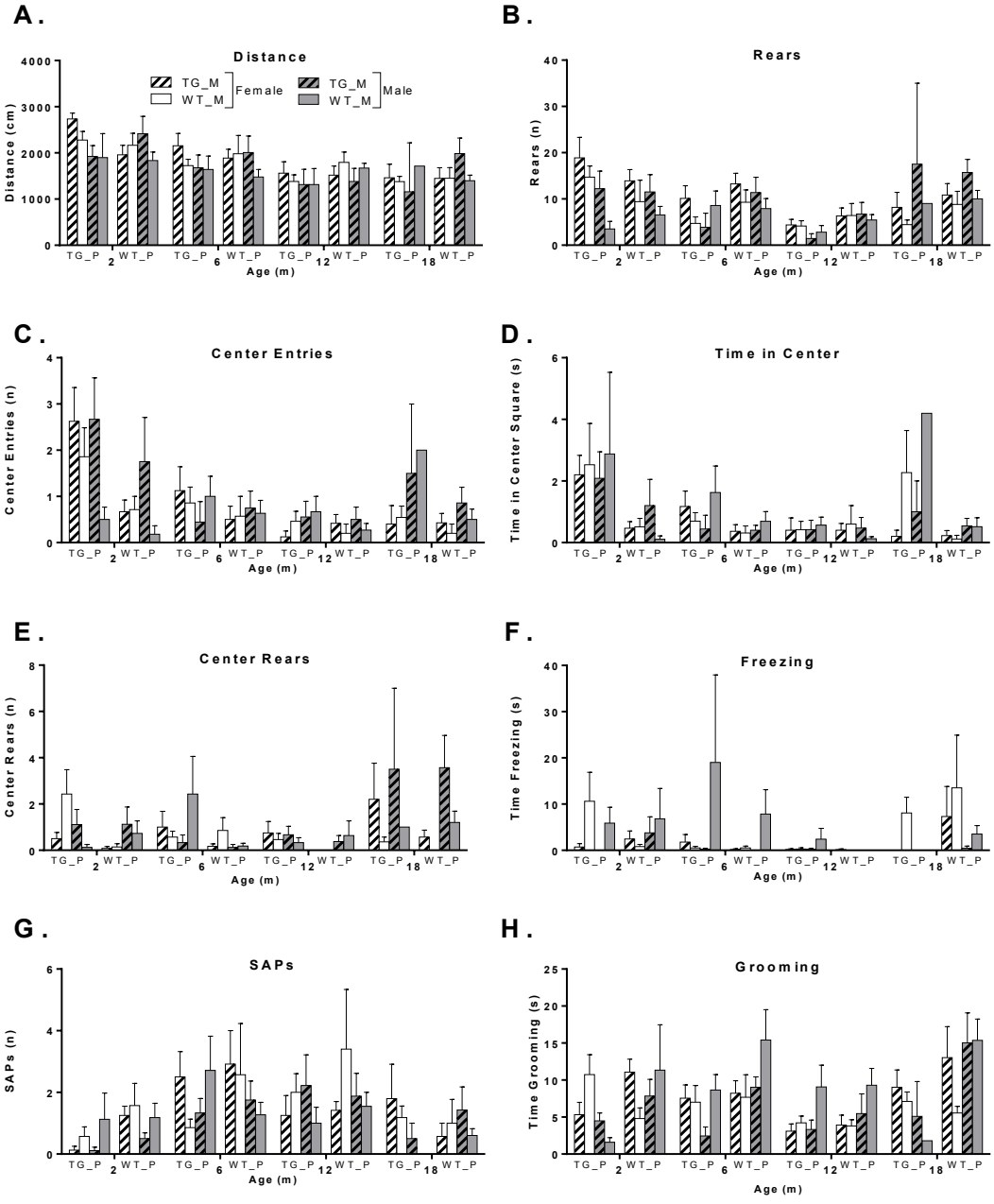
	Elevated Plus Maze			Rotarod
Neuropathology Measure	**Time Closed Arms (s)**	**Distance (cm)**	**Groom (s)**	**Latency (s)**
Hippocampus Tau Density (#/μm^3)	0.264	0.077	0.071	-0.005
Amygdala Tau Density (#/μm3)	0.487	0.407	0.092	-0.174
Hippocampus Aβ Coverage (%)	0.264	-0.303	0.256	-0.314
Amygdala Aβ Coverage (%)	0.201	-0.521	-0.307	-0.475
Cortex Aβ Coverage (%)	-0.083	-0.536	-0.263	0.013

	Open Field			
Neuropathology Measure	**Rear (n)**	**Center Rear (n)**	**Groom (s)**	**Center (s)**
Hippocampus Tau Density (#/μm^3)	0.055	0.236	0.256	-0.218
Amygdala Tau Density (#/μm3)	0.415	0.530	0.294	-0.021
Hippocampus Aβ Coverage (%)	-0.348	-0.385	0.205	-0.204
Amygdala Aβ Coverage (%)	-0.459	-0.371	-0.198	-0.189
Cortex Aβ Coverage (%)	-0.435	-0.282	-0.056	-0.354

2.9 SUPPLEMENTAL FIGURES



Supplemental Figure 2.1 The Elevated Plus Maze. The mean (± S.E.M) total distance travelled (A), number of rears (B) and head dips (C), percentage of distance (D) and time (E) spent in the closed arms, the time spent freezing (F), number of stretch attend postures (G) and time spent grooming (H) of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers in the elevated plus maze.



Supplemental Figure 2.2 The Open Field. The mean (± S.E.M) total distance travelled (A), number of rears (B), center entries (C), time spent in the center (D), number of center rears (E), time spent freezing (F), number of stretch attend postures (G), and time spent grooming (H) of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers in the open field.

CHAPTER 3 AGE-RELATED CHANGES IN ACOUSTIC STARTLE AND PREPULSE INHIBITION IN THE 3XTG-AD MOUSE MODEL OF ALZHEIMER'S DISEASE: A LONGITUDINAL STUDY

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3.1 ABSTRACT

Prepulse inhibition (PPI) occurs when a weak prepulse causes the inhibition of the startle response to a stronger stimulus. Deficits in PPI are associated with deficits in sensorimotor gating, and occur in schizophrenia and other neuropsychiatric disorders. The 3xTg-AD transgenic mouse model of Alzheimer's disease (AD) has three transgenes, two associated with familial AD and one with frontotemporal dementia and is commonly used in AD research as it develops amyloid beta plaques, neurofibrillary tangles, and cognitive deficits. The 3xTg-AD mice have been reported to have an increase in acoustic startle response and a decrease in prepulse inhibition relative to B6129SF2 control mice at 7 months of age. We tested male and female 3xTg-AD and B6129SF2 mice in acoustic startle and PPI in a longitudinal study at 2, 6, 12, and 18 months of age. Female 3xTg-AD mice had a larger acoustic startle response than males, and the magnitude of acoustic startle increased from 2 to 6 months of age, then decreased. All mice exhibited evidence of PPI, and at the highest prepulse intensity the 3xTg-AD mice exhibited more PPI than B6129SF2 mice. The amount of PPI increased with age for all mice. Overall there was an increase in acoustic startle response and no deficit in sensorimotor gating in 3xTg-AD mice relative to B6129SF2 control mice.

3.2 INTRODUCTION

The 3xTg-AD mouse model of Alzheimer's disease (AD) has three transgenes, two associated with familial AD and one with frontotemporal dementia. This strain is commonly used in AD research as it develops amyloid beta plaques, neurofibrillary tangles, and cognitive deficits. The breeding system requires that the 3xTg-AD females be bred with 3xTg-AD males and the wildtype control (B6129SF2) mice be bred with other wildtype mice. This results in the 3xTg-AD mice being be reared by 3xTg-AD mothers, and B6129SF2 controls being reared by B6129SF2 mothers (Oddo et al., 2003). Because differences in maternal care or early life environment can have a lasting effect on brain and behaviour (Priebe et al., 2005; Szyf et al., 2007), we cross-fostered litters of 3xTg-AD and B6129SF2 pups to create mixed genotype litters and assessed the development of reflexes, learning, memory, and activity before weaning (see Blaney et al., 2013). The present study examines the long-term effects of maternal genotype on acoustic startle and prepulse inhibition of startle in these mice.

Prepulse inhibition (PPI) occurs when a weak prepulse causes the inhibition of the startle response to a stronger stimulus. Prepulse inhibition is conserved across species; both humans and mice exhibit PPI (Geyer et al., 2002). Deficits in PPI are associated with deficits in sensorimotor gating, and occur in schizophrenia and other neuropsychiatric disorders, though there are mixed reports about PPI impairments in AD (Braff et al., 1978; Hejl et al., 2004; Ueki et al., 2006). Some transgenic mouse models of AD are reported to have deficits in prepulse inhibition (McCool et al., 2003; Esposito et al., 2006). In the only studies on 3xTg-AD mice, Pietropaolo et al., (2008) found an increased startle response at 6 months of age in male and female 3xTg-AD mice relative

to control mice, and García-Mesa et al. (2011) found an increase in acoustic startle response and a decrease in prepulse inhibition in male and female 3xTg-AD mice relative to B6129SF2 control mice at 7 months of age. To determine if there were maternal effects and age-related changes in acoustic startle and PPI in male and female 3xTg-AD mice, we conducted a longitudinal study to assess the effect of pup genotype, maternal genotype, sex, and age at 2, 6, 12, and 18 months of age.

3.3 METHODS

3.3.1 BREEDING, CROSS-FOSTERING & PRE-WEANING TREATMENT OF MICE

The mice in this experiment were bred from four pairs of 3xTg-AD mice (JAX # 004807) and four pairs of B6129SF2 mice (JAX# 101045), which were purchased from Jackson Laboratories (Bar Harbor, Maine). The 3xTg-AD and B6129SF3 are bred separately, so 3xTg-AD mice are always reared by 3xTg-AD mothers, and B6129SF2 mice are always reared by B6129SF2 mothers. To study the effect of maternal genotype we cross-fostered 3xTg-AD and B6129SF2 pups to create mixed genotype litters and no mother had a litter containing her own pups. The mice underwent a neurodevelopmental test battery from post natal day 0-24 to study pup and maternal differences in development (see Blaney et al., 2013). After weaning, the mice were housed in same sex mixed genotype groups of two to four mice, in clear plastic cages measuring 18.75 x 28 x 12.5 cm, with wood chip bedding, a PVC tube (4 cm diameter x 7 cm length) for enrichment, and metal wire covers. They were fed Purina rodent chow (#5001, Purina, St. Louis, Missouri) and tap water ad libitum, unless otherwise indicated. The colony room was maintained at 22±2 °C on a reversed 12:12 light:dark cycle, with lights off at

10:00am. All procedures used in this experiment was approved by the Dalhousie University Committee on Animal Care.

3.3.2 PROCEDURE

We tested a total of 78 mice, 40 3xTg-AD (17 male and 24 female) and 38 B6129SF2 (19 ale and 19 female), in a longitudinal study at 2, 6, 12, and 18 months of age. Because of age and sex differences in mortality rates (Rae and Brown, 2015), the sample sizes decreased unequally as mice aged (Table 3.1). The mice used in this study also underwent a test battery to assess anxiety-like, motor, cognitive and social behaviour as described by Stover et al. (2015a, 2015b, 2015c). The mice were tested in three cohorts of approximately 27 animals each. During behavioural testing the experimenters were kept blind to the pup genotype and foster mother genotype of the mice, however due to the longitudinal design it was not possible to blind the experimenters to the age of the mice. All testing took place during the dark phase of the light:dark cycle.

Table 3.1 Distribution of mice by pup genotype and maternal genotype at each age tested.

Pup Genotype	Maternal Genotype		
2 Months of Age	**B6129SF2**	**3xTg-AD**	**Total**
B6129SF2	11M, 7F	8M, 12F	38
3xTg-AD	8M, 14F	9M, 9F	40
Total	40	38	78
6 Months of Age			
B6129SF2	11M, 7F	8M, 12F	38
3xTg-AD	7M, 14F	9M, 8F	38
Total	39	37	76
12 Months of Age			
B6129SF2	11M, 5F	8M, 12F	36
3xTg-AD	6M, 13F	9M, 8F	36
Total	35	37	72
18 Months of Age			
B6129SF2	10M, 5F	7M, 7F	29
3xTg-AD	1M, 11F	2M, 5F	19
Total	27	21	48

3.3.3 ACOUSTIC STARTLE AND PREPULSE INHIBITION

The acoustic startle and prepulse inhibition (PPI) tests were performed as described by Martin and Brown (2010) using an SR-Lab system (San Diego Instruments, San Diego, California, USA). The PPI chamber consisted of a sound-attenuated box (38.1 x 40.6 x 58.4 cm) with a cylindrical restraining tube (12.8 x 5 cm, internal diameter 3.5 cm) mounted on a square platform (12.8 x 20.3 cm) and the speaker was mounted 28 cm above the restraint tube. The startle response was recorded by a piezoelectric accelerometer mounted under the platform. Each mouse was given one test consisting of 42 trials with a variable 10-20 second inter trial interval. Before the trials began there was a five minute acclimation period with 65dB background white noise which remained on throughout the test session. Following the acclimation period there were six acoustic startle trials with a 40ms 120dB tone; startle data were collected for 65ms after the tone

was presented. After the initial startle trials there were 30 prepulse inhibition and 6 no-stimulus trials (with only background white noise) presented in a semi-random order. Prepulse inhibition trials lasted 185ms and had a pairing of a lower prepulse tone and a 120dB startle tone. For the first 20ms of the trial a prepulse tone of 74, 78, 82, 86, or 90 dB was presented, followed by 100 ms background white noise, and then a final 40 ms 120 dB startle tone. During PPI trials, startle data were collected for 65 ms after the 120dB startle tone and the auditory startle (response to only the initial startle trials) and percentage of inhibition $\left(\left(\frac{Startle\ Response\ in\ PPI\ Trial}{Startle\ Response\ in\ 120dB\ Trial}\right) * 100\right)$ were analyzed.

3.3.4 STATISTICAL ANALYSES

Statistical analyses were performed with R (www.R-project.org) using linear mixed effects models, with pup genotype, foster mother genotype, sex, and age as possible predictors. For acoustic startle all of the possible models were constructed, and the second order Akaike information criterion (AIC_c), Akaike weight, and evidence ratio were calculated for each model. The model with the lowest AICc was chosen as the 'best' model. For prepulse inhibition the model was chosen using backwards elimination. The best model was compared to the null model with a χ^2 test (Akaike, 1974; Burnham and Anderson, 2002) and we calculated confidence intervals (95%) for all effects in that model. For measures with significant main effects of pup genotype, sex, or foster mother genotype, the effect size was calculated using Cohen's d with a Hedge's (d_{unb}) for an unbiased measure (Hedges, 1981; Cumming, 2014). The levels of amyloid beta and tau neuropathology were assessed after behavioural testing at 19 months of age (Stover et al., 2015e), and the data were correlated with acoustic startle and the percentage of PPI at the highest prepulse intensity using Pearson's-r.

3.4 RESULTS

For acoustic startle the best model had pup genotype, sex, age, a pup genotype by sex interaction, and a pup genotype by age interaction (AICc=3983.951, Figure 3.1A; See also Supplemental Table 3.1.1 and Supplemental Figure 3.1), which differed significantly from the null model ($\chi^2(9, N=273)= 171.03$ $p <0.0001$). The 3xTg-AD mice had a larger acoustic startle response than B6129SF2 mice (CI_{95}= 88.753 – 405.326 mV), female mice had a larger acoustic startle response than males (CI_{95}= 81.420 – 385.621 mV), and the magnitude of startle increased from 2 to 6 months of age (CI_{95}= 281.303 –494.168 mV), but decreased from 6 to 18 months of age (CI_{95}= -870.573 – -611.880 mV). The genotype by sex interaction occurred because female 3xTg-AD mice had a larger startle response than males (CI_{95}= 196.213 – 636.661 mV), while there was no sex difference in B6129SF2 mice (CI_{95}= -156.223 – 262.648 mV). The genotype by age interaction occurred as only at 6 months of age did the 3xTg-AD mice have a larger acoustic startle response than B6129SF2 mice (CI_{95}= 408.219 – 809.225 mV).

For prepulse inhibition (PPI) the best model had prepulse intensity, pup genotype, foster mother genotype, sex, age, a prepulse intensity by pup genotype interaction, a pup genotype by foster mother genotype by age interaction, and a pup genotype by sex by age interaction (AICc=12398.699, Figures 1 B,C,D, and E; See also Supplemental Table 3.1.2,) which differed significantly from the null model ($\chi^2(31,N= 1340)= 774.6$, $p < 0.001$). The percentage of PPI increased as the intensity of the prepulse tone increased (e.g. 74-90dB: CI_{95}= 48.778 – 56.455%) and increased from 2 to 18 months of age (CI_{95}= 10.198 – 25.192%). There was no main effect of pup genotype (CI_{95}= -14.457 – 8.514%), foster mother genotype (CI_{95}= -17.766 – 5.595%), or sex on PPI (CI_{95}= -19.432 –

3.367%). The pup genotype by prepulse intensity interaction occurred because at the highest level of prepulse intensity (90dB) the 3xTg-AD mice tended to have a higher percentage of PPI than B6129SF2 mice (CI_{95}= -2.112 – 22.540%), but there was no difference at lower prepulse intensities. The pup genotype by foster mother genotype by age interaction occurred because only at 2 months of age did the B6129SF2 mice reared by B6129SF2 mothers have a higher percentage of PPI than mice reared by 3xTg-AD mothers (CI_{95}= -1.906 – 20.676%), while the 3xTg-AD mice reared by 3xTg-AD mothers had a higher percentage of PPI than the mice reared by B6129SF2 mothers (CI_{95}= 13.968 – 39.465%. The pup genotype by sex by age interaction occurred because only at 6 months of age did the male 3xTg-AD mice have a higher percentage of PPI than females (CI_{95}= 7.676 – 34.024%), while there was no sex difference in B6129SF2 mice (CI_{95}= -22.570 – 11.728%).

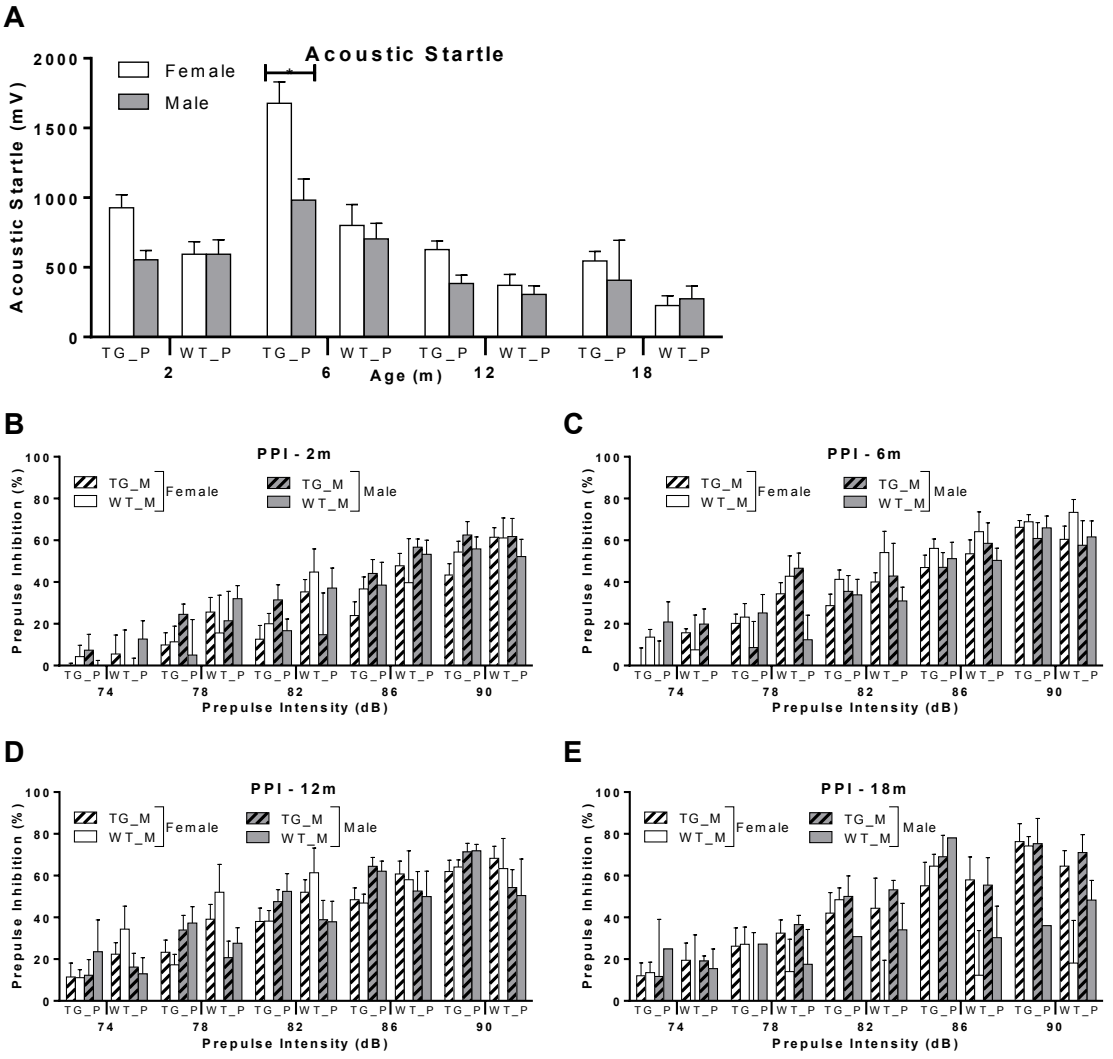

Figure 3.1 Mean (± S.E.M) startle response (A) of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice at 2, 6, 12, and 18 months of age. The mean (± S.E.M) percentage of prepulse inhibition at 2 (B), 6 (C), 12 (D), and 18 (E) months of age in 3xTg-AD and B6129SF2 mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers.

The effect sizes of models with a main effect of genotype, sex, or foster mother genotype are shown in Table 3.2, which shows that the acoustic startle had the only effect size that did not include zero.

Table 3.2 Effect size estimates calculated with a pooled SD and Hedges correction. A '#' indicates that the confidence interval includes zero

Measure	d_{unb}	95% Confidence Interval	
3xTg-AD higher than B6129SF2		Lower	Upper
Acoustic Startle	0.626	0.379	0.873
PPI (90dB)	0.211	-0.031	0.453 #
Female higher than Male			
Acoustic Startle	0.436	0.191	0.681

.

There were no significant correlations between the levels of amyloid beta or tau pathology and acoustic startle or PPI at the highest prepulse intensity (all $p > 0.05$, Supplemental Table 3.2).

3.5 DISCUSSION

In the 3xTg-AD mice the females had a larger acoustic startle response than the males, and the amount of acoustic startle increased from 2 to 6 months of age, then decreased. All mice exhibited evidence of PPI, and the amount of PPI increased with age. At the highest level of prepulse intensity, the 3xTg-AD mice tended to exhibit more PPI than B6129SF2 mice, though there was no difference at lower intensities, which indicates that 3xTg-AD mice do not have a deficit in PPI. Thus our data replicate the findings of Pietropaolo et al. (2008) and García-Mesa et al. (2011) who found an increased startle response in 3xTg-AD mice at 6-7 months of age but do not replicate the findings of García-Mesa et al. (2011) that there was a decrease in PPI in 3xTg-AD mice at 7 months of age. García-Mesa et al. (2011) used mice from breeding colonies at the University of Barcelona, while our mice were bred from mice acquired from the Jackson Laboratories. It is possible that genotype differences resulting from genetic drift or a founder effect could be responsible for the discrepant findings.

We found only one sex difference: At six months of age male 3xTg-AD mice had a higher percentage of PPI than females, but there was no sex difference in B6129SF2 mice. Willott et al. (2003) assessed PPI in 40 strains of inbred mice and found a sex difference in only two strains of mice, and neither of these were the background strains of the 3xTg-AD mice (C57BL/6J and 129S1/SVImJ), so the sex difference may be due to the transgenes, a spurious result, or due to some neurodevelopmental event that is particular to 6 month old mice, though it is unclear why it would only be present at six months of age. We analyzed the levels of amyloid beta and tau pathology in the brains of the mice used in this study at 19 months of age (See Stover et al., 2015c) and we found no correlation between PPI and levels of neuropathology.

There is considerable variation in the levels of both acoustic startle and PPI between strains of mice, though F1 hybrids generally have more homogenous PPI scores (Logue et al., 1997). The B6129SF2 mice used as a background strain for the 3xTg-AD mice and a control strain are F2 hybrids, so it is possible that there will be independent segregation of the genes related to PPI, for example disrupted in schizophrenia 1 (DISC1), a gene associated with schizophrenia in humans and schizophrenia-like symptoms in mice, which could lead to increased variability between mice. We observed fairly high levels of variability in PPI in both genotypes at all ages tested (see Figure 3.1 B-E). There is also variability between laboratories when assessing the levels of acoustic startle and PPI in the same strain (Bullock et al., 1997; Crawley et al., 1997; Willott et al., 2003). The 3xTg-AD mice are bred as a separate line from the B6129SF2 control mice, and so there is the possibility of several issues with genetic differences including genetic drift between 3xTg-AD and B6129SF2 mice, which are an approximate control strain, a

founder effect, or the influence of maternal genotype on development. The only difference that we found due to maternal genotype was a pup genotype by maternal genotype interaction at two months of age, where mice who were reared by the same genotype mothers had higher levels of PPI than those who were reared by mothers of different genotypes. Thus even at two months of age there was no overall effect of maternal genotype on acoustic startle or PPI, though during a neurodevelopmental test battery before weaning we found that maternal genotype affected the development of several reflexes and mice reared by B6129SF2 mothers had a higher frequency of rearing (vertical beam breaks in an automated open field) than mice reared by 3xTg-AD mothers (Blaney et al., 2013).

McCool et al. (2003) found no difference in PPI between AD model mice that were tested longitudinally and those that were tested cross-sectionally, indicating that there is no detectable habituation to PPI in mice. Willott et al., (2003) tested the PPI of 40 strains of inbred mice and found that with an immediate re-test there was no habituation in the majority of strains tested. These findings indicate that there is little effect of re-testing on PPI in mice. In our study we found that the percentage of PPI increased with age, which is likely a result of ageing, not habituation or sensitization to the test.

There have been mixed reports about whether humans with AD develop deficits in PPI (Hejl et al., 2004; Ueki et al., 2006), so it is unclear whether PPI is a desirable trait in a mouse model of AD, though at least two other mouse models of AD (TgCRND8 and hAPP), develop deficits in PPI (McCool et al., 2003; Esposito et al., 2006). Overall it appears that there is an increase in acoustic startle response and no deficit in sensorimotor

gating in both male and female 3xTg-AD mice relative to B6129SF2 control mice at all ages tested.

3.6 ACKNOWLEDGEMENTS

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3.8 SUPPLEMENTAL TABLES

Description of supplemental table 3.1

These supplemental tables provide the statistics used to determine the best model for each measure. The best five models for each measure are included. The model column describes the factors analyzed, terms separated by a '*' indicate both individual main effects and interactions between those two terms, terms separated by a '+' are both simple main effects, and terms separated by a ':' indicate an interaction alone. The 'AICc' column is the second order Akaike information criterion, which is a measure used to evaluate the models based on the complexity and how well the model fits the data; lower values are better. The "Δ AICc" column provides the difference between the given model's AICc and the model with the lowest AICc. The 'Wt' column is the Akaike weight, a measure of relative likelihood that the fit is the best, ranging from 0 (unlikely) – 1(likely). The 'ER' column is the evidence ratio which provides the likelihood that the model with the lowest AICc is better than the model in question.

Supplemental Table 3.1.1 PPI – Acoustic Startle

Model	AICc	Δ AICc	Wt	ER
(Genotype+Sex+Age)+(Genotype:Sex)+(Genotype:Age)	3983.951	0	0.183	1
(Genotype+Sex+Age)+(Genotype:Sex)+(Genotype:Age)+(Sex:Age)	3984.368	0.417	0.149	1.232
(Genotype+FMGenotype+Sex+Age)+(Genotype:Sex)+(FMGenotype:Sex)+(Genotype:Age)	3984.414	0.463	0.145	1.260
(Genotype+FMGenotype+Sex+Age)+(Genotype:Sex)+(FMGenotype:Sex)+(Genotype:Age)+(Sex:Age)	3984.791	0.840	0.120	1.522
(Genotype+FMGenotype+Sex+Age)+(Genotype:Sex)+(Genotype:Age)	3985.116	1.165	0.102	1.791

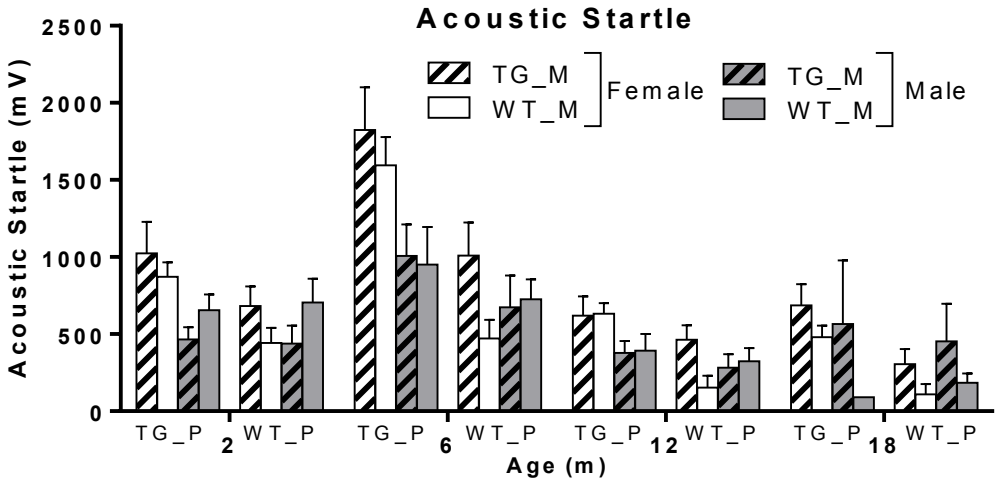
Supplemental Table 3.1.2 PPI – Prepulse Inhibition

Model	AICc	Δ AICc	AICcWt	ER
(dB+Genotype+FMGenotype+Sex+Age)+(dB:Genotype)+ (Genotype:FMGenotype:Age)+(Genotype:Sex:Age)	12398.699	0	0.865	1
(dB+Genotype+FMGenotype+Sex+Age)+(Genotype:FMGenotype:Age)+ (Genotype:Sex:Age)	12403.290	4.591	0.087	9.931
(dB+Genotype+FMGenotype+Sex+Age)+(dB:Genotype)+(FMGenotype:Age)+ (Genotype:Sex:Age)	12405.088	6.389	0.035	24.402
(dB+Genotype+FMGenotype+Sex+Age)+(dB:Genotype)+(Genotype:Sex:Age)+ (FMGenotype:Sex:Age)	12408.800	10.101	0.006	156.126
(dB+Genotype+FMGenotype+Sex+Age)+(FMGenotype:Age)+ (Genotype:Sex:Age)	12409.602	10.903	0.004	233.087

Supplemental Table 3.2 Correlations of Neuropathology and Behavior. Pearson's-r scores for correlations between measures levels of amyloid beta and tau in the brain of 3xTg-AD mice and behavioral measures at 18 months of age. There were no significant correlations (all $p > 0.05$).

Neuropathology Measure	PPI (90dB)	Acoustic Startle
Hippocampus Tau Density (#/μm3)	0.305	0.403
Amygdala Tau Density (#/μm3)	0.477	0.291
Hippocampus Aβ Coverage (%)	0.075	0.132
Amygdala Aβ Coverage (%)	-0.066	0.107
Cortex Aβ Coverage (%)	-0.152	0.120

3.9 SUPPLEMENTAL FIGURE



Supplemental Figure 3.1 Mean (± S.E.M) startle response (A) of male and female 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) at 2, 6, 12, and 18 months of age.

CHAPTER 4 LEARNING AND MEMORY IN THE 3XTG-AD MOUSE MODEL OF ALZHEIMER'S DISEASE AT 2, 6, 12, AND 18 MONTHS OF AGE

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4.1 ABSTRACT

To study the effects of maternal genotype on adult behaviour in the 3xTg-AD mouse model of Alzheimer's disease we cross fostered litters of 3xTg-AD and B6129SF2 control mice to create mixed genotype litters where no mother reared her own pup. We then conducted a longitudinal study on the mice at 2, 6, 12, and 18 months of age to assess spatial learning and memory. We found that the 3xTg-AD mice had a deficit in spatial learning and an age-dependant deficit in spatial memory in the Morris water maze. There was no genotype difference in short or long term olfactory-dependant memory. The mice reared by B6129SF2 mothers appear to have a deficit in both spatial and olfactory memory, though this finding was not consistent across ages. There were no consistent differences between the sexes in spatial memory, but male mice tended to have better olfactory memory than female mice. Overall the 3xTg-AD mice are useful for modeling the spatial memory deficits of AD as they had spatial memory deficits at 6 and 12 months of age in the MWM, and the effect of maternal genotype was not large enough to effect this deficit.

4.2 INTRODUCTION

The 3xTg-AD mouse is a commonly used model of Alzheimer's disease (AD) that has three transgenes which cause it to develop amyloid beta plaques, tau pathology, and behavioural deficits. The three transgenes are an amyloid precursor protein gene with the Swedish mutation associated with familial AD (APPswe), a presenilin gene with a mutation also associated with familial AD (PS1M146V), and a microtubule protein associated protein tau gene with a mutation (TauP301L) that causes tau pathology to develop (Oddo et al., 2003). These mutations cause the development of both amyloid beta plaques and tau tangles in the brain, which in turn are thought to cause a number of behavioural deficits.

The 3xTg-AD mice develop a deficit in spatial learning and memory on the Morris water maze (MWM), though there is some variability in the timeline of its development. The deficit has been reported as early as 2.5 months of age (Marchese et al., 2014), though there are reports of no deficit at 2 months of age with the defect developing at 4 months of age (Billings et al., 2005; Clinton et al., 2007), and one study found no deficit even at six months of age, though they used a protocol with only four trials, compared to many protocols which use four trials a day for several days (Giménez-Llort et al., 2010). There are many reports of deficits in the MWM at older ages (McKee et al., 2008; Movsesyan et al., 2008; Corona et al., 2010; García-Mesa et al., 2011; Chen et al., 2013). One longitudinal study found a deficit in MWM performance in the 3xTg-AD from 2.5 to 9 months of age (Marchese et al., 2014). Another study compared the deficit of the 3xTg-AD mice in the MWM using both cross-sectional and longitudinal methodology and found that at earlier ages the effect of

previous testing enhanced performance on the MWM, this masked the deficits in younger 3xTg-AD mice, but by 12 months of age the deficit was detectable in cross-sectional and longitudinal testing groups and by 15 months of age there was little effect of pervious learning in the longitudinal group (Billings et al., 2007).

Compared to spatial memory there are few studies on olfactory learning and memory in transgenic mice, though mice are largely olfactory animals. At least one study on olfactory function in the 3xTg-AD mice found a deficit in olfactory discrimination in the 3xTg-AD mice compared to B6129SF2 mice beginning at 10 months of age (Coronas-Sámano et al., 2014).

The breeding system of the 3xTg-AD requires that 3xTg-AD only be bred with 3xTg-AD, and B6129SF2 wildtype controls only be bred with B6129SF2 mice. This system causes mice to only be reared by mothers of the same genotype, and thus any genotypic differences in maternal behaviour could influence pup development. Several previous studies have shown that maternal care and early life environment can have a lasting effect on behaviour (Priebe et al., 2005; Szyf et al., 2007). In order to study the effect of maternal genotype we cross-fostered litters of both genotypes to make mixed genotype litters and first conducted a neurodevelopmental test battery from birth to weaning which has been published (Blaney et al., 2013). We then conducted a longitudinal study on the mice at 2, 6, 12, and 18 months of age to determine the long term effects of maternal genotype on adult behaviours. In addition to the experiments described in this study the mice also underwent a behavioural test battery at each age to assess prepulse inhibition, anxiety-like, motor, and social behaviours (Stover et al., 2015b, 2015d, 2015e) .

4.3 METHODS

4.3.1 BREEDING, CROSS-FOSTERING & PRE-WEANING TREATMENT OF MICE

We used 3xTg-AD mice (B6;129-Psen1tm1Mpm Tg(APPSwe,tauP301L)1Lfa/Mmjax) and B6129S/F2 mice, bred from animals purchased from Jackson Laboratories (Strain #004807 and #004807, respectively, Bar Harbor, Maine). All procedures used in this experiment were approved by the Dalhousie University Committee on Animal Care.

In order to examine maternal effects on development we cross fostered all litters at post natal day 0 (the day of birth) or 1, so that half the mice of each genotype had a 3xTg-AD foster mother and half had a B6129SF2 foster mother, and no mother reared her own pups. These mice were tested in a neurodevelopmental test battery as described by Blaney et al. (2013).

The mice were housed in same sex groups of two to four foster-littermates in clear plastic cages measuring (18.75 x 28 x 12.5 cm), with wire tops, wood chip bedding, and a PVC tube (4 cm diameter x 7 cm length) for enrichment. The colony room was kept at 22±2 °C on a reversed 12:12 light:dark cycle, with lights off at 10:00am.

The mice were fed Purina 5001 rodent chow (Purina, St. Louis, Missouri) and tap water ad libitum, except for when food restricted as desired in the procedure. This experiment was approved by the Dalhousie University Committee on Laboratory Animals.

4.3.2 PROCEDURE

Beginning at 2 months of age we tested 78 mice, 40 3xTg-AD and 38 B6129S/F2, with approximately equal numbers of each sex, however some mice died between the testing periods (Table 4.1). The mice were tested in three cohorts of approximately the same size at two, six, twelve, and eighteen months of age in a longitudinal design. Behavioural testing took place during the dark phase of the light:dark cycle. For the behavioural tasks the experimenters were kept blind to the genotype and foster mother genotype of the mice; it was not possible to blind the experimenters to the age due to the longitudinal design. The tests were performed in the order described below.

Table 4.1 Distribution of mice by pup genotype and maternal genotype at each age.

Pup Genotype	**Maternal Genotype**		
2 Months of Age	**B6129SF2**	**3xTg-AD**	**Total**
B6129SF2	11M, 7F	8M, 12F	38
3xTg-AD	8M, 14F	9M, 9F	40
Total	40	38	78
6 Months of Age			
B6129SF2	11M, 7F	8M, 12F	38
3xTg-AD	7M, 14F	9M, 8F	38
Total	39	37	76
12 Months of Age			
B6129SF2	11M, 5F	8M, 12F	36
3xTg-AD	6M, 13F	9M, 8F	36
Total	35	37	72
18 Months of Age			
B6129SF2	10M, 5F	7M, 7F	29
3xTg-AD	1M, 11F	2M, 5F	19

4.3.3 MORRIS WATER MAZE

We used the Morris water maze (MWM) to study visually dependent spatial learning and memory using the protocol of Wong and Brown (2007). The maze was a circular polypropylene pool (Canadian Tire, Toronto, ON) 100 cm in diameter and 20 cm

deep. The maze was filled with 14 cm of water made opaque to obscure the platform location using non-toxic white paint (Schola, Marieville, PQ). The pool had an escape platform (13.5 cm high, 9 cm diameter) which mice were trained to locate. Shapes and posters were placed on the walls to serve as extra-maze visual cues; these cues were not changed for the duration of testing. The mice were tested in squads of four to six. To begin a trial the mice were released from one of four possible release points, which were equally spaced around the perimeter of the pool (N, S, E, and W). All the mice in a group were released from the same point for each trial and the release point varied semi-randomly over trials, ensuring that mice were never released from the same point twice in a row. The maze was divided into four quadrants based on the four release points. For the acquisition trials the platform was placed in the middle of one of the quadrants and were given four trials a day for three days to find the platform. For the reversal phase, the platform was moved to the opposite side of the pool and mice were given another three days of training with four trials a day. If a mouse did not locate the platform within 60 seconds it was led to the platform using a plastic bucket and left on the platform for 30 seconds to learn its location. The latency to find the platform, swim speed, distance travelled, and amount of time spent within 10 cm of the side of the pool (thigmotaxis) were determined using an automated tracking system (WaterMaze, Actimetrics, Willamette, IL) with a camera placed 2.1 m above the pool. After acquisition and reversal trials were completed, a single probe trial was given on day seven. During the probe trials the platform was removed and the mice were placed in the pool for 60 seconds. The tracking system recorded the total amount of time spent in each quadrant, and the number of crossings of an imaginary annulus drawn around the locations of the platform during

acquisition and reversal. The following day the mice were given a visual cued platform trial with an attachment on the platform to raise it above the water level and a flag to indicate its location, as a simple test of visual ability. For this test the mouse was given four trials using the procedure used in acquisition and reversal.

4.3.4 CONDITIONED ODOUR PREFERENCE TASK

To assess olfactory learning and memory we used the conditioned odour preference task, using the protocol of Schellinck et al. (2001). In this task the mice were trained to associate an odour (CS+) with a buried sugar reward and trained to associate another odour (CS-) with no reward. The CS+ odour varied semi-randomly between cages; all mice in a cage were assigned the same CS+. The mice were food deprived to between 85% and 90% of their starting body weight over three days before training. The training boxes were clear plastic cages identical to their home cages with the bottoms covered with pine chip bedding. In each cage an odour pot consisting of 0.5ml of the odorant absorbed on piece of filter paper (55 mm diameter) in a plastic cup (1.5cm height, 6.25-cm diameter) covered by the cover of a plastic Petri dish with 10-12 holes. For the CS+ odours pieces of sugar were placed on top of the petri dish cover, but no sugar was included with the CS- odours, and the odour cups were buried in 2cm of the wood chip bedding. Four rooms were used for training, one for each odour, a third where mice were kept between trials, and a fourth where preference testing took place. For training, mice were given four ten-minute trials per day (2 trials per odour), alternating between CS+ and CS- odours with a ten minute inter-trial interval each day for four days (total 16 trials, 8 per odour). On day five the mice were given a memory test. The test apparatus was an open box (69 x 20 x 20 cm) made of clear plastic with three chambers

(each 23 x 20 x 20 cm) connected by openings in the walls (6 x 5.5 cm). First the mice were given a habituation trial lasting two minutes with cups containing no odour in each end chamber. The total amount of time spent in each end chamber was recorded. If the mice spent more than 75% time in one end chamber the CS+ was placed in the non-preferred end. Then 24 hours after the last habituation trial, the mice were given a test trial where an odour cup containing the CS+ (with no sugar) was placed in one end chamber and an odour cup containing the CS- (with no sugar) was placed in the other end chamber. The total amount of time spent digging (defined as displacing the wood chip bedding with the snout or paws) in each odour cup was recorded. At each age the mice trained using a new set of odours and were re-tested for the odour learned at previous ages. Therefore mice learned a new odour pair at two, six, twelve and eighteen months of age and were tested at six, twelve, and eighteen months of age on the odours learned at previous ages.

4.3.5 CORRELATIONS OF NEUROPATHOLOGY AND BEHAVIOUR

All measures that had genotype difference were correlated with levels of tau and amyloid beta neuropathology in the brains of the mice at 19 months of age. If there was more than one measure of the same underlying difference (e.g. latency and distance in the MWM) then only the one with the largest effect size was included in the analysis to prevent multiple comparisons of the same measure. The methodology used to determine the levels of neuropathology is described in Stover et al. (2015e)

4.3.6 STATISTICAL ANALYSES

The statistical analyses described in the following section were performed using R (www.R-project.org). We used linear regression to assess the long-term memory tasks in

the conditioned odour preference task, and for all other measures we used linear mixed effects regression. For measures without a repeated measures component all models were compared and the model with the lowest second-order Akaike's Information Criterion (AICc) were selected, the top five models for each of these measures are presented in supplemental tables. For repeated measures tasks this approach was no not feasible (it would require comparing roughly 67 million models), so models were selected using backward elimination. The best model was compared to the null model with a chi-square test (linear mixed effects regression) or an F-test (linear regression). When there were significant effects of pup genotype, sex, or foster mother genotype, we calculated unbiased effect sizes using Cohen's d with a hedges correction. The correlations between levels of neuropathology and behaviour were calculated using Pearson's r.

4.4 RESULTS

Several mice died over the testing period, and Table 4.1 gives the number of mice tested at each age.

4.4.1 MORRIS WATER MAZE

4.4.1.1 ACQUISITION

For the latency to reach the hidden platform in the acquisition phase of the Morris water maze the best model had all the factors as main effects and a day by age, foster mother genotype by age, and a pup genotype by sex by age interaction, and was significantly different from the null model (AICc = 5944.75; $\chi^2(27, N = 819)= 579.43$, $p < 0.001$; Figure 4.1.1 A-D). Latency decreased from day 1 to 3 (CI_{95}= -5.879 – -2.889 s), and B6129SF2 mice had a faster latency to reach the platform than 3xTg-AD mice (CI_{95}=

-6.502 – -2.167 s). Mice reared by B6129SF2 mothers had a longer latency to reach the platform than mice reared by 3xTg-AD mothers (CI_{95}= 0.394 – 4.355 s), and there was no difference between males and females (CI_{95}= -1.709 – 2.592 s). Latency to reach the platform decreased from 2 to 18 months of age (CI_{95}= -7.427 – -4.101 s). The day by age interaction occurred because the decrease in latency over days tended to diminish with age (2m day 1 – 3: CI_{95}= -30.181 – -24.59 s; 18m day 1-3:-7.340 – -0.582 s). The foster mother genotype by age interaction occurred because the increased latency of mice reared by B6129SF2 mice only occurred at 6 and 12 months of age (CI_{95}= 0.200 – 5.947 s, CI_{95}= 2.992 – 9.720 s). The pup genotype by sex by age interaction occurred because at six months of age in B6129SF2 mice only males took longer to reach the platform than females (CI_{95}= -9.879 – -2.116 s).

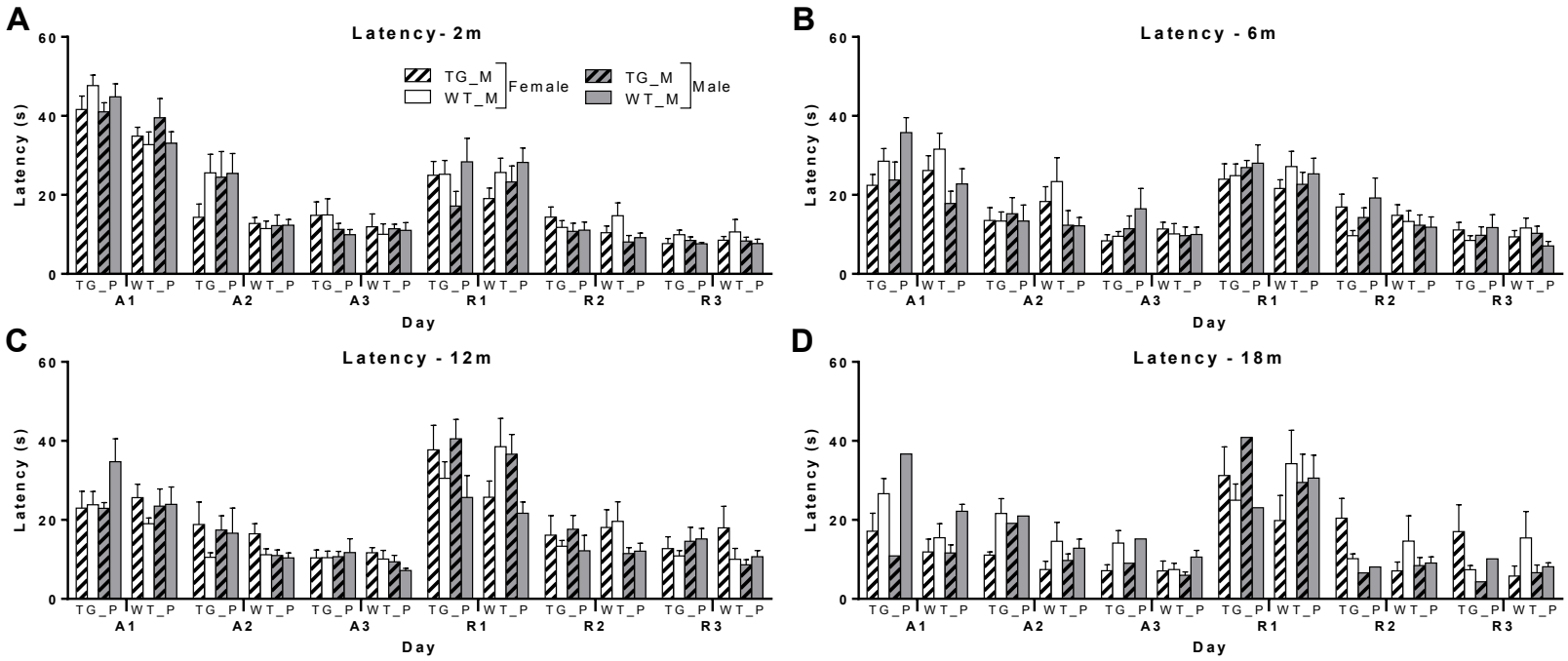

Figure 4.1.1 Latency in the Morris Water Maze. The mean (± S.E.M) latency to reach the hidden platform during each day of acquisition and reversal at 2 (A), 6 (B), 12 (C), and 18 (D) months of age of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers.

For the distance travelled during acquisition the best model had all the factors as main effects and day by foster mother genotype, pup genotype by foster mother genotype, foster mother genotype by age, day by pup genotype by age, and pup genotype by sex by age interactions. It was significantly different from the null model (AICc = 10521.93; $\chi^2(38, N = 819) = 374.68$, $p < 0.001$; Figure 4.1.2 A-D). The distance to reach the platform decreased over days (1 – 3: CI_{95}= -269.117 – -208.511 cm), and B6129SF2 mice took less distance than 3xTg-AD to reach the platform (CI_{95}= -121.10 – 47.836 cm), mice reared by B6129SF2 mothers travelled a greater distance to reach the platform than mice reared by 3xTg-AD mothers (CI_{95}= 15.841 – 86.553 cm). There was no difference in performance between males and females (CI_{95}= -30.943 – 46.758 cm). The distance to reach the platform during acquisition decreased with age (2 – 18m: CI_{95}= -145.399 – -49.924 cm). The day by foster mother genotype interaction occurred because only on day 1 mice reared by B6129SF2 mice travelled a greater distance to reach the platform than mice reared by 3xTg-AD mothers (CI_{95}= 64.357 – 163.180 cm). The pup genotype by foster mother genotype interaction occurred because only in mice reared by B6129SF2 mothers did the B6129SF2 mice take less distance to reach the platform (CI_{95}= -172.154 – -70.610 cm). The foster mother genotype by age interaction occurred because only at 2 and 18 months of age did the mice reared by B6129SF2 mothers travel a greater distance to reach the platform than mice reared by 3xTg-AD mothers (CI_{95}= 22.637 – 126.798 cm, CI_{95}= 52.946 – 178.576 cm). The day by genotype by age interaction occurred because the B6129SF2 mice only travelled a shorter distance to reach the platform than the 3xTg-AD mice on some combinations of days and ages (2m day 2: CI_{95}= -294.872 – -131.661 cm, 6m day 1: CI_{95}= -183.143 – -19.214 cm, 18m day 1: CI_{95}= -249.557 – -5.077 cm,

18m day 2: CI_{95}= -285.763 – -49.134 cm). The pup genotype by sex by age interaction occurred because in female mice only at every age except 6 months the B6129SF2 mice had a lower latency to reach the platform than the 3xTg-AD mice (2m: CI_{95}= -210.281 – -66.625 cm, 6m: CI_{95}= -60.937 – 84.461 cm, 12m: CI_{95}= -159.188 – -0.767 cm. 18m: CI_{95}= -193.680 – -31.976 cm).

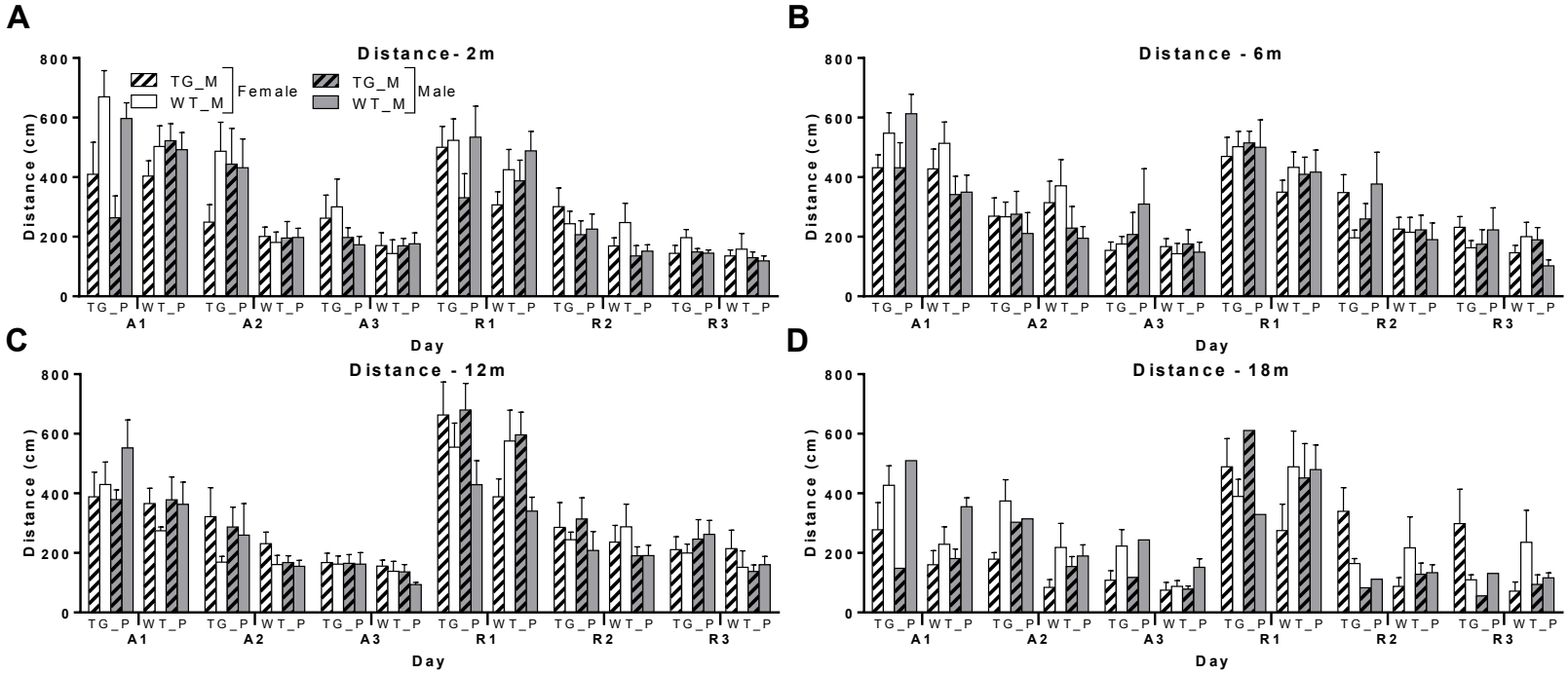

Figure 4.1.2 Distance in the Morris Water Maze. The mean (± S.E.M) distance to reach the hidden platform during each day of acquisition and reversal at 2 (A), 6 (B), 12 (C), and 18 (D) months of age of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers.

For the swim speed during acquisition the best model had all the factors as main effects and pup genotype by sex, foster mother genotype by age, day by pup genotype by foster mother genotype, and day by pup genotype by age interactions. This model was significantly different from the null model (AICc = 3959.164; $\chi^2(34\ N = 819) = 330.66$, $p < 0.001$; Figure 4.1.3 A-D). The swim speed increased from day 1 to 2 (CI_{95}= 0.576 – 1.517 cm/s), then decreased from day 2 to 3 (CI_{95}= -1.045 – -0.113 cm/s). The 3xTg-AD mice swam faster than the B6129SF2 mice (CI_{95}= 0.651 – 2.144 cm/s). There were no main effects of foster mother genotype (CI_{95}= -0.924 – 0.551 cm/s), or sex (CI_{95}= -0.475 – 1.019 cm/s). The swim speed increased from 2 to 6 months of age (CI_{95}= 1.552 – 2.528 cm/s), then decreased from 6 to 12 (CI_{95}= -2.432 – -1.423 cm/s), and 12 to 18 months of age (CI_{95}= -1.914 – -0.711 cm/s). The pup genotype by sex interaction occurred because only in female mice the 3xTg-AD swam faster than the B6129SF2 mice (CI_{95}= 1.818 – 3.816 cm/s). The foster mother genotype by age interaction occurred because at 2 and 18 months of age mice reared by B6129SF2 mothers swam faster than mice reared by 3xTg-AD mothers (CI_{95}= 0.144 – 1.967, CI_{95}= 0.113 – 2.363 cm/s), but at 6 months of age mice reared by 3xTg-AD mothers swam faster than mice reared by B6129SF2 mothers (CI_{95}= 0.643 – 2.480 cm/s). The day by pup genotype by foster mother genotype interaction occurred because 3xTg-AD mice swam faster than B6129SF2 mice except in mice reared by 3xTg-AD mothers on day 1, when there was no difference between genotypes (CI_{95}= -0.669 –1.893 cm/s). The day by age by pup genotype interaction occurred because the genotype difference was present at every day and age except day 1 at 2 months of age (CI_{95}= 0.869 – 3.555 cm/s).

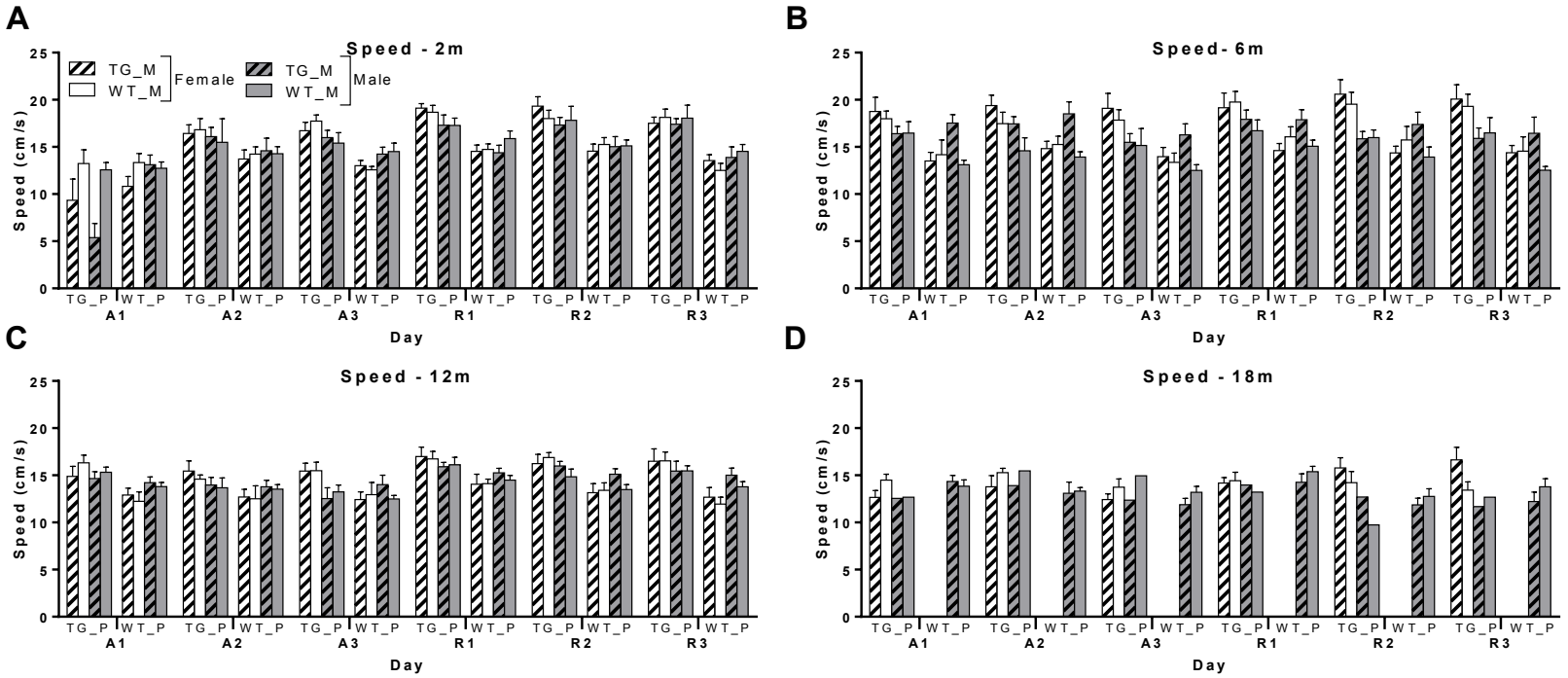

Figure 4.1.3 Swim Speed in the Morris Water Maze. The mean (± S.E.M) swim speed for each day of acquisition and reversal at 2 (A), 6 (B), 12 (C), and 18 (D) months of age of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers.

The best model for the percentage of time spent in thigmotaxis during acquisition had all the factors as main effects and foster mother genotype by age, sex by age, day by pup genotype by foster mother genotype, and day by pup genotype by age interactions, and was significantly different from the null model (AICc = 4850.757; χ^2(36 N = 798)= 387.2, $p < 0.001$; Figure 4.1.4 A-D). Thigmotaxis decreased from day 1 to 3 (CI_{95}= -4.737 – -2.978 %), and there was no difference in thigmotaxis between pup genotypes or sexes (CI_{95}= -1.637 – 0.304, CI_{95}= -1.669 – 0.335 %). Mice reared by B6129SF2 mothers exhibited more thigmotaxis than mice reared by 3xTg-AD mothers (CI_{95}= 0.620 – 2.499 %). The amount of thigmotaxis decreased with age (2 to 18m: CI_{95}= 0.869 6.014 – -3.715 %), and there was an age by foster mother genotype interaction as mice reared by B6129SF2 mothers exhibited more thigmotaxis than mice reared by 3xTg-AD mothers only at 2 months of age (CI_{95}= 2.358 – 5.258 %). The sex by age interaction occurred because only at two months of age females exhibited more thigmotaxis than males (CI_{95}= 0.420 – 3.312 %). The day by foster mother genotype by pup genotype interaction occurred because only on day one in mice reared by B6129SF2 mothers 3xTg-AD mice exhibited more thigmotaxis than B6129SF2 mice (CI_{95}= 3.136 – 7.019 %). The age by day by pup genotype interaction occurred because only on day 1 and 2 and 6 months did 3xTg-AD mice exhibit more thigmotaxis than B6129SF2 mice (CI_{95}= 2.400 – 7.019, CI_{95}= 1.231 – 5.866 %).

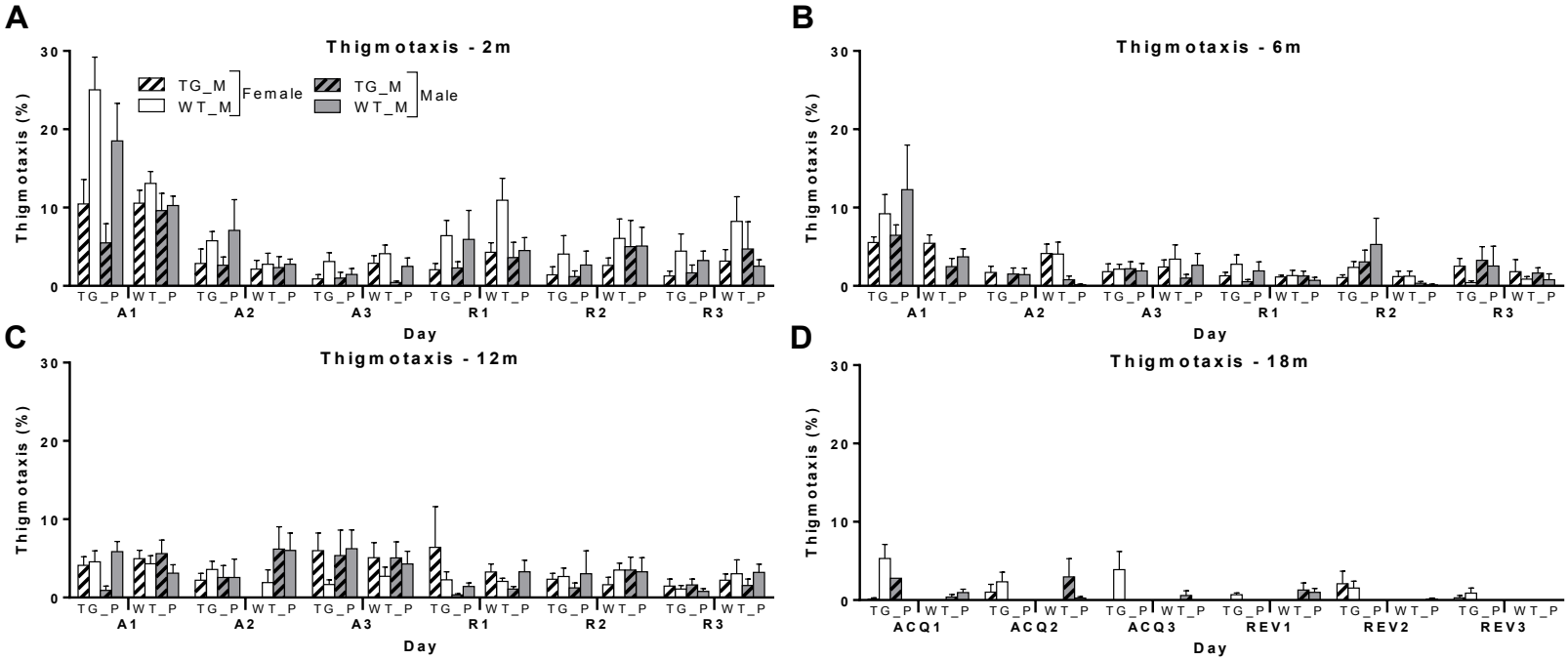

Figure 4.1.4 Thigmotaxis in the Morris Water Maze. The mean (± S.E.M) percentage of thigmotaxis for each day of acquisition and reversal at 2 (A), 6 (B), 12 (C), and 18 (D) months of age of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers.

4.4.1.2 REVERSAL

For the latency to find the hidden platform during the reversal phase the model had day, pup genotype, foster mother genotype, age, and a genotype by foster mother genotype by age interaction, and was significantly different from the null model (AICc = 6031.077; χ^2(17 N = 819)= 428.92,p < 0.001; Figure 4.1.1 A-D). The latency to the platform decreased during reversal (day 1 to 3: CI_{95}= -18.176 – -15.024 s) and there was no difference between pup genotypes (CI_{95}= -4.047 – 0.181 s), or foster mother genotypes (CI_{95}= -2.839 – 1.482 s). Latency generally increased with age (2 to 18m: CI_{95}= 0.341 – 4.490 s), and the age by foster mother genotype by pup genotype interaction occurred because only at 18 months of age in mice reared by 3xTg-AD mothers the 3xTg-AD mice exhibited more thigmotaxis than the B6129SF2 mice (CI_{95}= 4.549 – 16.084 s).

For the distance travelled during the reversal phase the best model had all the factors as main effects and age by pup genotype by foster mother genotype, and pup genotype by foster mother genotype by age interactions. The model was significantly different from the null model (AICc = 10533.74; χ^2(21 N = 819)= 450.16, p < 0.001; Figure 4.1.2 A-D).The distance to reach the platform decreased across days (day 1 to 3: CI_{95}= -317.22 – -263.819 cm), and the B6129SF2 mice travelled a shorter distance to the platform than the 3xTg-AD mice (CI_{95}= -104.057 – -32.309 cm). There was no difference in distance between foster mother genotypes or sexes (CI_{95}= -42.269 – 29.772, CI_{95}= -42.722 – 27.402 cm). The distance travelled increased from 2 to 12 months of age (CI_{95}= 24.992 – 87.110cm), then decreased from 12 to 18 months of age (CI_{95}= -98.887 – -27.052 cm). The age by foster mother genotype by pup genotype interaction occurred

because at every age after 2 months in mice reared by 3xTg-AD mothers the B6129SF2 mice travelled a shorter distance to the platform (6m: CI_{95}= -147.456 – -5.536, 12m: CI_{95}= -180.980 – -35.035, 18m: CI_{95}= -273.971 – -83.886 cm). The sex by foster mother genotype by pup genotype interaction occurred because in female mice there was a genotype effect in mice reared by 3xTg-AD mothers (CI_{95}=-323.618 – -119.423 cm), and some evidence for one in B6129SF2 mice (CI_{95}= -8.283 – 172.268cm), but in male mice the pup genotype effect was only present in mice reared by 3xTg-AD mothers (CI_{95}= -250.783 – -26.572 cm).

For the swim speed during reversal the best model again contained all the factors as main effects, and had pup genotype by age, pup genotype by sex, and foster mother genotype by sex by age interactions. The best model was significantly different from the null model (AICc = 3877.682; χ^2(22 N = 801)= 239.7, $p < 0.001$; Figure 4.1.3 A-D). The swim speed decreased across days (1 to 3: CI_{95}= -1.292 – -0.447 cm/s) and the 3xTg-AD swam significantly faster than the B6129SF2 mice (CI_{95}= 1.475 – 3.067 cm/s). There was no difference between foster mother genotypes (CI_{95}= -0.869 – 0.632 cm/s) or sexes (CI_{95}= -0.201 – 1.338 cm/s), and swim speed decreased with age (2 to 18m: CI_{95}= -3.450 – 2.297 cm/s). The pup genotype by age interaction occurred because the pup genotype effect was present at all ages except 18 months (CI_{95}= -1.562 – 0.884 cm/s). The pup genotype by sex interaction occurred because the faster swim speed of the 3xTg-AD was only present in female mice (CI_{95}= -4.538 – -2.480 cm/s). The age by sex by foster mother genotype interaction occurred because only at six months in males the mice reared by 3xTg-AD mothers swam faster than mice reared by B6129SF2 mothers (CI_{95}= -3.190 – -0.438 cm/s).

For the percentage of thigmotaxis the best model had pup genotype, foster mother genotype, sex, age, and genotype by age, and foster mother genotype by age by sex interactions, and it was significantly different form the null model (AICc = 4611.819; χ^2(19 N = 800)= 97.811, p < 0.001; Figure 4.1.4 A-D). Thigmotaxis decreased with age (2 to 18m: CI_{95}= -4.195 – -2.281 %), and mice reared by B6129SF2 mothers exhibited more thigmotaxis than mice reared by 3xTg-AD mothers (CI_{95}= 0.089 – 1.550 %). The pup genotype by age interaction occurred because only at 2 months of age the B6129SF2 mice exhibited more thigmotaxis than the 3xTg-AD mice (CI_{95}= 0.837 – 3.276 %). The foster mother by sex by age interaction occurred because only at two months of age in female mice the mice reared by B6129SF2 mothers exhibited more thigmotaxis than mice reared by 3xTg-AD mothers (CI_{95}= 2.374 – 5.690 %).

4.4.1.3 PROBE MEMORY TRIAL

The best model for the percentage of time spent in the correct quadrant had pup genotype, age, and a genotype by age interaction, and was significantly different from the null model (χ^2(7 N = 267)= 74.211, p < 0.001; Figure 4.1.5 A; Supplemental Table 4.1.1; Supplemental Figure 4.1 A). There was no overall effect of genotype (CI_{95}= -0.964 – 4.628 %), and the time in the correct quadrant was stable from 2 to 12 months of age (CI_{95}= -3.022 – 4.313 %), then decreased at 18 months of age (12 to 18: CI_{95}= -14.889 – -6.607 %). The age by genotype interaction occurred because there was some evidence that B6129SF2 mice spent a greater percentage of time in the correct quadrant at 6 months of age (CI_{95}= -0.165 – 9.935 %), and strong evidence that the B6129SF2 mice spent a greater percentage of time in the correct quadrant at 12 months of age (CI_{95}=

1.810 – 12.793 %) as the confidence interval did not include 0, but there was no difference at 2 or 18 months of age.

For the number of crossings of the correct annulus the best model had only age and was significantly different from the null model ($\chi^2(3\ N = 273)= 63.9$, $p < 0.001$; Figure 4.1.5 B; Supplemental Table 4.1.2; Supplemental Figure 4.1 B). The number of correct annulus crossings decreased with age after 6 months (2 to 6m: CI_{95}= -0.909 – 0.376, 6 to 18m: -3.557 – -2.156 crossings).

Figure 4.5

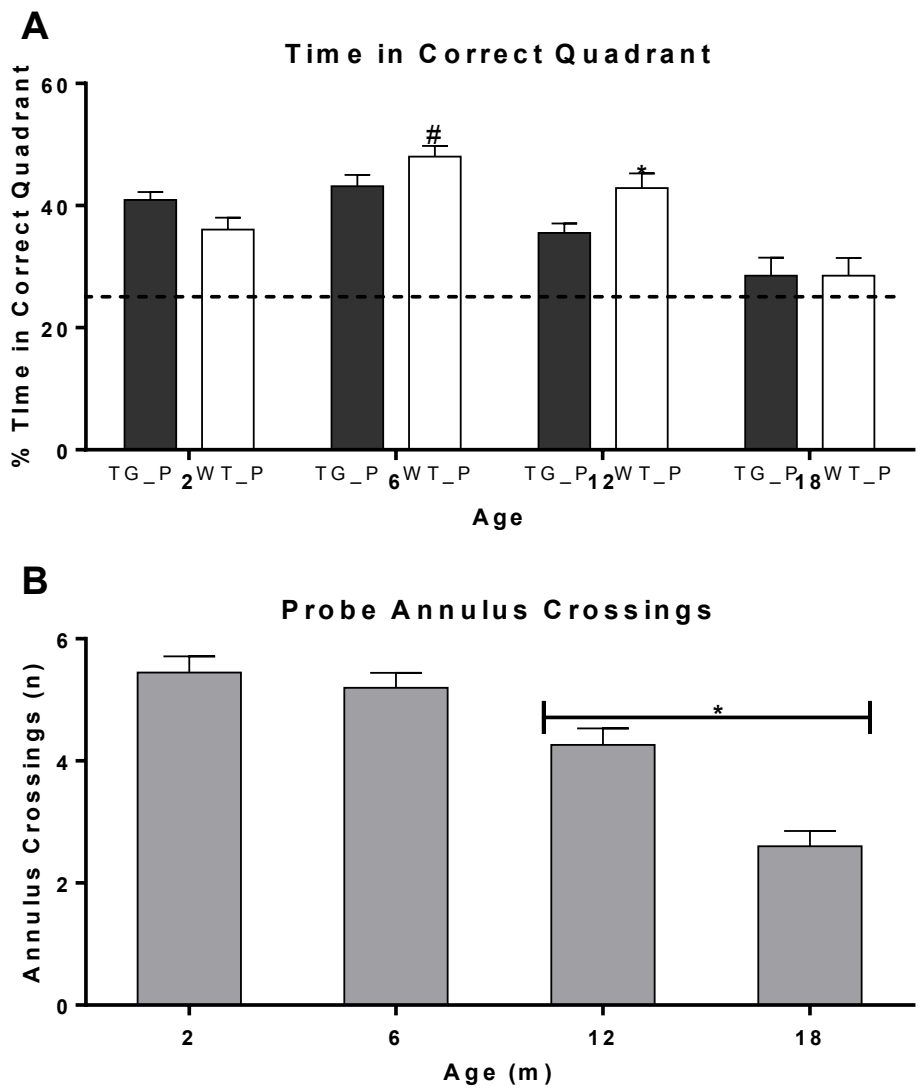

Figure 4.1.5 Morris Water Maze Probe Trial. The mean (± S.E.M) percentage of time spent in the correct quadrant (A) and of the Morris water maze during the probe trial of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice at each age, and the number of correct annulus crossings (B) at each age.

4.4.2 CONDITIONED ODOUR PREFERENCE TASK

The best model for short term memory was the null model (AICc = 2649.318, Figure 4.2 A; and Supplemental Table 4.1.1), which indicates there was no difference between any groups. For the long term memory of the 2 month odour at 6 months of age

the best model had foster mother genotype and sex, which was significantly different from the null model (AICc = 108.301, ($F(2,75) = 4.776$, $p < 0.05$; Figure 4.2 B; Supplemental Figure 4.2 A; and Supplemental Table 4.2.2). Mice reared by 3xTg-AD mothers had better long term memory retention from 2 to 6 months of age (CI_{95}= 6.215 – 48.087 %) but there was no difference between male and female mice (CI_{95}= -3.482 – 39.406 %). For memory of the 2 month odor at 12 months of age the best model had foster mother genotype and sex, which was significantly different from the null model (AICc = 724.593, ($F(2,69) = 9.775$, $p < 0.001$; Figure 4.2 C; Supplemental Figure 4.2 B; and Supplemental Table 4.2.3). Mice reared by 3xTg-AD mothers had better long term memory from 2 to 12 months of age (CI_{95}= 17.95 – 56.61 %) and male mice had better retention than female mice (CI_{95}= -3.482 – 39.406 %). The best model for memory of the 2 month odour at 18 months was the null model (AICc= 480.892, Figure 4.2 D; and Supplemental Table 4.2.5), which indicates there was no difference between groups.

The best model for the memory of the 6 month odour at 12 months of age had only sex and was significantly different from the null model (AICc= 75.503; $F(1, 69)=5.612$, $p < 0.05$; Figure 4.2 E; Supplemental Figure 4.2 C; and Supplemental Table 4.2.4), as males performed better than females (CI_{95}= 4.845 – 41.546 %). For memory of the 6 month odour at 18 months of age the best model had only sex, though it was not different from the null model and there was no difference between the sexes (AICc= 475.468; $F(1,45)= 3.306$, $p > 0.05$; Figure 4.5 F; CI_{95}= -1.572 – 42.734; Supplemental Figure 4.2 D; and Supplemental Table 4.2.6). For memory of the 12 month odour at 18 months the best model had foster mother genotype, sex, and an interaction between foster mother genotype and sex and was significantly different from the null model (AICc =

454.430; $F(3,45)= 6.650$, $p < 0.001$; Figure 4.2 G; Supplemental Figure 4.2 E; and Supplemental Table 4.2.7). Mice reared by 3xTg-AD mothers had worse memory for the 12 month odour at 18 months of age (CI_{95}= -38.364 – -13.947 %). Males performed better than females (CI_{95}= 2.201 – 36.962 %), though the foster mother genotype by sex interaction indicated that males performed better than females only in mice reared by 3xTg-AD mothers (CI_{95}= 28.592 – 77.378 %).

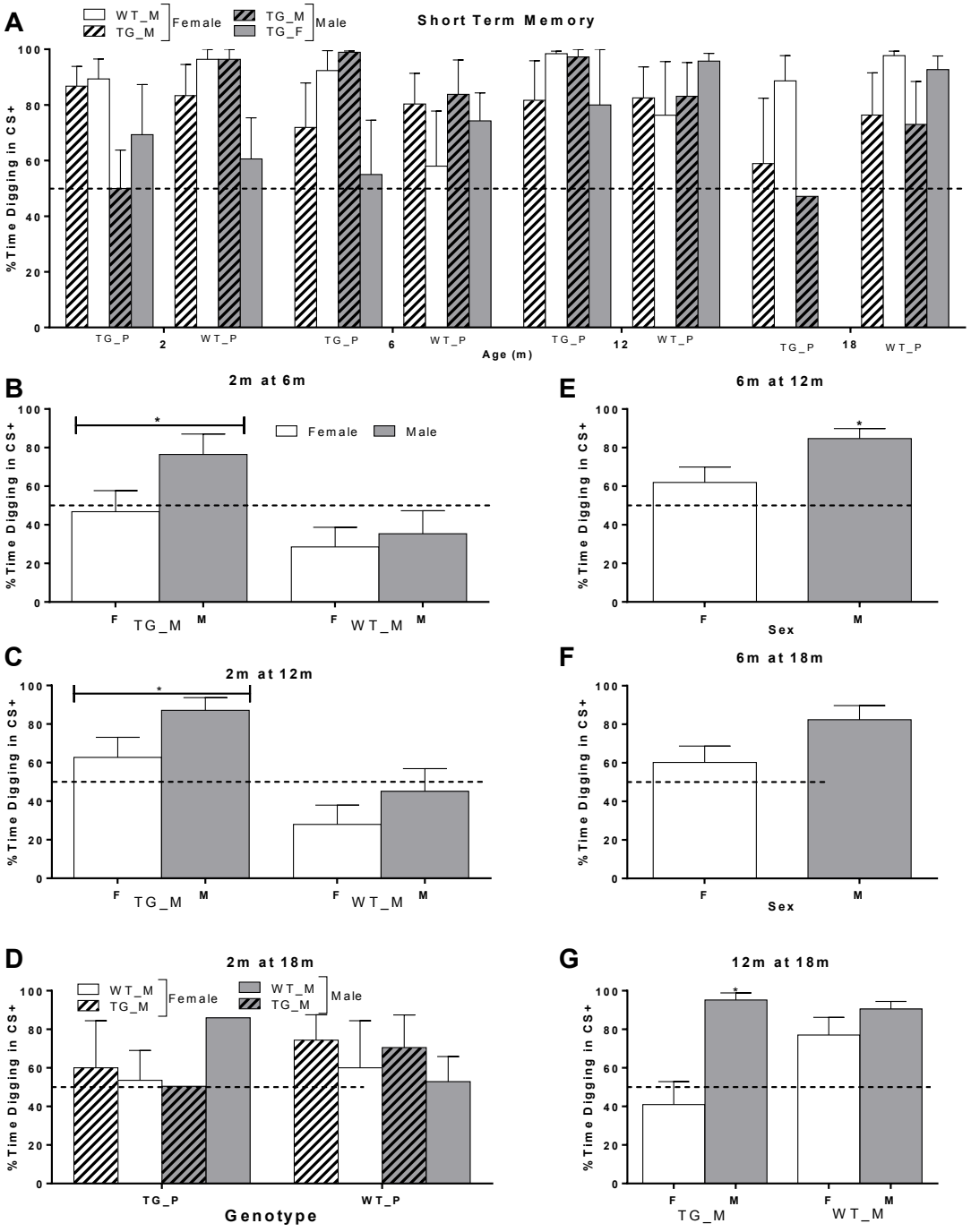

Figure 4.2 Conditioned Odour Preference Task. The mean (± S.E.M) percentage of time spent digging the correct odour cup at each age for short-term memory (A), the 2 month odour at 6 months (B), the 2 month odour at 12 months (C), the 6 month odour at 12 months (D), the 2 month odour at 12 months (E), the 6 month odour at 18 months (F), and the 12 month odour at 18 months (G) in in 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers.

4.4.3 CORRELATIONS OF NEUROPATHOLOGY AND BEHAVIOUR

We correlated behavioural measures at 18 months of age with a genotype difference with levels of amyloid beta staining in the cortex, hippocampus, and amygdala, and tau staining in the hippocampus and amygdala at 19 months of age (Table 4.2). The only significant correlation was a negative relationship between the density of tau positive neurons in the amygdala and swim speed the acquisition phase of the MWM ($r = -0.530$, $p = 0.042$, $n = 15$).

Table 4.2 Correlation of Neuropathology and Behaviour. The Pearson's-r values for correlations between measures levels of amyloid beta and tau in the brain of 19 month old 3xTg-AD mice and behavioral measures with a genotype difference at 18 months of age. A '*' indicates a significant correlation ($p < 0.05$).

	MWM Acquisition		**MWM Probe**
Neuropathology Measure	**Distance**	**Swim Speed**	**Correct Quadrant time**
Hippocampus Tau Density (#/µm3)	0.122	-0.257	-0.404
Amygdala Tau Density (#/µm3)	-0.298	-0.530*	-0.342
Hippocampus Aβ Coverage (%)	0.245	-0.080	0.264
Amygdala Aβ Coverage (%)	0.352	-0.057	0.110
Cortex Aβ Coverage (%)	0.313	0.195	0.045

4.4.4 EFFECT SIZES

We calculated a Cohen's d with a Hedge's correction for an unbiased measure of effect size for all measures with a CI that indicated there was a difference between pup genotype, foster mother genotype, or sex (Tables 4.3, 4.4, and 4.5, respectively). For genotype the largest effect was that the 3xTg-AD swam faster than the B6129SF2 mice during acquisition and reversal in the MWM ($d_{unb} = 0.544$, $CI_{95} = 0.402 – 0.686$; $d_{unb} = 0.921$, $CI_{95} = 0.775 – 1.068$), followed by the decreased amount of time spent in the

correct quadrant during the probe of the MWM relative to B6129SF2 mice at 12 months of age (d_{unb} = -0.601, CI_{95}= -1.105 – 0.097).

Table 4.3 Genotype Effect Size Estimates. Cohen's d calculated with a pooled SD and Hedges correction for all models that included genotype and had a CI that provided evidence for an effect. Positive values indicate 3xTg-AD mice had higher scores than B6129SF2 wildtype mice and negative scores indicate B6129SF2 wildtype mice had higher scores than 3xTg-AD mice. A '#' indicates that the confidence interval includes zero.

Measure	d_{unb}	95% Confidence Interval	
3xTg-AD higher than B6129SF2		Lower	Upper
MWM - Reversal Swim Speed	0.921	0.775	1.068
MWM - Acquisition Swim Speed	0.544	0.402	0.686
MWM - Acquisition Distance	0.417	0.277	0.556
MWM - Acquisition Latency	0.334	0.196	0.473
MWM - Reversal Distance	0.321	0.182	0.460
B6129SF2 higher than 3xTg-AD			
MWM - Probe Correct Quadrant Time (12m)	-0.601	-1.105	-0.097

For sex the largest effects were the better long term memory of male mice compared to female mice at 12 and 18 months of age for previous odours in the COPT (12m odour at 18m: d_{unb} = 0.898, CI_{95}= 0.245 – 1.551; 6m odour at 12m: d_{unb} = 0.560, CI_{95}= 0.066 – 1.054).

Table 4.4 Sex Effect Size Estimates. Cohen's d calculated with a pooled SD and Hedges correction for all models that included sex and had a CI that provided evidence for an effect. Positive values indicate male mice had higher scores than female mice and negative scores indicate female mice had higher scores than male mice. A '#' indicates that the confidence interval includes zero.

Measure	d_{unb}	95% Confidence Interval	
Male higher than Female		Lower	Upper
COPT - 12m odour at 18m	0.898	0.245	1.551
COPT - 6m odour at 12m	0.560	0.066	1.054
COPT - 2m odour at 12m	0.459	-0.032#	0.951

For foster mother genotype the largest effects were again in the COPT long term olfactory memory, with the mice reared by 3xTg-AD mothers performing better than the

mice reared by B6129SF2 mothers at on the 2 month odour at 6 and 12 months (12m: $d_{unb} = 0.894$, $CI_{95} = 0.386 – 1.403$; 6m: $d_{unb} = 0.585$, $CI_{95} = 0.111 – 1.058$).

Table 4.5 Foster Mother Genotype Effect Size Estimates. Cohen's d calculated with a pooled SD and Hedges correction for all models that included foster mother genotype and had a CI that provided evidence for an effect. Positive values indicate mice reared by 3xTg-AD mothers had higher scores than mice reared by B6129SF2 mothers and negative scores indicate mice reared by B619SF2 mothers had higher scores than mice reared by 3xTg-AD mothers. A '#' indicates that the confidence interval includes zero.

Measure	d_{unb}	95% Confidence Interval	
3xTg-AD Mothers higher than B6129SF2 Mothers		Lower	Upper
COPT - 2m odour at 12m	0.894	0.386	1.403
COPT - 2m odour at 6m	0.585	0.111	1.058
B6129SF2 Mothers higher than 3xTg-AD Mothers			
COPT - 12m odour at 18m	-0.601	-1.232	0.030#
MWM - Acquisition Distance	-0.237	-0.375	-0.100
MWM - Acquisition Thigmotaxis	-0.232	-0.372	-0.093
MWM - Acquisition Latency	-0.156	-0.294	-0.019

4.5 DISCUSSION

Due to the longitudinal nature of this study, and the repeated measures design of the tasks we focus here on findings that followed a consistent pattern to decrease the influence of spurious results from multiple tests.

In the Morris water maze all mice learned the task as latency, distance, and thigmotaxis decreased over days in both phases of the task. The 3xTg-AD mice had a deficit in learning as they had longer distances to reach the platform during both acquisition and reversal, which is consistant with previous research (Billings et al., 2005, 2007; Clinton et al., 2007; McKee et al., 2008; Movsesyan et al., 2008; Corona et al., 2010; García-Mesa et al., 2011; Chen et al., 2013; Marchese et al., 2014). The 3xTg-AD mice swam faster than the B6129SF2 mice during both phases, though the difference was less consistent in reversal, where it was only present in females and at 18 months of age it

was no longer present. The enhanced swim speed of the 3xTg-AD mice is consistent with previous findings of enhanced motor performance in the Rotarod (Blanchard et al., 2010; Chen et al., 2013; Oore et al., 2013; Stover et al., 2015a). Mice reared by 3xTg-AD foster mothers also swam faster than those reared by B6129SF2 foster mothers which suggests a long-term maternal effect of motor behaviour. The increased swim speed of the 3xTg-AD mice relative to the B6129SF2 mice can confound the latency measure, so distance should be used instead of latency as a measure of learning in this strain. The amount of thigmotaxis decreased after the first day of acquisition and with age, which may explain why many of the differences in thigmotaxis were only present on day 1. The 3xTg-AD mice exhibited more thigmotaxis than B6129SF2 mice on day 1 and 2 of acquisition at 6 months of age, which provides further evidence of a deficit in spatial learning, as thigmotaxis is a strategy that demonstrates no knowledge of the task. At two months of age only, mice reared by B6129SF2 mothers exhibited more thigmotaxis, and at 2 and 18 months of age mice reared by B6129SF2 foster mothers took a longer distance to reach the platform in acquisition which may indicate that they have a deficit in spatial learning.

Although there is at least one report of an age-dependant sex difference in 3xTg-AD mice in the MWM where females have a larger deficit than males before 12 months of age, with the deficit disappearing at later ages (Clinton et al., 2007), we found no overall sex effects in learning or memory across the lifespan. It is possible that the effect of previous training in the MWM was large enough to mask any sex difference. Billings et al. (2007) compared MWM performance between cross-sectional and longitudinal designs and found that the effect of previous learning improved MWM performance to the point where at earlier ages there was no deficit in 3xTg-AD mice, but by 12 months

of age the deficit was detectable and by 15 months of age there was no longer an improvement in the longitudinal group relative to the cross-sectional group. Marchese et al. (2014) also performed and longitudinal study on the 3xTg-AD mice in the MWM and found deficits from 2.5 to 12 months of age. In our study we found a consistent deficit in learning in the MWM, though the deficit in memory was only present at 6 and 12 months of age. It is possible that that at 18 months of age any memory deficit in the 3xTg-AD mice was masked by the effect of previous training, though Billings et al. (2007) found little effect of previous training at 15 months of age. Another possible explanation is that the age-related decline in cognitive function of the B6129SF2 mice has caused their memory to degrade to the point where they perform similarly to the 3xTg-AD at 18 months of age, as both groups performed just above chance (Figure 4.4.5). Lastly the low number of animals surviving at 18 months of age (Table 4.1) may have decreased our statistical power to the point where we were not able to detect a genotype difference.

In the conditioned odor preference short term memory task all mice performed well and there were no differences between any groups in short term memory. There were several differences in the measures of long term memory, but overall males tended to perform better than females. Mice reared by B619SF2 mice had a deficit relative to mice reared by 3xTg-AD mothers in two long term olfactory memory test (2 month odour at 6 months, and 2 month odour at 12 months), and in one test (12 month odour at 18 months) the effect was reversed and mice reared by 3xTg-AD mothers had a deficit relative to mice reared by B6129SF2 mothers.

We found a negative correlation between the density of tau positive neurons in the amygdala and swim speed the acquisition phase of the MWM. The implication of this

relationship is unclear; it may be a spurious result given the number of correlations calculated. Though some studies have shown that in humans there is little relationship between amyloid beta plaque levels and behavioural deficits, there is some evidence that tau pathology is correlated with behavioural deficits as there is a temporal correlation with the development of tau pathology and behavioural deficits as well as a correlation between the amount of tau pathology and the severity of the behavioural deficits (Snowdon, 1997; Tiraboschi et al., 2004). However, there is also evidence that soluable Aβ itelf causes synaptic dysfunction and behavioural deficits, and that the Aβ we currently measure are not necessicaly representative of soluable Aβ levels (Cleary et al., 2005). In the 3xTg-AD mice there is also evidence of a temporal correlation between the development of amyloid beta neuropathology in certain areas of the brain and deficits associated with those areas (Billings et al., 2005), though the longitudinal design of our study prevented us from evaluating the temporal progression of neuropathology in our animals.

The act of cross fostering itself can have lasting effects on mice. Bartolomucci et al. (2004) compared cross fostered CD-1 mice to normally reared mice and found that the cross fostered mice had increased weight and at three months of age the male mice exhibited less anxiety-like behaviour. Several other studies have shown that cross fostering between strains can influence learning and other behaviours later in life (Zaharia et al., 1996; Francis et al., 2003; Priebe et al., 2005). It is therefore possible that some of the effects we observed could be due to the stress of cross fostering itself. A cross-sectional study performed in our lab on the 3xTg-AD mice found that the 3xTg-AD mice had deficits in reference and working memory in the 8-arm radial maze from 2 to 15

months of age, which demonstrates the effect of our longitudinal design and cross fostering did not mask the cognitive deficits found in the 3xTg-AD mice (Stevens and Brown, 2014).

The 3xTg-AD mice have a deficit in spatial learning, an age-dependent deficit in spatial memory, and the mice reared by B6129SF2 mothers appear to have a deficit in both spatial and olfactory memory, though this finding was not consistent. In the MWM there was evidence of age-related cognitive decline in all mice by 18 months of age. Despite the deficit of the 3xTg-AD mice in spatial memory, there was no genotype difference in short or long term olfactory-dependent memory. Short term olfactory-dependent memory did not change with age. There was no consistent difference between the sexes in spatial memory (but male mice tended to have better olfactory memory than female mice). The 3xTg-AD are useful for modeling the spatial learning deficits of AD across the life span and the memory deficits of AD at 6 and 12 months of age in the MWM and learning deficits across the life span, though the effect of maternal genotype was not large enough to mask these differences.

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4.8 SUPPLEMENTAL TABLES

These supplemental tables provide the statistics used to determine the best model for each measure. The top five models for each measure are included. The model column describes the factors included in the model, terms separated by a '*' that there were main effects and interactions for the terms, those separated by a '+' are simple main effects, and those separated by a ':' indicate an interaction alone. The 'AICc' column contains the second order Akaike information criterion, which is a measure used to evaluate the models based on the complexity and goodness of fit of the model to the data, with lower values being better. The "Δ AICc" column is difference between that model's AICc and the model with the lowest AICc. The 'Wt' column is the Akaike weight, a measure of relative likelihood that the model is the best, ranging from 0, meaning unlikely, to 1, meaning likely. The 'ER' column is the evidence ratio, it is the likelihood that the model with the lowest AICc is better than that model.

Supplemental Table 4.1.1 MWM – Time in Correct Quadrant

Model	**AICc**	**Δ AICc**	**AICcWt**	**ER**
Genotype*Age	2057.589	0	0.087	1
(Genotype+Sex+Age)+(Genotype:Age)	2059.525	1.935	0.033	2.632
(Genotype*Age+FMGenotype	2059.679	2.090	0.030	2.843
(Genotype+Sex+Age)+(Genotype:Age)+ (Sex:Age)	2060.416	2.827	0.021	4.110
(Genotype+Sex+Age)+(Genotype:Sex)+ (Genotype:Age)	2060.43	2.841	0.021	4.140

Supplemental Table 4.1.2 MWM – Correct Annulus Crossings

Model	**AICc**	**Δ AICc**	**AICcWt**	**ER**
Age	1195.234	0	0.126	1
Genotype+Age	1196.791	1.558	0.058	2.179
Sex+Age	1196.914	1.681	0.054	2.317
FMGenotype+Age	1197.199	1.965	0.047	2.672
Sex*Age	1197.583	2.350	0.038	3.237

Supplemental Table 4.2.1 COPT – Short Term Memory

Model	AICc	Δ AICc	AICcWt	ER
Null	2649.318	0	0.089	1
Sex	2650.116	0.798	0.060	1.490
Age	2651.058	1.740	0.037	2.387
FMGenotype*Sex	2651.133	1.815	0.036	2.478
FMGenotype	2651.257	1.939	0.034	2.637

Supplemental Table 4.2.2 COPT – 2m Odour at 6m

Model	AICc	Δ AICc	AICcWt	ER
FMGenotype+Sex	108.301	0	0.233	1
FMGenotype	108.896	0.595	0.173	1.347
FMGenotype*Sex	109.453	1.152	0.131	1.779
Genotype+FMGenotype+Sex	110.212	1.911	0.090	2.599
Genotype+FMGenotype	110.574	2.273	0.075	3.115

Supplemental Table 4.2.3 COPT – 2m Odour at 12m

Model	AICc	Δ AICc	AICcWt	ER
FMGenotype+Sex	724.593	0	0.287	1
Genotype+FMGenotype+Sex	726.170	1.577	0.130	2.200
Genotype*FMGenotype+Sex	726.240	1.648	0.126	2.279
FMGenotype*Sex	726.772	2.179	0.096	2.973
FMGenotype	726.903	2.310	0.090	3.174

Supplemental Table 4.2.4 COPT – 6m odour at 12m

Model	AICc	Δ AICc	AICcWt	ER
Sex	75.503	0	0.372	1
Genotype+Sex	77.313	1.810	0.151	2.472
FMGenotype+Sex	77.628	2.125	0.129	2.894
Null	78.869	3.366	0.069	5.383
Genotype * Sex	79.459	3.956	0.051	7.229

Supplemental Table 4.2.5 COPT – 2m odour at 18m

Model	AICc	Δ AICc	AICcWt	ER
Null	480.892	0	0.350	1
FMGenotype	482.240	1.349	0.178	1.963
Genotype	482.945	2.053	0.125	2.791
Sex	483.182	2.290	0.111	3.143
Genotype+FMGenotype	484.507	3.615	0.057	6.096

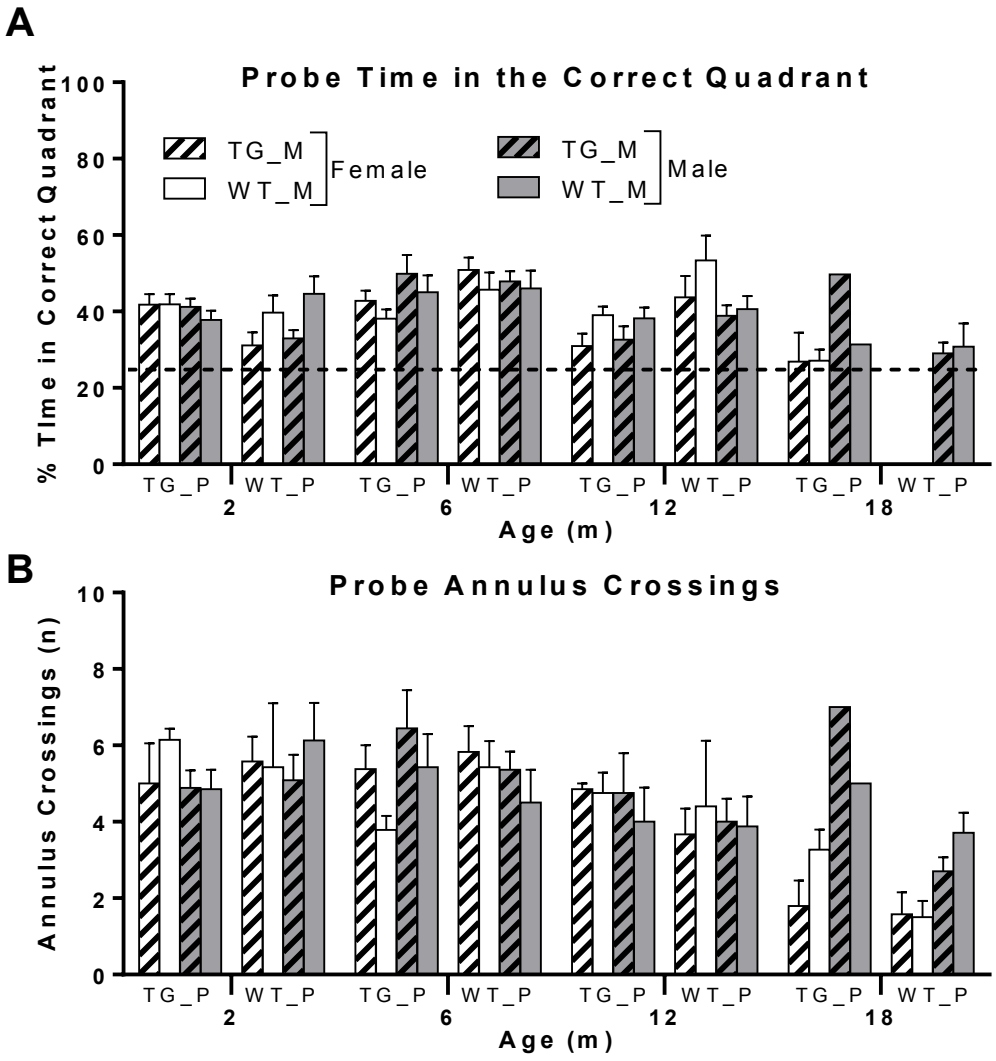
Supplemental Table 4.2.6 COPT – 6m odour at 18m

Model	**AICc**	**Δ AICc**	**AICcWt**	**ER**
Sex	475.468	0	0.237	1
Null	476.508	1.040	0.141	1.682
FMGenotype+Sex	476.591	1.123	0.135	1.753
Genotype	477.324	1.856	0.094	2.529
Genotype+Sex	477.674	2.207	0.079	3.014

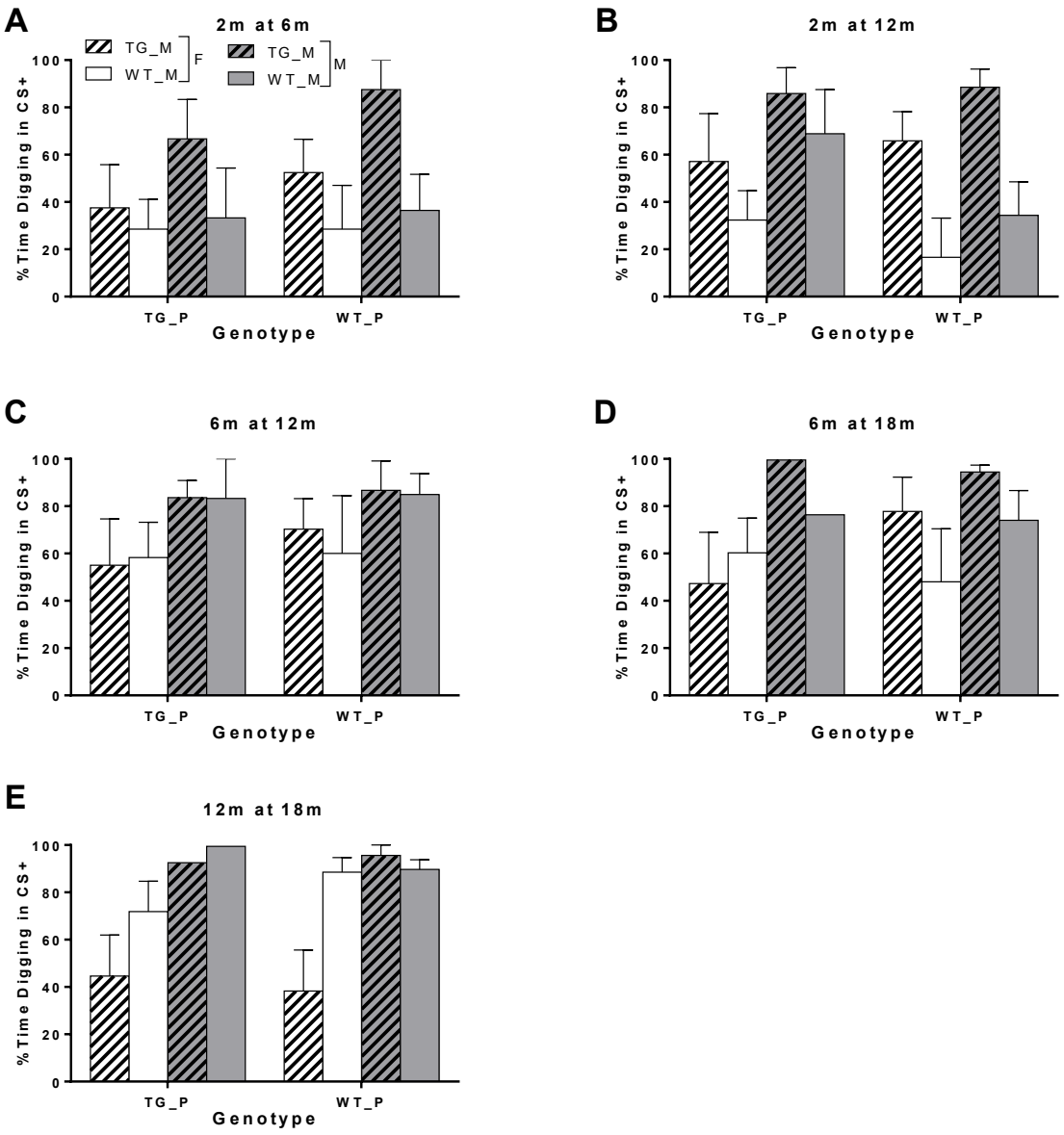
Supplemental Table 4.2.7 COPT – 12m odour at 18m

Model	**AICc**	**Δ AICc**	**AICcWt**	**ER**
FMGenotype*Sex	454.430	0	0.450	1
FMGenotype+Sex	456.583	2.153	0.153	2.934
Genotype+FMGenotype*Sex	456.938	2.508	0.128	3.504
Sex	458.695	4.264	0.053	8.434
Genotype+FMGenotype+Sex	459.107	4.676	0.043	10.363

4.9 SUPPLEMENTAL FIGURES



Supplemental Figure 4.1 Morris Water Maze Probe Trial. The mean (± S.E.M) percentage of time spent in the correct quadrant (A) and number of annulus crossings at each age of the probe trial in the Morris water maze mouse of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers.



Supplemental Figure 4.2 Conditioned Odour Preference Task. The mean (± S.E.M) percentage of time spent digging the correct odour cup at each age for the 2 month odour at 6 months (A), the 2 month odour at 12 months (B), the 6 month odour at 18 months (C), and the 6 month odour at 18 months (D) and the 12 month odour at 18 months (E) in in 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers.

CHAPTER 5 AGE-RELATED CHANGES IN SOCIAL BEHAVIOUR IN THE 3XTG-AD MOUSE MODEL OF ALZHEIMER'S DISEASE FROM 2 TO 18 MONTHS OF AGE

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5.1 ABSTRACT

Patients with AD develop a number of behavioural symptoms associated with a decrease in social behaviour including apathy, depression, and anxiety. Additionally the cognitive deficits of AD can make maintaining social interactions difficult as symptoms progress. Ideally a mouse model of AD would replicate these deficits. We tested the 3xTg-AD mouse model of AD longitudinally at 2, 6, 12, and 18 months of age using a behavioural test battery to assess social behaviour. We found that male mice were more aggressive than female mice and that there was no consistent change in social behaviour with age. There was some indication that mice reared by B6129SF2 mothers had reduced aggression compared to mice reared by 3xTg-AD mothers in home cage observations. Interestingly neither the 3xTg-AD nor the B6129SF2 mice exhibited a preference for social novelty, which indicates that this is not an appropriate test to assess social behaviour in this strain. Overall the 3xTg-AD mice do not appear to have a deficit in social behaviour compared to the B6129SF2 mice.

5.2 INTRODUCTION

Patients with AD develop a number of behavioural symptoms associated with a decrease in social behaviour including apathy, depression, and anxiety. Additionally the cognitive deficits of AD can make maintaining social interactions difficult as symptoms progress (Devanand et al., 1996; Teri et al., 1999; Ferretti et al., 2001; Landes et al., 2001). Ideally a mouse model of AD would replicate these deficits. A study of social behaviour in the 3xTg-AD mice using a social recognition task found that the 3xTg-AD mice had impaired social recognition compared to control mice at 18 months of age (Medeiros et al., 2011). Other studies have found deficits in social behaviours in the APPswe/PS1dE9 and 5xFAD mouse models of AD (Filali et al., 2011; Flanigan et al., 2014). These deficits may be the result of either decreases in social behaviour or deficits in cognitive function that impair the memory required for social recognition.

The 3xTg-AD mouse is a commonly used model of Alzheimer's disease (AD) with three transgenes, two associated with familial AD (APP_{swe} and $PS1_{M146V}$) and one with tau pathology (Tau_{P301L}). As a result of these transgenes the 3xTg-AD develops a number of behavioural and neuropathological deficits (Oddo et al., 2003). To create homozygous genotypes 3xTg-AD females are bred with 3xTg-AD males, and the in the B6129SF2 control strain, the B6129SF2 females are bred with B6129SF2 mice. This results in pups only being reared by mothers of the same genotype. Maternal care can have a long term effect on pup behaviour, and strain differences in maternal care could result in neural and behavioural differences in adulthood (Priebe et al., 2005; Szyf et al., 2007). We cross-fostered litters of 3xTg-AD and B6129SF2 mice to study the effect of maternal genotype. We then assessed the mice on a neurodevelopmental test battery

before weaning (Blaney et al., 2013). In this study we conducted a longitudinal study on the mice at 2, 6, 12, and 18 months. The mice also underwent a behavioural test battery to assess prepulse inhibition and anxiety-like, motor, and cognitive behaviours (Stover et al., 2015c, 2015d, 2015e).

5.3 METHODS

2.3.1 BREEDING, CROSS-FOSTERING & PRE-WEANING TREATMENT OF MICE

We used four breeding pairs each of 3xTg-AD mice (B6;129-Psen1tm1Mpm Tg(APPSwe,tauP301L)1Lfa/Mmjax) and B6129S/F2 mice, which were purchased from Jackson Laboratories (Strain #004807 and #004807, respectively, Bar Harbor, Maine). All procedures used in this experiment were approved by the Dalhousie University Committee on Animal Care.

In order to examine maternal effects on development we cross fostered all litters at post natal day 0 (the day of birth) or 1, so that half the mice of each genotype had 3xTg-AD foster mothers and half had B6129SF2 foster mothers, and no mother reared her own pups. These mice were tested in a neurodevelopmental test battery (Blaney et al., 2013).

The mice were housed in same sex groups of two to four foster-littermates in clear plastic cages measuring (18.75 x 28 x 12.5 cm), with wire tops, wood chip bedding, and a PVC tube (4 cm diameter x 7 cm length) for enrichment. The colony room was kept at 22±2 °C on a reversed 12:12 light:dark cycle, with lights off at 10:00am. The mice were fed Purina 5001 rodent chow (Purina, St. Louis, Missouri) and tap water ad libitum.

5.3.2 PROCEDURE

Beginning at 2 months of age we tested 78 mice, 40 3xTg-AD and 38 B6129SF2, with approximately equal numbers of each sex, however some mice died between the testing periods (Table 5.1). The mice were tested in three cohorts of approximately the same size at two, six, twelve, and eighteen months of age in a longitudinal design. Behavioural testing took place during the dark phase of the light:dark cycle. For the behavioural tasks the experimenters were blind to the genotype and foster mother genotype of the mice; it was not possible to blind the experimenters to the age due to the longitudinal design. The tests were performed in the order described below, except between home cage observations and social preference / novelty the mice underwent a test battery to assess motor and anxiety-like behaviour (See Stover et al., 2015c). The tests were ordered from least to most stressful to attempt to decrease the effect of stress.

Table 5.1 Distribution of mice by pup genotype and maternal genotype at each age.

Pup Genotype	**Maternal Genotype**		
2 Months of Age	**B6129SF2**	**3xTg-AD**	**Total**
B6129SF2	11M, 7F	8M, 12F	38
3xTg-AD	8M, 14F	9M, 9F	40
Total	40	38	78
6 Months of Age			
B6129SF2	11M, 7F	8M, 12F	38
3xTg-AD	7M, 14F	9M, 8F	38
Total	39	37	76
12 Months of Age			
B6129SF2	11M, 5F	8M, 12F	36
3xTg-AD	6M, 13F	9M, 8F	36
Total	35	37	72
18 Months of Age			
B6129SF2	10M, 5F	7M, 7F	29
3xTg-AD	1M, 11F	2M, 5F	19

5.3.4 HOME CAGE OBSERVATIONS

Home cage observations were completed to study social behavior using a procedure adapted from D'Andrea et al. (2007). To aid in identification of the mice we marked them with a black non-toxic maker in unique patterns. The home cage observations took place in the colony room during the dark phase of the light:dark cycle and the cages were illuminated using a red 60 W light. Behaviours were scored using one-zero sampling with time sampling every two minutes. No more than five cages were scored at one time and there were 10 minutes between each set of observations per cage. If fewer than five cages were observed breaks were added to maintain the 10 minute break between observations. We completed three sets of observations in one day which were evenly spaced over the 12 hours of the dark phase of the light:dark cycle. Three categories of behaviour were recorded: affiliative, agonistic, and non-social (Grant and Mackintosh, 1963). The affiliative behaviours were: sniffing (touching a cage mate with their snout) and social grooming (stroking with paws or licking the fur of a cage mate). The agonistic behaviours were: attacking (biting, striking with paws), aggressive grooming (aggressively grooming a cage mate), offensive upright posture (on hind limbs facing a cage mate), defensive upright posture (on hind limbs pushing against an attacking cage mate), submissive upright posture (on hind limbs turned away from an attacking cage mate), crouched posture (lying on floor of cage with head touching cage), freezing (only respiration), fleeing (quickly moving away from a cage mate), and tail rattling (pointing the tail upwards and moving it from side to side). The non-social behaviours were: wall rearing (placing one or both fore paws against the wall of the cage with the hind paws on the floor of the cage), self-grooming, immobility, brief contact

with a cage mate, eating, and drinking. The frequency of each category of behaviour was analyzed at the three time points at each age.

5.3.5 SOCIAL NOVELTY/PREFERENCE

To measure social behaviour we used the social novelty/preference test using a procedure adapted from Moy et al. (2004) and (Pearson et al., 2010). The apparatus was an open box (69 x 20 x 20 cm) made of clear plastic with three chambers (each 23 x 20 x 20 cm) connected by openings in the walls (6 x 5.5 cm). We used two male C57BL/6J (Stock Number: 000664, Jackson Laboratories, Bar Harbour, ME) as stimulus mice (Mouse A & B). There were two phases: in phase one mouse A was placed in a wire cylinder in the center of one end chamber and a small plastic toy was placed in a wire cylinder in the center of other end chamber. To begin the trials the mouse being tested was placed in the centre chamber, and was allowed to explore the apparatus for ten minutes. In phase two mouse A was moved to the opposite end chamber and a novel stimulus mouse, mouse B, was placed in the other end chamber. The same procedure was repeated and the amount of time spent interacting with each mouse was recorded. Mice will normally express a preference for the novel mouse and spend more time interacting with the novel mouse. There was a one hour interval between phase one and phase two. The mice were tested individually in groups of four, all four mice completed phase one before beginning phase two. The apparatus was cleaned with ethanol between mice. A novel mouse preference score was calculated for the amount of time spent interacting with each mouse in phase two $\left(\frac{A-B}{A+B}\right) * 100$.

5.3.6 TUBE TEST OF SOCIAL DOMINANCE

To study social behaviour and aggression we used the social dominance tube method adapted from Koh et al. (2008) and Lijam et al. (1997). The tube test apparatus was made of clear Plexiglas, had two holding chambers (10 x 10 cm) which were connected by a tube (30 long x 3 cm diameter), blocked by a removable piece of Plexiglas. The apparatus was mounted on a wooden board (12.5 x 58 cm) painted white. To begin a trial the two mice were placed in the opposing holding chambers and the tube was opened. If one mouse forced the other to back out of the tube it was considered the "winner", if no mouse forced the other out of the tube within 10 minutes the trial was considered a draw. Only mice of the same sex were tested, they were only tested against mice who were not cage mates and were of different genotypes. Each pair was given two trials. The mice were matched on weight.

5.3.7 CORRELATIONS OF NEUROPATHOLOGY AND BEHAVIOUR

All measures that had genotype differences were correlated with levels of tau and amyloid beta neuropathology in the brains of the mice at 19 months of age. The methodology used to determine the levels of neuropathology is described in (Stover et al., 2015e). Briefly, the levels of tau pathology were calculated using unbiased stereology and the levels of Aβ were calculated using a thresholding technique.

5.3.8 STATISTICAL ANALYSES

The statistical analyses described in the following section were performed using R (www.R-project.org). For the tube test of social dominance the differences between genotypes were analyzed using a chi-squared test at each age. We used linear mixed effects regression to assess difference between genotype, sex, foster mother genotype,

and age. For social novelty / recognition all models were compared and the model with the lowest second-order Akaike's Information Criterion (AICc) were selected, the top five models for is presented in Supplemental Table 5.1. For home cage observations, which has a repeated measures design, models were selected using backward elimination. The best model was compared to the null model with a chi-square test. When there were significant effects of genotype, sex, or foster mother genotype, we calculated unbiased effect sizes using Cohen's d with a hedges correction. The correlations between levels of neuropathology and behaviour were calculated using Pearson's r.

5.4 RESULTS

Several mice died over the testing period, see Table 5.1 for the total number of mice at each age.

5.4.1 HOME CAGE OBSERVATIONS

The best linear mixed effects model for the number of affiliative behaviours had pup genotype, foster mother genotype, age, and a foster mother genotype by age interaction, which was significantly different from the null model (AICc=1523.094; $\chi 2(8, N= 768)= 106.67$, $p < 0.001$; Figure 5.1 A-D; and Supplemental Figure 5.1 A). Overall B6129SF2 mice exhibited more affiliative behaviours than 3xTg-AD mice (CI_{95}= 0.018 – 0.215 behaviours), and there was some evidence that mice reared by B6129SF2 mothers exhibited more affiliative behaviours than mice reared by 3xTg-AD mothers (CI_{95}= -0.002 – 0.212 behaviours). The number of affiliative behaviours decreased from 2-6 months of age (CI_{95}= -0.311 -0.083 behaviours), and increased from 2-12 (CI_{95}= 0.189 – 0.427 behaviours) and 2-18 (CI_{95}= 0.220 – 0.515 behaviours) months of age. The foster mother genotype by age interaction occurred because at both 2 and 12 months of age

mice reared by B6129SF2 mice exhibited more affiliative behaviours than mice reared by 3xTg-AD mice (CI_{95}= 0.005 – 0.344 behaviours, and CI_{95}= 0.114 – 0.472 behaviours, respectively).

For the number of agonistic behaviours the best model had time point, pup genotype, foster mother genotype, sex, age, a time point by foster mother genotype interaction, a foster mother genotype by age interaction, and a pup genotype by sex by age interaction, which was significantly different from the null model (AICc=971.4635; $\chi 2(23, N= 768)= 78.033$, $p < 0.001$; Figure 5.1 F-H). Confidence intervals provided little evidence of a main effect of time point, genotype (CI_{95}= -0.150 –0.178), or foster mother genotype (CI_{95}= -0.122 – 0.005 behaviours). Males exhibited more agonistic behaviour than females (CI_{95}=.0497 – 0.374 behaviours), and mice exhibited more agnostic behaviours at six months than at any other age (6-2: CI_{95}= 0.130 – 0.444, 6-12: CI_{95}= 0.069 – 0.393, 6-18: CI_{95}= 0.034 – 0.368 behaviours). The time point by foster mother genotype interaction occurred because only at the first time point mice reared by B6129SF2 mothers exhibited more agonistic behaviours than mice reared by 3xTg-AD mothers (CI_{95}= 0.005 – 0.292 behaviours). The foster mother genotype by age interaction occurred because at 2 and 12 months only the mice reared by B6129SF2 mothers exhibited fewer agonistic behaviours than mice reared by 3xTg-AD mothers (2: CI_{95}= -0.293 – -0.062, 12: CI_{95}= -0.271 – -0.034 behaviours).

The best model for non-social behaviours had time point, pup genotype, foster mother genotype, sex, age, a time point by sex by age interaction, and a pup genotype by foster mother genotype by sex interaction, and was significantly different from the null model (AICc = 2321.035; $\chi^2(39, N= 768)= 128.37$, $p > 0.001$); Figure 5.1 I-L). There

were no overall pup genotype, foster mother genotype, or sex differences (CI_{95}= -0.250 – 0.429, CI_{95}= -0.131 – 0.451, and CI_{95} = -0.729 – 0.393 behaviours). There were fewer non-social behaviours at the first time point than at the second (CI_{95}= -1.091 – -0.036 behaviours). The number of non-social behaviours was higher at 12 months of age than at 6 or 18 months of age (CI_{95}= 0.416 – 1.420, CI_{95}= 0.280 – 1.535 behaviours). The sex by foster mother genotype by pup genotype interaction occurred because in male mice reared by B6129SF2 mothers only, the B6129SF2 mice exhibited more non-social behaviours than the 3xTg-AD mice (CI_{95}= 0.305 – 0.910 behaviours). The time point by age by sex interaction occurred because at six months of age during the first time point only, females exhibited more non-social behaviours than male mice (CI_{95}= 0.470 – 1.423 behaviours).

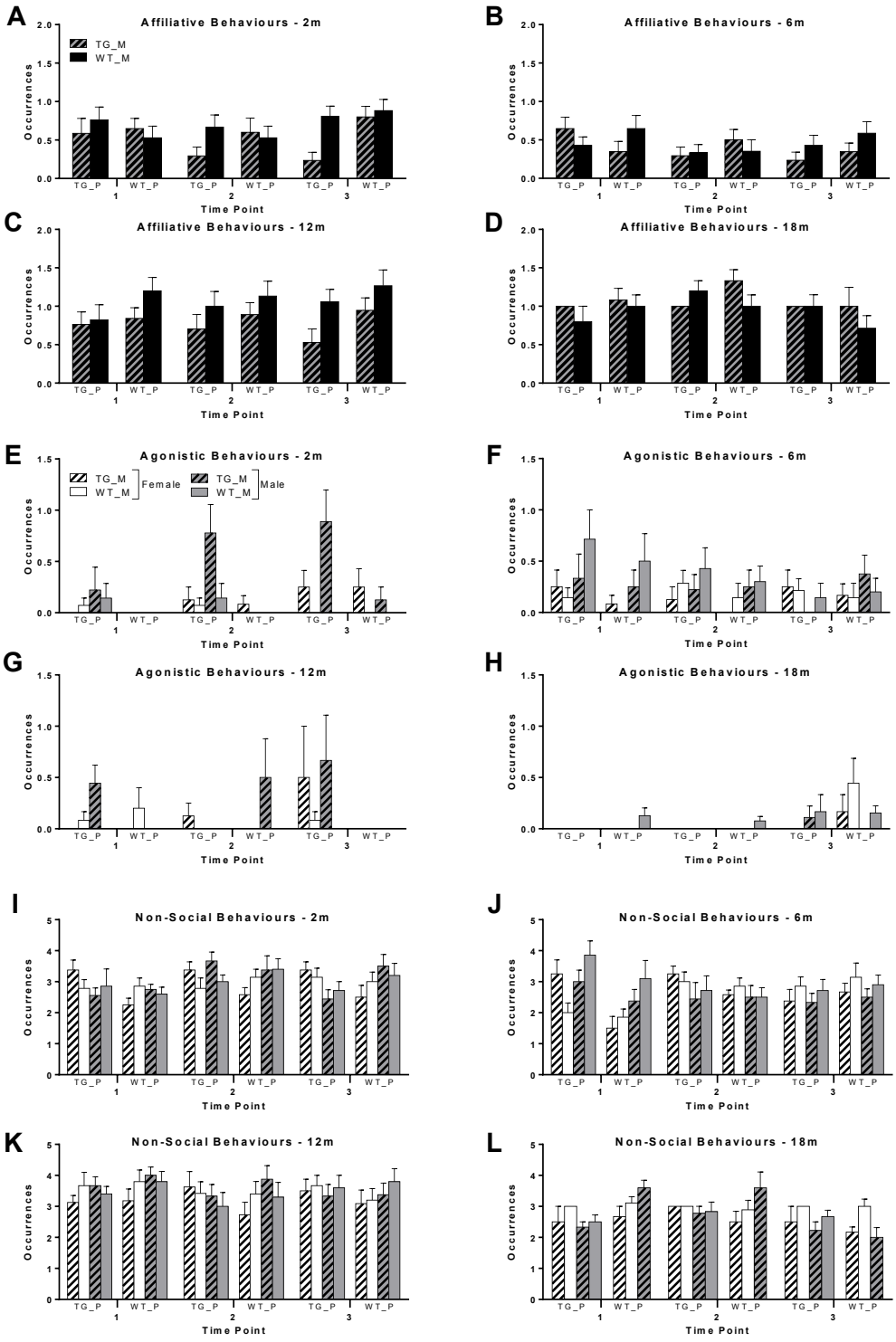

Figure 5.1 Home Cage Observations. The mean (± S.E.M) number of affiliative (A-D), agonistic (E-H), and non-social (I-L) behaviours at each age of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers during home cage observations.

5.4.2 SOCIAL NOVELTY/PREFERENCE

The best model for the preference score for the amount of time spent interacting with the novel or familiar mouse had both pup genotype and foster mother genotype, and was significantly different from the null model ($\chi^2(2, N= 254)= 8.087$, $p < 0.05$; Figure 5.2; Supplemental Figure 5.2; and Supplemental Table 5.1). The B6129SF2 mice had a higher preference for the novel mouse (CI_{95}= 1.842 – 19.996 %), and there was some evidence that mice reared by B6129SF2 mice had a higher preference for the novel mouse (CI_{95}= -0.369 – 18.748 %), though most mice slightly preferred the familiar mouse over the novel mouse.

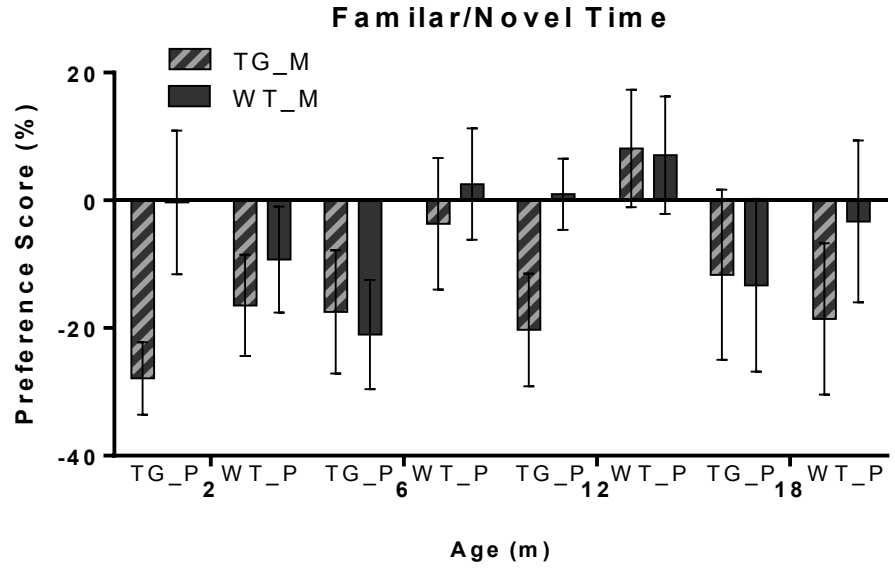

Figure 5.2 Social Novelty / Preference. The mean (± S.E.M) preference score for interacting with the novel mouse of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers.

5.4.3 TUBE TEST OF SOCIAL DOMINANCE

At 2 months of age there was no significant difference between the number of wins between genotypes ($\chi^2(1, N= 76)= 0.819$, $p > 0.05$), and at 6 months of age each genotype had the same number of wins (Figure 5.3). At 12 months of age the B6129SF2 mice won significantly more often than the 3xTg-AD mice ($\chi^2(1, N= 62)= 4.129$, $p < 0.05$), but at 18 months of age there was again no difference between the genotypes ($\chi^2(1, N= 35)= 0.714$, $p < 0.05$, Figure 5.3).

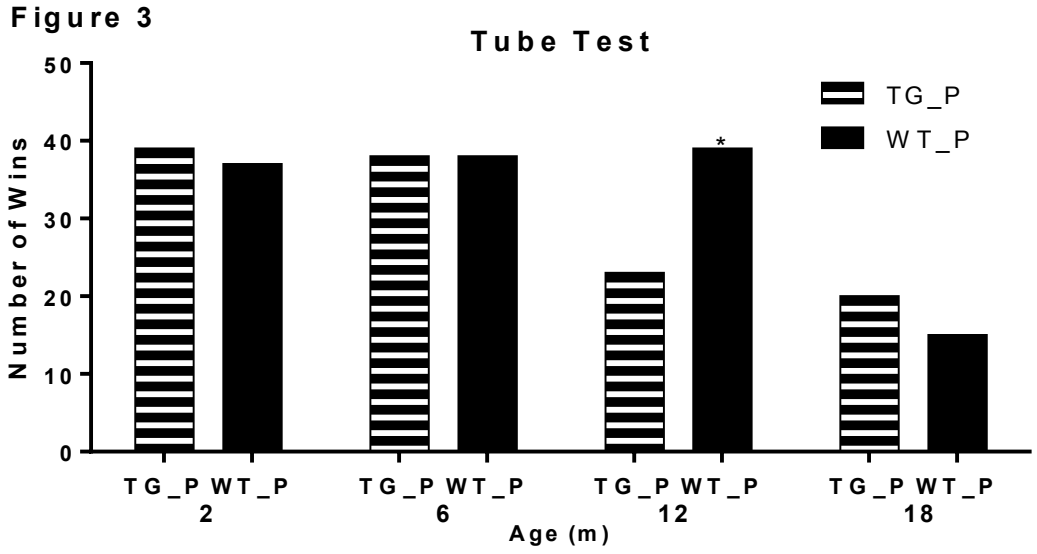

Figure 5.3 Tube Test of Social Dominance. The number of wins of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice at each age. * = $p < 0.05$.

5.4.5 CORRELATIONS OF NEUROPATHOLOGY AND BEHAVIOUR

We correlated behavioural measures at 18 months of age with a genotype difference with levels of amyloid beta staining in the cortex, hippocampus, and amygdala, and tau staining in the hippocampus and amygdala at 19 months of age (Table 5.2). The only significant correlation was a negative correlation between the level of

amyloid beta plaque staining in the cortex and the preference for the novel mouse in the SNSP task ($r = -0.643$, $p = 0.045$, $n = 15$).

Table 5.2 Correlation of Neuropathology and Behaviour. The Pearson's-r values for correlations between measures levels of amyloid beta and tau in the brain of 19 month old 3xTg-AD mice and behavioral measures with a genotype difference at 18 months of age. A '*' indicates a significant correlation ($p < 0.05$).

Neuropathology Measure	HCO Affiliative Behaviours	SNSP Preference Score
Hippocampus Tau Density (#/µm3)	-0.133	0.015
Amygdala Tau Density (#/µm3)	0.036	0.520
Hippocampus Aβ Coverage (%)	-0.450	-0.607
Amygdala Aβ Coverage (%)	-0.515	-0.566
Cortex Aβ Coverage (%)	-0.289	-0.643*

5.4.6 EFFECT SIZES

We calculated a Cohen's d with a Hedge's correction for an unbiased measure of effect size for all measures with a CI that indicated there was a difference between genotype, foster mother genotype, or sex (Table 5.3). For genotype the largest effect was that the B6129SF2 mice had a higher preference for the novel mouse than the 3xTg-AD mice ($d_{unb} = -0.264$, CI_{95}=-0.514 - -0.015). For sex the only effect was the increase in agonistic behaviours in male mice compared to female mice in HCO ($d_{unb} = 0.255$, CI_{95}= 0.112 – 0.398. For foster mother genotype the only effect which had a CI that did not include zero was the increase in affinitive behaviours in mice reared by B6129SF2 mothers compared to mice reared by 3xTg-AD mothers in HCO ($d_{unb} = -0.193$, CI_{95}= -0.336 – -0.051).

Table 5.3 Effect Size Estimates. Cohen's d calculated with a pooled SD and Hedges correction for all models that had a CI that provided evidence for an effect. A '#' indicates that the confidence interval includes zero. HCO = home cage observations, SNSP = social novelty / preference.

Measure	d_{unb}	95% Confidence Interval	
B6129SF2 higher than 3xTg-AD		Lower	Upper
SNSP - Preference Score	-0.264	-0.514	-0.015
HCO - Affiliative Behaviours	-0.208	-0.350	-0.065
Male higher than B6129SF2			
HCO - Agonistic Behaviours	0.255	0.112	0.398
B6129SF2 Mothers higher than 3xTg-AD Mothers			
SNSP - Preference Score	-0.218	-0.466	0.031#
HCO - Affiliative Behaviours	-0.193	-0.336	-0.051

5.5 DISCUSSION

Due to the longitudinal nature of this study, and the repeated measures design of several of the tasks we have chosen to focus on findings that followed a consistent pattern to decrease the influence of spurious results from the multiple tests.

In our assessment of social behaviour using home cage observations we found that the 3xTg-AD mice exhibited less affiliative behaviour, which may indicate that the 3xTg-AD mice have decreased social behaviours, though there were no other genotype differences with other tests. Male mice exhibited more agnostic behaviours than females, which may be an indication of increased aggression in male mice relative to female mice. Interestingly at both 2 and 12 months of age mice reared by B6129SF2 mothers exhibited more affiliative behaviours and fewer agnostic behaviours which may indicate that mice reared by B6129SF2 mice have reduced aggression compared to mice reared by 3xTg-AD mothers. In general the mice did not exhibit a preference for social novelty, as most mice spent more time with the familiar mouse or had no preference (Figure 5.2). The lack of a preference for the novel mouse and high level of variability in this tests indicates that it did not function as intended, as the mice were expected to show a preference for the

novel mouse (Moy et al., 2004). One possible confound in our study of home cage behaviour is the number of mice in each cage, which varied from 2 to 4. Mice who were housed with more cage mates have more opportunity for social interactions (both affiliative and agonistic), which could influence the results. In the tube test of social dominance we found no consistent genotype difference. Male mice were more aggressive than female mice, and there was some evidence that the 3xTg-AD mice have decreased social behaviour relative to B6129SF2 controls. Social withdrawal is a common symptom in early AD (Jost and Grossberg, 1996; Förstl and Kurz, 1999), and the decreased social behaviour of the 3xTg-AD relative to B6129SF2 controls may be a manifestation of this symptom in mice.

We found a negative correlation between the level of amyloid beta plaque staining in the cortex and the preference for the novel mouse in the SNSP task, though that task did not work as intended so interpreting the correlation result is difficult. The correlation may be a spurious effect due to the number of correlations we calculated.

Overall male mice were more aggressive than female mice, and there was some evidence that the 3xTg-AD mice have decreased social behaviour relative to B6129SF2 controls. There was no consistent change in social behaviour with age. There was some indication that mice reared by B6129SF2 mice had reduced aggression compared to mice reared by 3xTg-AD mothers in home cage observations. Interestingly neither the 3xTg-AD nor the B6129SF2 mice exhibited a preference for social novelty, which indicates that this is not an appropriate test to assess social behaviour in this strain. The 3xTg-AD do not appear to have a pronounced difference in social behaviour compared to the B6129SF2 mice.

5.6 ACKNOWLEDGEMENTS

This research was funded by an NSERC grant to REB. The authors would like to thank Rhian Gunn and Daniel Ikpi for their assistance in this project.

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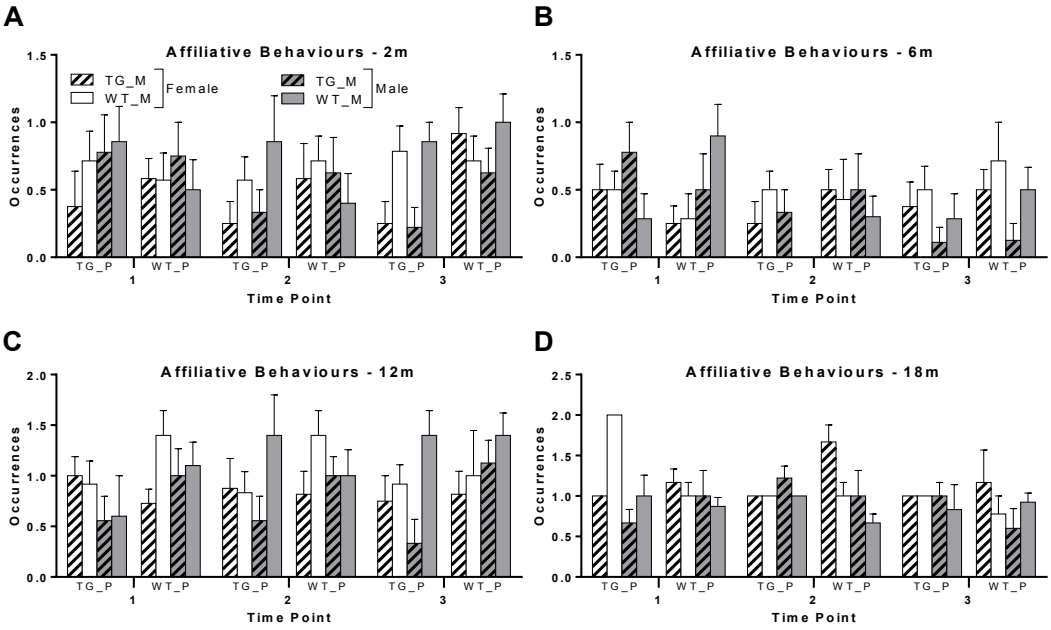
5.8 SUPPLEMENTAL TABLE

Supplemental Table 5.1 provides the statistics used to determine the best model for each measure. The top five models are included. The model column describes the factors included in the model, terms separated by a '*' that there were main effects and interactions for the terms, those separated by a '+' are simple main effects, and those separated by a ':' indicate an interaction alone. The 'AICc' column contains the second order Akaike information criterion, which is a measure used to evaluate the models based on the complexity and goodness of fit of the model to the data, with lower values being better. The "Δ AICc" column is difference between that model's AICc and the model with the lowest AICc. The 'Wt' column is the Akaike weight, a measure of relative likelihood that the model is the best, ranging from 0, meaning unlikely, to 1, meaning likely. The 'ER' column is the evidence ratio, it is the likelihood that the model with the lowest AICc is better than that model.

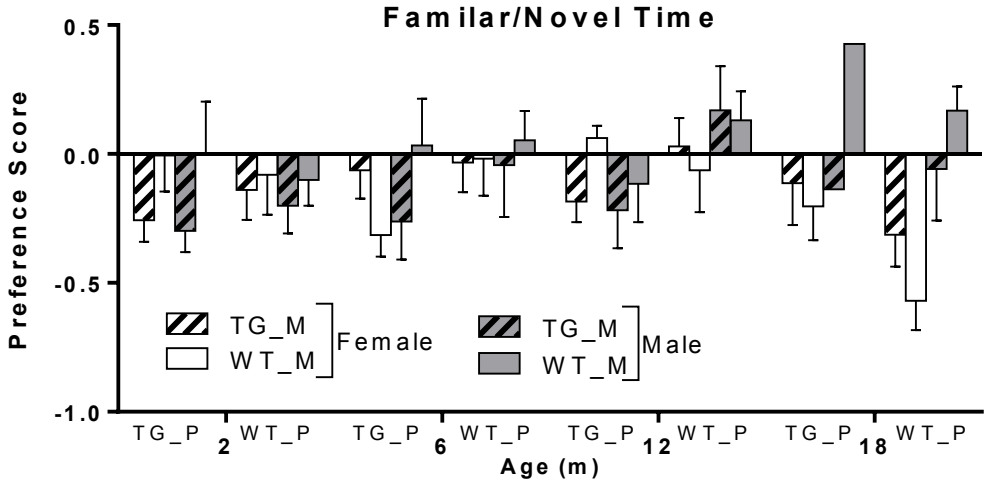
Supplemental Table 5.1 SNSP Preference Score

Model	**AICc**	**Δ AICc**	**AICcWt**	**ER**
Genotype+FMGenotype	236.857	0	0.064	1
Genotype+FMGenotype*Sex	237.686	0.822	0.042	1.514
Genotype+FMGenotype+Sex	237.853	0.996	0.039	1.646
Genotype*FMGenotype*Sex	238.249	1.391	0.032	2.01
Genotype*FMGenotype	238.404	1.547	0.029	2.168

5.9 SUPPLEMENTAL FIGURES



Supplemental Figure 5.1 Home Cage Observations. The mean (± S.E.M) number of affiliative behaviours at 2 (A), 6 (B), 12 (C), and 18 (D) months of age in 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers during home cage observations.



Supplemental Figure 5.2 Social Novelty / Preference. The mean (± S.E.M) preference score for interacting with the novel mouse of 3xTg-AD (TG_P) and B6129SF2 (WT_P) mice reared by 3xTg-AD (TG_M) and B6129SF2 (WT_M) foster mothers at each age.

CHAPTER 6 ANALYSIS OF MOTOR FUNCTION IN 6 MONTH OLD MALE AND FEMALE 3XTG-AD MICE

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6.1 ABSTRACT

The 3xTg-AD mouse has high validity as a model of Alzheimer's disease (AD) because it develops both amyloid beta plaques and neurofibrillary tangles. Human patients with AD typically develop motor deficits, which worsen as the disease progresses, but 3xTg-AD mice have been reported to show enhanced motor abilities. We investigated the motor behaviour phenotype of male and female 3xTg-AD and B6129SF2 wildtype mice on a battery of motor behaviours at 6 months of age. Compared to wildtype mice, the 3xTg-AD mice had enhanced motor performance on the Rotarod, but worse performance on the grid suspension task. In gait analysis 3xTg-AD mice had a longer stride length and made more foot slips on the balance beam than wildtype mice. There was no overall difference in voluntary wheel-running activity between genotypes, but there was a disruption in circadian activity rhythm in 3xTg-AD mice. In some motor tasks, such as the Rotarod and balance beam, females appeared to perform better than males, but this sex differences was accounted for by differences in body weight. Our results indicate that while the 3xTg-AD mice show enhanced performance on the Rotarod, they have poorer performance on other motor behaviour tasks, indicating that their motor behaviour phenotype is more complex than previously reported. The presence of the P301L transgene may explain the enhancement of Rotarod performance but the poorer performance on other motor behaviour tasks may be due to other transgenes.

6.2 INTRODUCTION

The 3xTg-AD mouse is one of many mouse models of Alzheimer's disease (AD) (Chin, 2011; LaFerla and Green, 2012; Webster et al., 2014). It has been rated as having the highest face and construct validly among mouse models by Bilkei-Gorzo (2014) because it harbours three transgenes: a human amyloid precursor protein associated with familiar AD (APP_{swe}); a mouse Presenilin1 (PS1) gene carrying a human mutation also associated with familiar AD ($PS1_{M146V}$); and a human gene associated with tau pathology (Tau_{P301L}). The 3xTg-AD mouse was created by adding the tau and APP mutations to a mouse with the PS1M146V mutation (Oddo et al., 2003). This combination of transgenes produces a strain that develops both amyloid beta (Aβ) plaques and tau tangles, the classic hallmarks of human AD neuropathology (Oddo et al., 2003; Billings et al., 2005; Mastrangelo and Bowers, 2008). The 3xTg-AD mouse develops neuropathology as a result of its transgenes, beginning with abnormal myelination in the Schaffer collateral fibers at two months of age (Desai et al., 2009), followed by intracellular Aβ in the neocortex at three months of age and in the hippocampus between three and six months of age. Extracellular plaques develop in the neocortex by six months of age (Billings et al., 2005; Mastrangelo and Bowers, 2008). Tau pathology develops later than Aβ pathology in 3xTg-AD mice, with the first phosphorylated tau, which is associated with neurofibrillary tangles, detectable in the hippocampus by six months of age and spreading to the motor cortex by nine months of age (Mastrangelo and Bowers, 2008).

Alzheimer's disease in humans begins with memory deficits, which are followed by deficits in language, vision, and motor function (McKhann et al., 1984; Carrillo et al.,

2013). The motor deficits in AD manifest early in the progression of the disease as mild problems as complex motor control and fine motor skills, which progress to problems with gross motor functioning (Kluger et al., 1997; Pettersson et al., 2005). Motor learning appears to remain intact in AD patients (Eslinger and Damasio, 1986; Dick et al., 1995). There are, however, indications that impairments in motor function may be a common feature of pre-clinical AD and that age-related decline may occur in both motor and cognitive behaviour in AD patients (Buchman and Bennett, 2011; Albers et al., 2014). Because there are a wide range of motor behaviours that can be measured including balance, motor coordination, strength, and gait, a battery of tests is required to accurately assess age-related changes in motor function in Alzheimer's and non-Alzheimer's patients (Reuben et al., 2013; Gras et al., 2014). Similarly, motor behaviour test batteries are available for mice (Brooks and Dunnett, 2009; Justice et al., 2014), and we have used such a test battery to assess motor function in 3xTg-AD mice at six months of age, a time when they begin to show the onset of AD-like symptoms (Billings et al., 2007; Clinton et al., 2007; Blanchard et al., 2010; España et al., 2010; Chen et al., 2013).

Previous results from our lab found that 3xTg-AD mice had improved performance on the Rotarod compared to wildtype mice beginning at two months of age and continuing until at least fifteen months of age (Oore et al., 2013), and results from a neurodevelopmental test battery demonstrated that newborn 3xTg-AD mice reach physical milestones earlier than wildtype mice (Blaney et al., 2013). Other researchers have also found that the 3xTg-AD mice show improved motor functioning compared to control mice on the Rotarod (Blanchard et al., 2010; Filali et al., 2012; Chen et al., 2013). However, Sterniczuk et al. (2010a) found no difference between 3xTg-AD and C57BL/6J

control mice on Rotarod performance, and neither Sterniczuk et al. (2010a) nor Arsenault et al. (2011) found any deficits in 3xTg-AD mice on the wire hang task. The 3xTg-AD mice performed more voluntary wheel-running than wildtype control mice over a one month period (García-Mesa et al., 2011), and, although Sterniczuk et al. (2010b) found no difference in total amount of wheel running between 3xTg-AD and C57BL/6J mice, they did find that the 3xTg-AD mice had disrupted circadian running rhythms compared to C57BL/6J mice.

Because motor behaviour can include activity level, gait, strength, balance, coordination, and endurance (Justice et al., 2014), we used a battery of tests to characterize the motor behaviour phenotype of 3xTg-AD mice compared to B6129SF2 wildtype controls at six months of age. Grip strength was examined using the wire hang and grid suspension tasks, motor coordination and motor learning using the Rotarod, balance using the balance beam, and spontaneous activity using a running wheel in the home cage. We hypothesized that the 3xTg-AD mice would have enhanced performance on the Rotarod relative to wildtype mice, and that this difference would be associated with enhanced performance on other motor behaviour tasks.

6.3 METHODS

6.3.1 ANIMALS

Eighty-five mice, forty-two 3xTg-AD (21 male and 21 female) and forty-three B6129SF2 (22 male and 21 female), were tested at six months of age in three cohorts of approximately 28 mice each. The mice were bred at Dalhousie University from parents purchased from the Jackson Laboratory in Bar Harbor, Maine (Stock #'s 004807 & 101045). After weaning at 21 days of age the mice were housed in same sex groups of 2

to 4 littermates in plastic cages (18.75 x 28 x 12.5 cm), with a PVC tube (4 cm diameter x 7 cm length) for enrichment, wood chips for bedding, and metal wire covers. The mice were fed rodent chow (Purina #5001) and tap water ad libitum and they were housed in a colony room at 22±2ºc on a reversed 12:12 light:dark cycle with lights off at 10:00 am. The tests were performed during the dark phase of the light:dark cycle (10:00-22:00) in the order described. The mice were genotyped by Dr. Chris Sinal (Pharmacology Department, Dalhousie University) using polymerase chain reaction of an ear punch tissue sample which was taken when the mice were ear-punched for identification at weaning. This experiment was approved by the Dalhousie University Committee on Laboratory Animals.

6.3.2 BODY WEIGHT

Each day before testing on the Rotarod the mice were weighed using an OHAUS CS 200 scale (Parsippany, NJ). Body weight was analyzed separately and used as a factor for the analysis of performance in other tasks.

6.3.3 ROTAROD

To assess motor coordination and motor learning as well as endurance, mice were trained for five days on the accelerating Rotarod (Accuscan Instruments, Columbus, Ohio), as described by Brown and Wong (2007). The apparatus consisted of a PVC rotating rod (3cm diameter) with four opaque Plexiglas barriers (15cm diameter) dividing the rod into four 11cm sections which had individual holding chambers located 39cm below the rod. The Rotarod was housed in a small room which was illuminated with a 60w red light bulb. Mice were placed on the rod facing away from the experimenter and the rod began to accelerate from 0-48rpm over 360 seconds. After the

last mouse fell from the rod, or if 360 seconds elapsed, the mice were given a one minute rest and then placed back on the rod for the next trial. Mice were trained for six trials per day for five days and the mean latency to fall over the six trials each day was analyzed.

3.3.4 GRIP STRENGTH

Grip strength was assessed using two tasks. In the wire hang task, mice were suspended by their forepaws from a wire 26 cm above a padded cushion and the latency to fall was recorded (to a maximum of 60 seconds). In the grid suspension task, mice were placed on a wire grid (15 by 20 cm) which was inverted 26 cm above a padded cushion and the latency to fall (up to a maximum of 60 seconds) was recorded (Sterniczuk et al., 2010a). Mice were tested on three consecutive trials for each test with an inter-trial interval of approximately 20 minutes, and the mean latency to fall over the three trials was used for analysis.

6.3.5 GAIT ANALYSIS

Mice were trained to walk down a narrow corridor for a food reward and, once trained, their hind paws were marked with paint (non-toxic liquid tempera, Schola, Marieville, Quebec, Canada), and they were placed in the corridor for a single trial with the floor covered by a paper sheet (21cm wide by 55cm long). Mean stride length and width from four to sixteen footsteps were analyzed by measuring the distance between paw prints on the paper using ImageJ (http://imagej.nih.gov/ij/) (Fleming et al., 2004).

6.3.6 BALANCE BEAM

To assess balance and motor coordination, the mice were placed on a balance beam (100cm by 2cm, elevated 40cm) for a single trial lasting a maximum of two

minutes and the distance travelled, number of foot slips, number of turns, and latency to fall (if applicable) were recorded and analyzed (Carter et al., 1999).

6.3.7 VOLUNTARY WHEEL-RUNNING

In order to assess activity levels mice were housed individually in the colony room for seven days in a cage with a running wheel (15.5cm diameter) using the procedure described by Wright and Brown (2002). The number and timing of the rotations was recorded automatically by hardware and software developed in our laboratory. The number of rotations and percentage of the total rotations that took place in the light phase of the light:dark cycle were analyzed.

6.3.8 STATISTICAL ANALYSES

The data were analyzed using linear mixed effects models for the Rotarod, and linear regression models for all other tasks. Genotype, sex, and weight were used as possible predictor variables. Body weight was included in the analyses as a possible predictor variable in order to control for weight as a confounding factor in sex effects. The second order Akaike information criterion (AIC_c), Akaike weight, and evidence ratio for all possible models was calculated, and the model with the lowest AIC_c was selected and compared to the null model with either an F-test (linear regression models), or a χ^2 test (linear mixed effects models). The AIC is a measure used to evaluate statistical models based on the complexity of the model and how well the model fits the data. The second order AIC (AICc) applies a correction for small sample sizes and does not differ from AIC for larger sample sizes, so was used for our analyses (Akaike, 1974; Burnham and Anderson, 2002). Confidence intervals (95%) for the effects in the models were calculated and, to determine which behavioural measures had the largest genotype

effects, Cohen's d with a Hedge correction for an unbiased measure of effect size (d_{unb}) was calculated for all of the selected models which included significant genotype effects (Hedges, 1981; Cumming, 2014).

6.4 RESULTS

6.4.1 BODY WEIGHT

The best model for describing differences in body weight was the full model with genotype, sex, and a genotype by sex interaction (AIC_c=441.794, weight = 0.950, Figure 6.1, Supplemental Table 6.1), which differed significantly from the null model ($F(3,81)=29.076$, $p<0.001$). Confidence intervals indicated that wildtype mice weighed more than transgenic mice (CI_{95}= 1.117 – 3.716g), and that males weighed more than females (CI_{95}= 4.147 – 6.776g). The genotype by sex interaction showed no genotype difference in body weight in females (CI_{95}= -1.465 – 2.357g), but male wildtype mice weighed more than male transgenic mice (CI_{95}= 2.438 – 6.154).

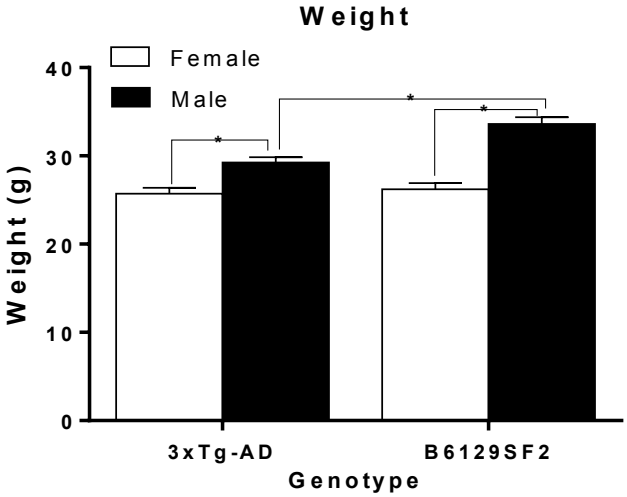

Figure 6.1 Body Weight. Mean (± SEM) body weight (g) at the beginning of the testing period in male and female 3xTg-AD and B6129SF2 mice. Male mice weighed more than females and male B6129SF2 mice weighed more than male 3xTg-AD mice. * = a difference between groups at a 95% confidence interval.

6.4.2 ROTAROD

The best model for the average latency to fall from the Rotarod over the five days of testing had day, genotype, weight, and day by genotype and day by weight interactions (AIC_c= 4233.164, weight=0.146, Figure 6.2A and B, Supplemental Table 6.2), and this model was significantly different than the null model (χ^2 (14, N=85) = 389.39, p=<0.0001). When we tested models which did not include body weight, sex was a significant predictor of latency to fall but with the inclusion of body weight, sex differences no longer explained performance on the Rotarod, because differences in body weight accounted for differences in performance better than sex. We also included stride length (as measured in gait analysis) as a possible predictor variable but it was not included in the best model. Confidence intervals showed that 3xTg-AD mice had longer latencies to fall than wildtype mice (CI_{95}= 35.399– 68.004s), and that lighter mice had longer latencies to fall than heavier mice (CI_{95}= 0.331 – 4.278s/g). The latency to fall increased over days (Day 1 to 5: CI_{95}= 87.854 – 198.901s), and the genotype by day interaction showed that 3xTg-AD mice had a greater latency to fall over days than wildtype mice. For example, the increase in latency to fall from day 1 to 5 was larger in 3xTg-AD mice (CI_{95}= 109.071 – 217.401s) than in wildtype mice (CI_{95}= 63.218 – 179.537s). The day by weight interaction showed little effect of weight on day 1 (CI_{95}= Day1: -1.785 – 1.978s/g), but by day 5 the lighter mice were performing better than the heavier mice regardless of sex or genotype (CI_{95}= 0.306 – 4.240s/g).

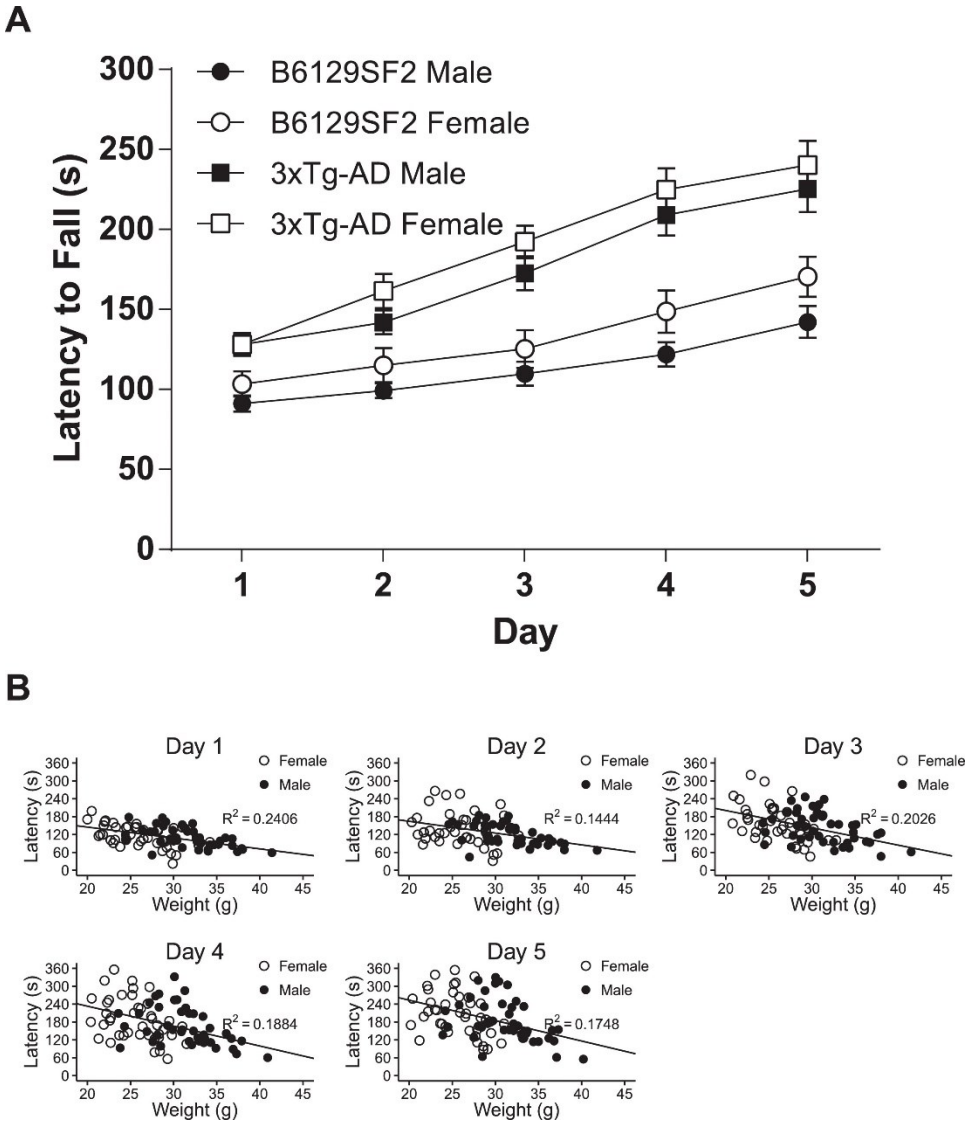

Figure 6.2 Rotarod. Mean (± SEM) latency (s) to fall from the Rotarod (A) for male and female 3xTg-AD and B6129SF2 mice over five days of testing. (B) The latency to fall from the Rotarod given the weight of each mouse for both genotypes on each of the five days of testing. The 3xTg-AD mice had a longer latency to fall from the Rotarod, and by day 5 lighter mice were performing better than heavier mice.

6.4.3 GRIP STRENGTH

For latency to fall in the wire hang task the model with the lowest AIC_c was the null model, indicating that neither genotype, sex, nor weight predicted latency to fall from the wire (AIC_c=736.504, weight = 0.299, Figure 6.3A, Supplemental Table 6.3.1). For latency to fall in the grid suspension test the model with the lowest AIC_c had only genotype as a predictor (AIC_c = 667.058, weight = 0.394, Figure 6.3B, Supplemental Table 6.3.2). This model was significantly different from the null model (F(1,83)=6.128, p=0.0153), and the confidence interval indicated that that 3xTg-AD mice had a shorter

latency to fall than wildtype mice (CI_{95}= -12.509 – -2.125s).

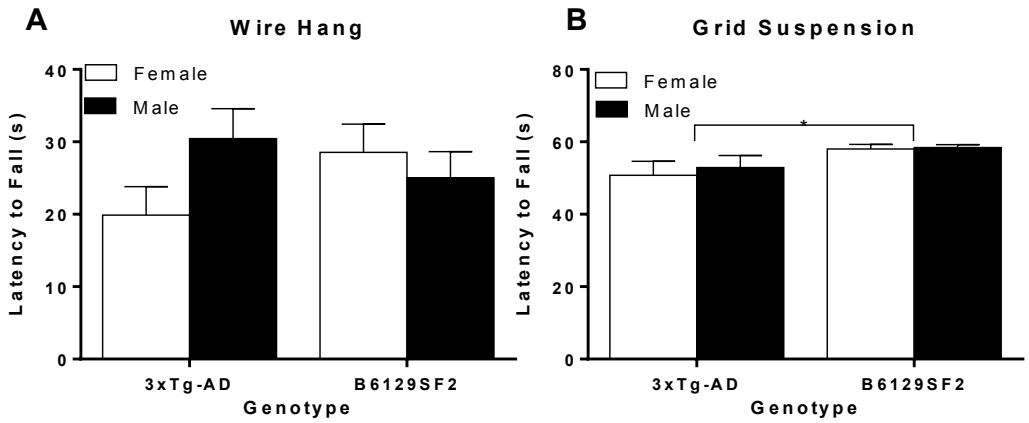

Figure 6.3 Grip Strength. Mean (± SEM) latency (s) to fall during the wire hang (A) and grid suspension (B) tasks for male and female 3xTg-AD and B6129SF2 mice. There were no genotype or sex differences in wire hang task, while B6129SF2 mice had a longer latency to fall than the 3xTg-AD mice in the grid suspension task. * = a difference between groups at a 95% confidence interval.

6.4.4 GAIT ANALYSIS

The gait of three mice (two male wildtypes and one male 3xTg-AD) could not be analyzed because the mice did not walk down the corridor, so no footprints could be measured. An average of nine steps per mouse was analyzed. For stride length the best model had only genotype as a predictor (AIC_c=146.940, weight= 0.389, Figure 6.4A, Supplemental Table 6.4.1), which differed significantly from the null model ($F(3,78)$= 4.695, $p < 0.005$). Confidence intervals indicated that transgenic mice had a longer stride length than wildtype mice (CI_{95}= -0.713 – -0.217cm). For stride width the full model with genotype, sex, and a genotype by sex interaction (AIC_c=62.222, weight = 0.263, Figure 6.4B, Supplemental Table 6.4.2) differed significantly from the null model ($F(3,78)$= 7.217, $p < 0.001$). Confidence intervals showed little evidence for a genotype difference (CI_{95}= -0.063 – 0.228cm), although there was a larger stride width in males compared to females (CI_{95}= 0.163 – 0.460cm), and there was a genotype by sex interaction as

wildtype male mice had a wider stride than females (CI_{95}= 0.228 – 0.697cm), but there was no sex difference in the 3xTg-AD mice (CI_{95}= -0.019 – 0.340cm).

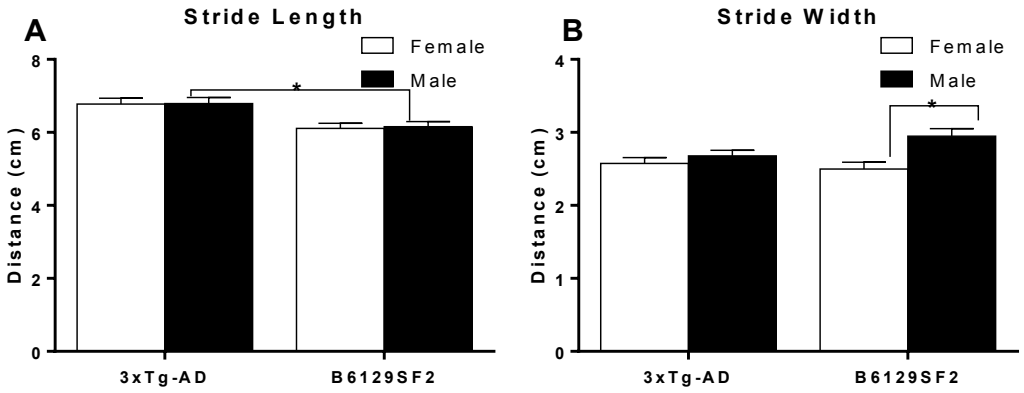

Figure 6.4 Gait Analysis. Mean (± SEM) stride length (A) and width (B) in cm in male and female 3xTg-AD and B6129SF2 mice. The 3xTg-AD mice had a longer stride than the B6129SF2 mice, and the B6129SF2 males had a wider stride than the B6129SF2 females. * = a difference between groups at a 95% confidence interval.

6.4.5 BALANCE BEAM

For the latency to fall from the balance beam, distance travelled, and locomotor speed the null model was the best model (AIC_cs= 820.773, 1086.210, 280.621, weights = 0.288, 0.292, 0.297, Figures 6.5 A, B, C, and Supplemental Tables 6.5.1, 6.5.2, 6.5.3, respectively), indicating that there were no genotype, sex, or weight effects. For the number of foot slips the best model had genotype, weight, and a genotype by weight interaction (AIC_c=303.970, weight = 0.628, Figure 6.5 D and E, Supplemental Table 6.5.4), and differed significantly from the null model ($F(1,84)= 5.86$, $p = 0.0001$). The confidence interval analysis showed that the 3xTg-AD mice had a greater number of foot slips than wildtype controls (CI_{95}= 0.0193 – 1.1687 slips). There was also an interaction between body weight and genotype as the number of foot slips decreased with weight in

3xTg-AD mice (CI_{95}= -0.522 – -0.075 slips/g), but there was little effect of weight on the number of foot slips in wildtype mice (CI_{95}= -0.013 – 0.085 slips/g).

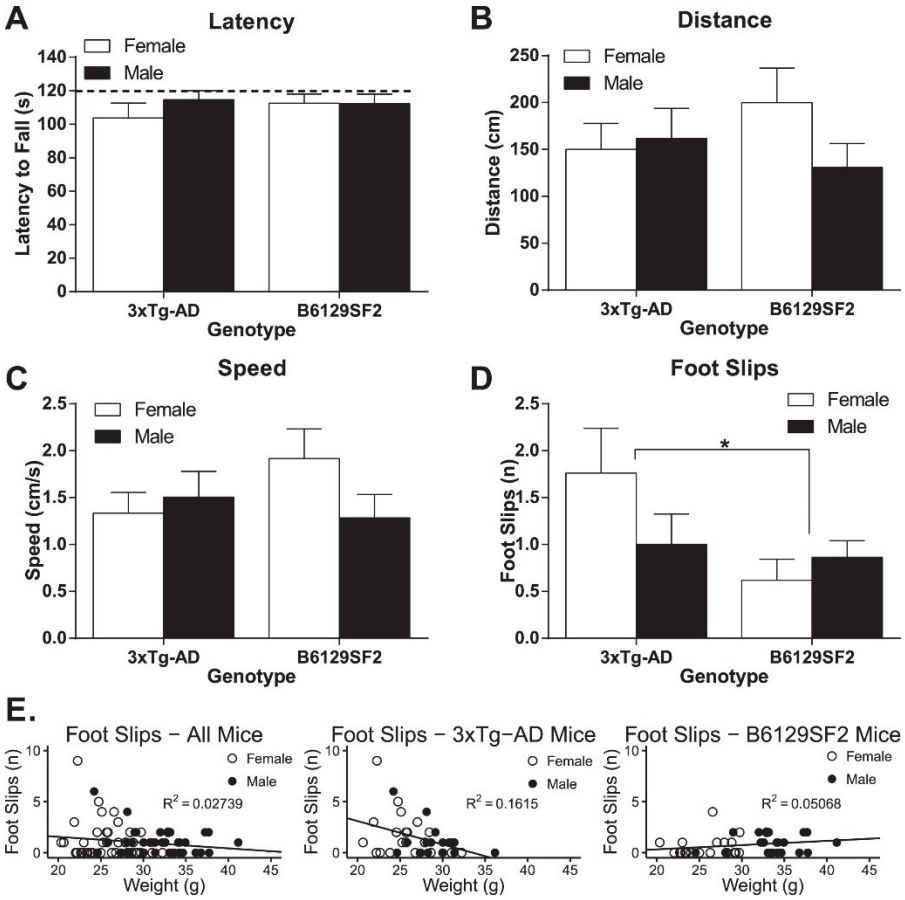

Figure 6.5 Balance Beam. Mean (± SEM) latency to fall (s) (A), distance travelled (cm) (B), speed (cm/s) (C), and number of foot slips (D) on the balance beam for male and female 3xTg-AD and B6129SF2 mice. The number of foot slips by weight (g) (E) for all mice and 3xTg-AD and B6129SF2 mice separately. The dotted line on (A) represents the maximum trial length. The 3xTg-AD made more foot slips than the B6129SF2 mice and the lighter 3xTg-AD mice had more foot slips than the heavier 3xTg-AD mice, but there was no effect of weight in the B6129SF2 mice. * = a difference between groups at a 95% confidence interval.

6.4.6 VOLUNTARY WHEEL-RUNNING

Five mice (two male 3xTg-AD mice, one male wildtype, and two female wildtypes) did not use their running wheels therefore no data was collected from them. For the number of total rotations the best model had genotype, weight, and a genotype by weight interaction (AIC_c=1948.834, weight = 0.432, Figure 6.6 A and C, Supplemental

Table 6.6.1), and was significantly different from the null model ($F(3,76)=3.298$, $p=0.0251$). Transgenic mice did not differ in number of total rotations from wildtype mice (CI_{95}= -32165 – 14104 rotations), but the weight by genotype interaction showed that the 3xTg-AD mice had an increased number of rotations as body weight increased (CI_{95}= 272 – 9239 rotations/g), but the wildtype mice had a decrease in number of rotations with increased weight (CI_{95}= -5496 – 136 rotations/g). For the percentage of rotations that took place in the light portion of the light:dark cycle, the best model had only genotype (AIC_c=635.748, weight = 0.299, Figure 6.6B, Supplemental Table 6.6.2). This model fell just short of differing significantly from the null model, ($F(1,78)=3.936$, $p=0.051$), however confidence interval analysis indicated that the transgenic mice had a greater percentage of their rotations during the light portion of the light:dark cycle than the wildtype mice (CI_{95}= 0.67 – 11.99%).

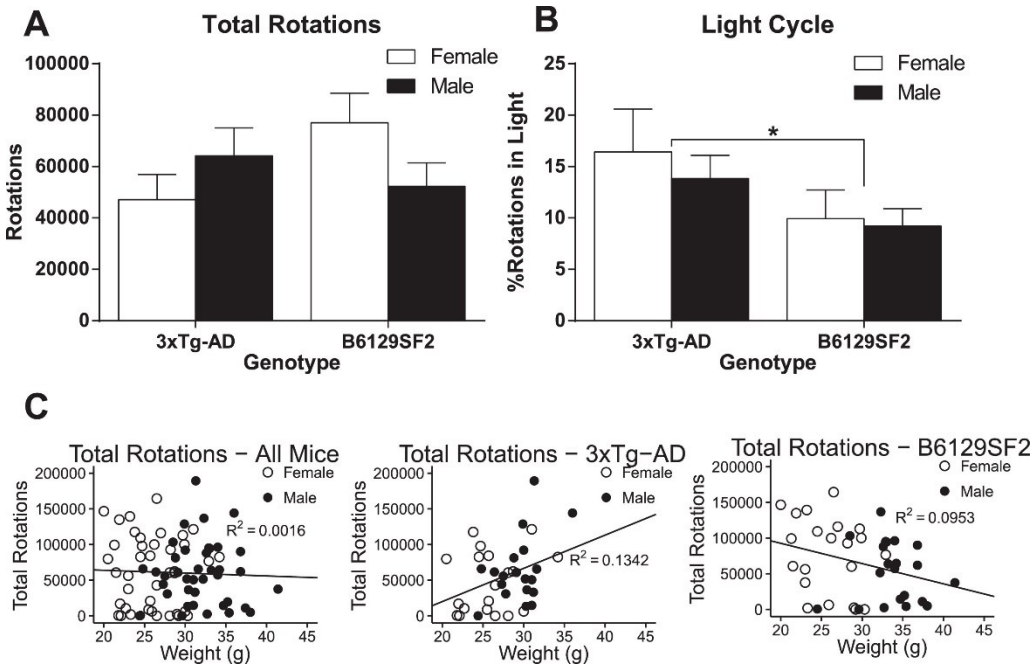

Figure 6.6 Wheel Running. Mean (± SEM) total rotations over the seven day period (A), and percentage of total rotations which occurred during the light phase of the light:dark cycle (B) for male and female 3xTg-AD and B6129SF2 mice. (C) Total rotations by weight for all mice and both genotypes separately. Female 3xTg-AD mice had more rotations than female B6129SF2 mice, and 3xTg-AD mice had more rotations in the light phase than B6129SF2. In 3xTg-AD mice the number of rotations increased with weight while in B6129SF2 mice the number of rotations decreased with weight. . * = a difference between groups at a 95% confidence interval.

6.4.7 EFFECT SIZE COMPARISON

The effect sizes for all of the measures which had genotype as a significant predictor of performance were compared using Cohen's d with a Hedges correction for an unbiased measure of effect size (d_{unb}) (Table 6.1). Positive effect sizes indicate higher scores in 3xTg-AD mice relative to wildtypes and negative scores indicate higher scores in wildtype mice. Latency to fall from the Rotarod on day 5 (when the genotype effects were largest, Figure 6.1) had the largest effect size (d_{unb}=1.252, CI_{95}= 0.774 – 1.731) as 3xTg-AD mice had a longer latency to fall than wildtype mice. Gait length had the next largest effect size (d_{unb}=0.807, CI_{95}= 0.344 – 1.271cm), as 3xTg-AD mice had a longer

gait length than wildtype mice. The largest deficit in motor behaviour in 3xTg-AD mice relative to wildtype mice was in the latency to fall in the grid suspension task (d_{unb}=-0.532, CI_{95}= 0.977 – 0.088).

Table 6.1 Genotype Effect Size Estimates. Cohen's d was calculated with a pooled SD and Hedges correction (dunb) for all models that included genotype. Positive values indicate that 3xTg-AD mice had higher scores than wildtypes and negative scores indicate that wildtype mice had higher scores than transgenic mice.

Measure	d_{unb}	95% Confidence Interval	
3xTg-AD higher than Wildtype		Lower	Upper
Rotarod – Latency to fall (day 5)	1.252	0.774	1.731
Gait – Length	0.807	0.344	1.271
Wheel Running – Percent in Light	0.440	-0.017	0.897
Balance Beam – Foot Slips	0.426	-0.016	0.868
Wildtype higher than 3xTg-AD			
Body Weight	-0.581	-1.027	-0.136
Grid Suspension – Latency to fall	-0.532	-0.977	-0.088
Gait – Width	-0.188	-0.634	-0.258

6.5 DISCUSSION

We found that 3xTg-AD mice had a greater latency to fall from the Rotarod than wildtype mice, and that this difference increased over days, which indicated that the 3xTg-AD mice have enhanced motor coordination and learning on this test. We also found that body weight played a significant role in Rotarod performance, with lighter mice outperforming heavier mice and that sex differences were accounted for by body weight (Figure 6.2). This finding is supported by other studies which found that 3xTg-AD mice had better motor performance on the Rotarod than controls, even when different protocols were used (Blanchard et al., 2010; Sterniczuk et al., 2010a; Filali et al., 2012; Chen et al., 2013). This enhanced motor phenotype had the largest effect size of any genotype difference in motor behaviour (Table 6.1), and appears to be a robust finding, as it occurs as early as two months of age (Oore et al., 2013).

In order to further investigate the motor phenotype of the 3xTg-AD mouse we used tests of strength, gait, balance, and voluntary activity. In the wire hang test there was no difference between genotypes or sexes in grip strength, which replicates what others have found (Sterniczuk et al., 2010a; Arsenault et al., 2011) (Figure 6.3A). In the grid suspension task, 3xTg-AD mice had poorer grip strength than wildtype mice independently of body weight (Figure 6.3B). To our knowledge this is the first time the 3xTg-AD mice have been assessed on this task. Different results from these two measures of grip strength may occur because the wire hang test requires mice only to use their forepaws while the grid suspension test requires mice to use both their hind and forepaws, which may be a more valid measure of the grip strength. There was also much less variability in performance in the grid suspension task suggesting that it has higher reliability than the wire hang task (Figure 6.3 A and B). Based on these findings it appears that the 3xTg-AD mice have a deficit in grip strength by six months of age.

In terms of gait, the 3xTg-AD mice had a longer stride than wildtype mice (Figure 6.4A. Male wildtype mice had a wider stride than females, but there was no sex difference in gait width in 3xTg-AD mice. This may be the result of a difference in overall body size, though body weight had no effect on gait. The only previous assessment of gait in the 3xTg-AD mice was qualitative and all mice assessed had normal gait (Filali et al., 2012). Differences in gait could influence motor tasks that involve locomotion and balance, such as the Rotarod or balance beam.

On the balance beam the 3xTg-AD mice made more foot slips than wildtype mice, but did not fall off the beam faster than wildtype mice (Figure 6.5 A and D), which may be the result of their better motor coordination. There was also a genotype-

dependant weight effect, in which the number of foot slips increased with weight in wildtype mice and decreased with weight in transgenic mice (Figure 6.5E). An increased ability to recover from a foot slip would enhance performance on the Rotarod, however having more foot slips would decrease performance, so it is unclear how this finding could explain performance on the Rotarod. Other mice with the P301L mutation have been reported to have better performance on the balance beam than control mice (Morgan et al., 2008).

In voluntary wheel-running there were no genotype or sex differences in total number of rotations, but there was a genotype-dependant weight effect; the heavier 3xTg-AD mice had a higher number of rotations but lighter wildtype mice had the higher number of rotations (Figure 6.6C). The 3xTg-AD mice appeared to have a disrupted circadian rhythm, as they spent a greater percentage of time running during the light phase of the light:dark cycle than wildtype mice. These findings are similar to those of Sterniczuk et al. (2010b), who found that the 3xTg-AD have disrupted circadian rhythms, and this is also a common symptom of AD.

There was only one sex difference in motor function: females had a narrower stride width than males; however there were several effects of body weight. The heavier 3xTg-AD mice had better motor functioning than lighter mice, as they had fewer foot slips on the balance beam and a higher number of rotations in voluntary wheel running. In wildtype mice weight had the opposite effect, as heavier mice performed more poorly than lighter mice on the Rotarod in the later days of training. These results indicate that body weight is an important factor in motor function and should be taken into consideration when examining motor ability. Male mice weighed more than female mice

and if weight was not taken into account then spurious sex differences would be detected. Figures 6.2B and 6.5E show that male and female mice with the same weight did not differ in performance, and since females were lighter than males weight was often a useful predictor of performance in our models while sex was not.

Overall, we found that the 3xTg-AD mice had enhanced performance on the Rotarod at six months of age, which may be explained by their longer gait and their better ability to recover from foot-slips on the balance beam. But the 3xTg-AD mice had poorer grip strength on the grid suspension task and a disrupted circadian rhythm in voluntary wheel running. It seems likely that the enhanced motor performance of the 3xTg-AD mice on the Rotarod is a result of the P301L transgene, since other strains of mice with the P301L mutation as well as the 3xTg-AD have the same enhancement of motor behavior performance on the Rotarod (Morgan et al., 2008; Blanchard et al., 2010; Sterniczuk et al., 2010b; Filali et al., 2012; Chen et al., 2013). There are no reports of enhanced motor performance in mice with the APPswe or PS1M146V mutations, however some mice with APP or PS1 mutations have motor deficits on the Rotarod (Peters et al., 2013; Héraud et al., 2014; Kuwabara et al., 2014). Another transgenic strain harbouring the P301L mutation, JNPL3 mice, also have enhanced performance on the Rotarod, and this enhanced motor phenotype remained intact when the strain was crossed with the Tg2576AD model mouse (Morgan et al., 2008). The JNPL3 and other P301L mutant mice also have enhanced cognitive performance before seven months of age, suggesting that while the P301L mutation causes tau pathology with motor and cognitive deficits at older ages, it may confer some benefits at younger ages, before the mice develop motor deficits (Boekhoorn et al., 2006; Morgan et al., 2008). Most mouse models

with the P301L mutation develop motor deficits with age (Lewis et al., 2000; Morgan et al., 2008), however the 3xTg-AD does not (Blanchard et al., 2010; Sterniczuk et al., 2010a; Chen et al., 2013), and it appears that the enhanced motor phenotype on the Rotarod is maintained until at least 15 months of age (Oore et al., 2013).

In the 3xTg-AD mice the separate locus of the APPswe and TauP301L mutations from the PS1M146V mutation means that two groups of transgenes would segregate independently and produce a number genotypes, so the transgenic mice are bred together and the wildtype control mice (B6129SF2) are bred separately. This can lead to maternal effects in the development of these mice (Blaney et al., 2013). Irrespective of their maternal genotype the 3xTg-AD mice reach physical milestones earlier than wildtype mice (Blaney et al., 2013). The lack of age-related development of motor deficits in 3xTg-AD mice on the Rotarod (Oore et al., 2013) may be related to the inclusion of the other two transgenes or to the background strains of these mice (B6129SF2). The tau P301L mutation may also provide an explanation for the relatively mild cognitive deficits in this strain, as it has been reported have a positive effect on some aspects of cognition early in life (Boekhoorn et al., 2006; Morgan et al., 2008). Future studies should examine the possible role of the tau P301L in motor phenotype enhancement and examine whether it has a protective effect on cognitive function in the 3xTg-AD mice.

Given that there are age-related changes in motor abilities and gait in AD patients (Cedervall et al., 2014) we would expect to find an age-related decline in motor performance in 3xTg-AD mice, however the Rotarod performance in the 3xTg-AD mice is considerably better than that of wildtype controls from two months of age and there is no decline in Rotarod performance with age (Oore et al., 2013). It is therefore important

to examine age-related changes in the entire motor test battery in the 3xTg-AD mice, as motor behaviour has been found to decline in activity level, strength, and endurance in C57BL/6 mice after 20 months of age (Justice et al., 2014). Future studies in the 3xTg-AD should examine the motor phenotype of these mice across the lifespan. The enhanced motor performance of the 3xTg-AD mice on the Rotarod appears to be a robust finding and should be taken into account when selecting a cognitive task in future experiments. However it appears that the motor phenotype of this strain is more complex than a simple enhancement of Rotarod performance, and other aspects of motor performance, such as grip strength, may show an age related decline.

6.6 ACKNOWLEDGEMENTS

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6.8 SUPPLEMENTAL TABLES

Description of supplemental tables.

These tables summarize the statistics used to analyze each behaviour. The model column describes the model used, terms separated by a '*' indicate both individual main effects and interactions between those terms, terms separated by a '+' are simple main effects, and terms separated by a ':' indicate an interaction. The top five models are included. The 'AICc' is the second order Akaike information criterion, which is a measure used to evaluate statistical models based on the complexity of the model and how well the model fits the data, with lower values indicating a better fit. The "Δ AICc" column provides the difference in AICc compared to the lowest AICc, 'Wt' is the Akaike weight, a measure of relative likelihood from 0 (unlikely) – 1(likely). The 'ER' column provides the evidence ratio, which compares each model to the model with the lowest AICc and provides the likelihood that the model with the lowest AICc is better than the model in question.

Supplemental Table 6.1 Body Weight.

Model	AICc	Δ AICc	Wt	ER
Genotype*Sex	441.794	0	0.950	1
Genotype+Sex	447.682	5.888	0.050	18.994
Sex	457.001	15.207	0.000	2004.993
Genotype	492.274	50.480	0.000	9.152E+10
Null	497.305	55.511	0.000	1.133E+12

Supplemental Table 6.2 Rotarod – Latency to Fall.

Model	AICc	Δ AICc	Wt	ER
Day+Genotype+Weight+(Day:Genotype)+(Day:Weight)	4233.164	0	0.146	1
Day+Genotype+Weight+(Day:Genotype)	4233.920	0.756	0.100	1.459
Day+Genotype+Weight+(Day:Genotype)+(Day:Weight)+(Genotype:Weight)	4234.483	1.318	0.076	1.933
Day+Genotype+Weight+(Day:Genotype)+(Genotype:Weight)	4235.148	1.984	0.054	2.697
Day+Genotype+Sex+Weight+(Day:Genotype)+(Day:Weight)	4235.329	2.164	0.050	2.951

Supplemental Table 6.3.1 Wire Hang – Latency to Fall.

Model	AICc	Δ AICc	Wt	ER
Null	736.505	0	0.299	1
Sex	737.857	1.353	0.152	1.967
Weight	738.342	1.837	0.119	2.506
Genotype	738.484	1.979	0.111	2.690
Genotype*Sex	738.799	2.294	0.095	3.149

Supplemental Table 6.3.2 Grid Suspension – Latency to Fall.

Model	AICc	Δ AICc	Wt	ER
Genotype	667.058	0	0.394	1
Genotype+Sex	669.023	1.966	0.147	2.672
Genotype+Weight	669.194	2.136	0.135	2.910
Null	670.962	3.905	0.056	7.046
Genotype*Weight	671.096	4.039	0.052	7.533

Supplemental Table 6.4.1 Gait Analysis – Length.

Model	AICc	Δ AICc	Wt	ER
Genotype	146.940	0	0.389	1
Genotype+Sex	149.026	2.086	0.137	2.838
Genotype+Weight	149.143	2.203	0.129	3.008
Genotype*Weight	149.574	2.633	0.104	3.731
Genotype*Sex	150.690	3.750	0.060	6.520

Supplemental Table 6.4.2 Gait Analysis – Width.

Model	AICc	Δ AICc	Wt	ER
Genotype*Sex	62.222	0	0.263	1
Sex	62.711	0.488	0.206	1.277
Genotype+Sex+Weight+(Genotype:Sex)	63.080	0.858	0.171	1.535
Genotype+Sex	63.817	1.594	0.118	2.219
Genotype+Sex+Weight+(Genotype:Sex)+(Weight:Sex)	65.171	2.948	0.060	4.368

Supplemental Table 6.5.1 Balance Beam – Latency to Fall.

Model	AICc	Δ AICc	Wt	ER
Null	820.773	0	0.288	1
Sex	822.248	1.474	0.138	2.090
Genotype	822.669	1.895	0.112	2.580
Weight	822.796	2.023	0.105	2.749
Genotype+Sex	824.204	3.431	0.052	5.559

Supplemental Table 6.5.2 Balance Beam – Distance Travelled.

Model	AICc	Δ AICc	Wt	ER
Null	1086.210	0	0.292	1
Weight	1087.195	0.985	0.178	1.637
Sex	1087.450	1.240	0.157	1.859
Genotype	1088.278	2.069	0.104	2.813
Genotype+Weight	1089.012	2.802	0.072	4.060

Supplemental Table 6.5.3 Balance Beam – Speed.

Model	AICc	Δ AICc	Wt	ER
Null	280.621	0	0.297	1
Sex	282.000	1.379	0.149	1.992
Genotype	282.349	1.728	0.125	2.373
Weight	282.746	2.124	0.103	2.893
Genotype*Sex	283.703	3.082	0.064	4.670

Supplemental Table 6.5.4 Balance Beam – Foot Slips.

Model	AICc	Δ AICc	Wt	ER
Genotype*Weight	303.970	0	0.628	1
Genotype+Weight+Sex+(Genotype:Weight)	306.286	2.316	0.197	3.183
Genotype+Sex+Weight+(Weight:Sex)+(Weight:Genotype)	308.573	4.603	0.063	9.988
Genotype+Sex+Weight+(Genotype:Sex)+(Weight:Genotype)	308.596	4.626	0.062	10.105
Genotype	312.273	8.303	0.010	63.536

Supplemental Table 6.6.1 Voluntary Wheel Running – Total Rotations.

Model	AICc	Δ AICc	Wt	ER
Genotype*Weight	1948.834	0	0.432	1
Genotype+Weight+Sex+(Genotype:Weight)	1950.988	2.154	0.147	2.935
Null	1951.944	3.110	0.091	4.734
Genotype+Sex+Weight+(Weight:Sex)+(Weight:Genotype)	1952.646	3.812	0.064	6.726
Genotype+Sex+Weight+(Genotype:Sex)+(Weight:Genotype)	1953.288	4.454	0.047	9.273

Supplemental Table 6.6.2 Voluntary Wheel Running – Percentage During Light Cycle.

Model	AICc	Δ AICc	Wt	ER
Genotype	635.748	0	0.299	1
Null	637.526	1.778	0.123	2.433
Genotype+Sex	637.632	1.885	0.116	2.566
Genotype+Weight	637.842	2.094	0.105	2.850
Genotype+Sex+Weight	638.974	3.226	0.060	5.019

CHAPTER 7 EARLY DETECTION OF COGNITIVE DEFICITS IN THE 3XTG-AD MOUSE MODEL OF ALZHEIMER'S DISEASE

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7.1 ABSTRACT

At what age can cognitive deficits first be detected in mouse models of Alzheimer's disease? The 3xTg-AD mouse model of Alzheimer's disease (AD) has three transgenes (APPswe, PS1M146V, and Tau P301L) which cause the development of amyloid beta plaques, neurofibrillary tangles, and cognitive deficits with age. However, the published literature is in disagreement about the age at which cognitive deficits are first detected in these mice. In order to determine which task is the most sensitive in the early detection of cognitive deficits, we compared 3xTg-AD and B6129SF2 wildtype mice at 6.5 months of age on a test battery including spontaneous alternation in the Y-Maze, novel object recognition, spatial memory in the Barnes maze, and cued and contextual fear conditioning. The 3xTg-AD mice had impaired learning and memory in the Barnes maze but performed better than B6129SF2 wildtype mice in the Y-Maze and in contextual fear conditioning. Neither genotype demonstrated a preference in the novel object recognition task nor was there a genotype difference in cued fear conditioning but females performed better than males. From our results we conclude that the 3xTg-AD mice have mild cognitive deficits in spatial learning and memory and that the Barnes maze was the most sensitive test for detecting these cognitive deficits in 6.5 month old mice.

7.2. INTRODUCTION

The 3xTg-AD mouse model of familial Alzheimer's disease (AD) was created by inserting the Swedish amyloid precursor protein (APP_{swe}) and tau (Tau_{P301L}) genes into the embryo of a $PS1_{M146V}$ transgenic mouse (Oddo et al., 2003). The APPswe gene is a human gene with a mutation associated with familial AD and the $PS1_{M146V}$ gene is a mouse presenilin 1 (PS1) gene which has a human mutation associated with familial AD inserted. The Tau_{P301L} mutation is associated with human tau pathology. With these transgenes the 3xTg-AD mouse develops both amyloid and tau pathology. The first detectable pathology is the development of intracellular amyloid beta at three months of age followed by the development of extracellular plaques in the neocortex and hippocampus at six months of age (Billings et al., 2005; Mastrangelo and Bowers, 2008). The development of tau pathology begins at six months of age, when phosphorylated tau is detectable in the hippocampus. The phosphorylated tau develops into neurofibrillary tangles between 18 and 26 months of age (Mastrangelo and Bowers, 2008).

There have been several studies delineating the cognitive behaviour phenotype of the 3xTg-AD mouse and using behavioural bio-assays to evaluate novel drug treatments for AD. However the results have not been consistent; differing according to the age and sex of the mice and the test procedures used. In the Y-Maze test of spontaneous alternation, a commonly used measure of short term memory, some studies reported that female 3xTg-AD mice have no deficit at six months of age, some reported a deficit at six months of age, and others found no deficits until seven months of age (Rosario et al., 2006; Carroll et al., 2007; Zhang et al., 2010). In the novel object recognition task, some studies found deficits in six month old female 3xTg-AD mice compared to B6129SF2

mice in a 15 minute delay task (Blanchard et al., 2010; Chen et al., 2013). However Clinton et al. (2007) did not find a deficit in novel object recognition in either male or female 3xTg-AD mice until 9 months of age in either the 1.5 hour or 24 hour delay task, likewise Martinez-Coria et al. (Martinez-Coria et al., 2010) found a deficit in both 1.5 hour and 24 hour retention in the novel object recognition task in 9 month old male and female 3xTg-AD mice compared to age-matched B6129SF2 wildtypes. In the Barnes maze, six month old 3xTg-AD mice of both sexes had significantly longer escape latencies and made more errors than control mice (Clinton et al., 2010). In the radial arm maze the 3xTg-AD mice were impaired in both working and reference memory relative to B6129SF2 control mice starting at two months of age (Stevens and Brown, 2014). Some studies have reported deficits in contextual fear conditioning by six months of age in 3xTg-AD mice of both sexes (Billings et al., 2005; España et al., 2010). Others found no difference between six month old 3xTg-AD and B6129SF2 wildtype mice in contextual fear conditioning with a 24 hour delay, but the 3xTg-AD were impaired in in longer term memory (Pietropaolo et al., 2008). On the other hand Chu et al. (2012) reported no difference between 3xTg-AD and B6129SF2 mice of either sex in cued or contextual fear conditioning at 13-14 months of age.

The 3xTg-AD mice are commonly used to assess potential therapies for the treatment of AD (Adlard et al., 2005; Oddo et al., 2006; Caccamo et al., 2007; Corona et al., 2010; Hasegawa et al., 2010). In order to determine the effectiveness of novel therapeutic agents on cognition in the 3xTg-AD mice, one or more of many behavioural tasks, which differ in sensitivity to detect cognitive impairments in this strain, have been used. Although there is variability in the ages of the 3xTg-AD mice to test potential AD

treatments, cognitive deficits have been reported by 6.5 months of age. However, the effect size for the cognitive deficits are seldom reported in these studies. If the effect sizes for the cognitive deficits is very small it may not be replicable and the effect of any drug treatments will be non-significant; if there are no deficits then cognition cannot be improved with any drug treatment. The purpose of the present experiment, therefore, was to test male and female 3xTg-AD mice on a number of commonly used behavioural tests of cognitive function: spontaneous alternation in the Y-maze, novel object recognition (NORT), the Barnes maze of spatial memory, and cued and contextual fear conditioning tasks, in order to determine which test is the most sensitive to the cognitive deficits of these mice at this age. The sensitivity of each test was evaluated by comparing the effect sizes for the genotype differences detected in that test. Knowing which test is the most sensitive will allow researchers to more efficiently screen new therapies by decreasing the number of animals required to detect a difference and increasing the likelihood of detecting any differences. Because sex differences in memory have been found in transgenic mice (Mizuno and Giese, 2010), including the 3xTg-AD mice (Blázquez et al., 2014), we also examined the effect sizes of sex differences in these cognitive tasks.

7.3 METHODS

7.3.1 ANIMALS

Eighty-five mice, 42 3xTG-AD (21 female and 21 male, Stock # 004807), and 43 B6129SF2 (22 male and 21 female, Stock #101045), were bred in our lab from parents purchased from the Jackson Laboratory (Bar Harbor, Maine). The mice were weaned at 21 days of age and tested at 6.5 months of age in three cohorts of approximately 28 mice. The mice were housed in groups of 2-4 same sex littermates in plastic cages (18.75 x 28 x

12.5 cm), with a PVC tube (4 cm diameter x 7 cm length) for enrichment, wood chip bedding, and metal wire covers. They were provided with rodent chow (Purina 5001) and tap water ad libitum. The mice were individually identified by ear punch and were genotyped using polymerase chain reaction of the ear punch tissue samples, by Dr. Chris Sinal (Pharmacology Department, Dalhousie University). The mice were housed in a colony room maintained at 22±2ºc with a reversed 12:12 light:dark (L:D) cycle (lights off at 10:00 am). They were tested during the dark phase of the L:D cycle in the order described below. These mice had completed a motor assessment battery prior to cognitive testing (Stover et al., 2015a).This research was approved by the Dalhousie University Committee on Laboratory Animals.

7.3.2 Y-MAZE TEST OF SPONTANEOUS ALTERNATION

The spontaneous alternation test was performed in a symmetrical black Plexiglas Y-maze with three arms (20 cm long by10 cm wide and 20 cm high) at 120° angles, designated A, B, and C. Based on the procedure of Carroll et al. (2007), the mice were placed in the distal end of arm A and allowed to explore the maze for 8 minutes. A video camera mounted above the maze recorded the movements of the mice for analysis. The arm entries were recorded and the percentage of alternations (entry in to an arm that differs from the previous two entries) was calculated with the following formula: $\left(\frac{Alternations}{Arm\ Entries-2}\right) * 100$. The percentage of alternate arm returns (AAR, ex. ABA), and same arm returns (SAR, ex. AA) were also calculated.

7.3.3 NOVEL OBJECT RECOGNITION TASK

The novel object recognition task (NORT) was performed in an open field (38x38 cm wide by 38 cm tall) with three white walls, and one clear Plexiglas wall for

observation , as described by Yan et al. (2004). A camera mounted above the open field recorded the movements of the mouse throughout the trial. Mice were first given a five minute habituation trial with no objects in the open field, and then a test phase that consisted of two trials beginning twenty-four hours later. In the first trial two different objects were placed in diagonally opposite corners of the open field and the mice were allowed to explore them for five minutes. Fifteen minutes later one of the objects was replaced with a novel object and the mouse was again placed in the maze for a second five minute trial. The objects used were small plastic toys of similar sizes. The maze was cleaned using 70% ethanol between each trial. The discrimination score for novel object exploration was calculated with the following formula:

$$\left(\frac{time\ exploring\ novel\ object}{time\ exploring\ novel\ object + time\ exploring\ familiar\ object}\right) * 100\ .$$

7.3.4 BARNES MAZE TEST OF SPATIAL LEARNING AND MEMORY

The Barnes maze was a white polyethylene platform (122 cm diameter) elevated 48.4 cm from the floor with sixteen holes (4.45 cm diameter) equally spaced around the perimeter 1.3 cm from the edge, as described by O'Leary and Brown (2012). Four of the holes (4, 8, 12 and 16) were capable of having a black plastic escape box beneath them. A buzzer (0–37.2 kHz, 89 dB) and two 150W flood lamps placed 155 cm above the maze were used as aversive stimuli. A polyvinylchloride tube (8 cm diameter, 12.5cm height) was used to hold the mouse in the center of the maze before the trial began. A camera was mounted 1.7 m above the maze and the Limelight tracking system (Actimetrics) used to track the location of the mice.

Mice were tested in groups of 3-4 and each mouse in the group was assigned a different escape hole location. There were five phases in the test procedure: habituation,

acquisition training, acquisition probe, reversal training, and reversal probe (O'Leary and Brown, 2013). During the habituation phase, mice were placed in a 2L glass beaker which was inverted over the assigned escape hole. The mice were then free to explore the escape hole, escape box, and the adjacent area for two minutes. The acquisition training phase consisted of two trials per day for 15 days. On each trial, mice were placed in the center tube and after an interval of 5-10 seconds the tube was lifted and the buzzer was turned on. The mice were given of 300 seconds to locate the escape hole and if they did not enter the escape box within this time they were led to the escape hole with a plastic cup which was used to transport the mice. The maze was cleaned between trials to prevent odour cues from developing around the escape holes. The latency to enter the escape hole, distance travelled, and average speed were analyzed for each trial using Ethovision (Noldus, Wageningen, The Netherlands). The number of errors (when a mouse dips its head into a hole that is not the escape hole) were recorded by the experimenter. Repeated head-dips into the same hole were recorded as one error.

The day after acquisition training the mice were given a five minute memory probe trial with no buzzer turned on. During this trial the escape box was removed and the maze was rotated 45º so that a non-escape hole was in the correct escape hole location. For analysis of spatial memory the maze was divided into 16 pie shaped zones and the number of entries and time spent in each zone were recorded. The following day mice were given a curtain probe trial using the same procedure with no spatial cues, as the maze was surrounded by a black curtain. The mice were then given two more days of acquisition training to counter any effect of the probe trials and then a five day reversal training phase the escape hole was moved to the opposite side of the maze and. Finally a

reversal probe trial (with no curtain) was given using the same procedure as during the acquisition probe trial.

7.3.5 CONTEXTUAL AND CUED FEAR CONDITIONING

Cued and contextual fear conditioning and testing took place in two identical MED Associates Inc. (St. Albans, VT) fear conditioning chambers, as described in Martin and Brown (2010). The front, top, and back of the chamber were transparent Plexiglas and the other two sides were stainless steel. The floor of the chamber consisted of 36 3.2 mm stainless steel rods that were capable of delivering a shock. A speaker was attached to one of the stainless steel walls and a video camera was mounted in front of one of the Plexiglas walls to record the behaviour of the mouse.

The procedure consisted of a training and test phase, which took place on two consecutive days. During the training phase, mice were placed in the chamber and their levels of baseline freezing were recorded for 120 seconds. The mice were then presented with an 80dB tone for 30 seconds, co-terminating with a two second 0.7mA foot shock, followed by another 120 second interval and a second tone and foot-shock pairing. Thirty seconds after the second shock the mice were removed from the chamber and returned to their home cage. In the memory test phase, mice were given two trials, first contextual then cued memory. For the contextual memory test, mice were placed in the same chamber used during training for five minutes, with no tones or shocks delivered, and the duration of freezing behaviour was recorded using a stopwatch. Differences between groups in time spent freezing were analyzed. For cued fear memory testing, black Plexiglas was placed over the floor of the chamber to cover the steel rods, the inside walls of the testing chamber were covered with black and white striped plastic, and a

novel orange odor was introduced into the chamber. The mice were then placed in the modified chamber and their freezing time was recorded for three minutes, and then a continuous three minute 80dB tone identical to the tone presented during training was presented and their level of freezing behaviour was recorded for a second three minutes. The time spent freezing during the three minute baseline measurement and during the three minute cued portion of the testing trial were analyzed.

7.3.6 STATISTICAL ANALYSES

For the repeated measures designs (Barnes maze acquisition and reversal tasks and the cued fear conditioning tasks) linear mixed effects modeling was used to analyze the measures described. For all other tasks, linear regression models were used. The second order Akaike information criterion (AICc), the Akaike weight, and the evidence ratio for all models was calculated, and the model with the lowest AICc was selected and compared to the null model with an F-test (linear regression models), or a $\chi 2$ test (linear mixed effects models). Confidence intervals (95%) of the coefficients in the models were calculated. The AIC takes into account the complexity of the model and how well the model fits the data, while the second order AIC (AICc) corrects for small sample sizes, but approaches the same value as the the AIC with larger sample sizes (Akaike, 1974; Burnham and Anderson, 2002). For models with genotype or sex effects, an unbiased effect size was calculated (Cohen's d with a Hedge correction, d_{unb}) to determine which measures showed the largest effects (Cumming, 2014).

7.4 RESULTS

7.4.1 Y-MAZE TEST OF SPONTANEOUS ALTERNATION

Three measures of alternation behaviour were calculated: the percentage of spontaneous alternation behaviours (SAB), the percentage of alternate arm returns (AAR), and the percentage of same arm returns (SAR). For the percentage of SABs the linear regression model with the lowest AICc was the model with only genotype (AICc=714.038, weight=0.429, Supplemental Table 7.1.1, Figure 7.1A), which differed significantly from the null model ($F(1,83)=6.273$, $p=0.014$). Confidence intervals indicated that the 3xTg-AD mice exhibited significantly more spontaneous alternations than B6129SF2 (CI_{95}= 1.745 – 15.002 SABs). There were no differences between genotypes or sexes in the percentage of AARs (Figure 7.1B, Supplemental Table 7.1.2) or SARs (Figure 7.1C, Supplemental Table 7.1.3).

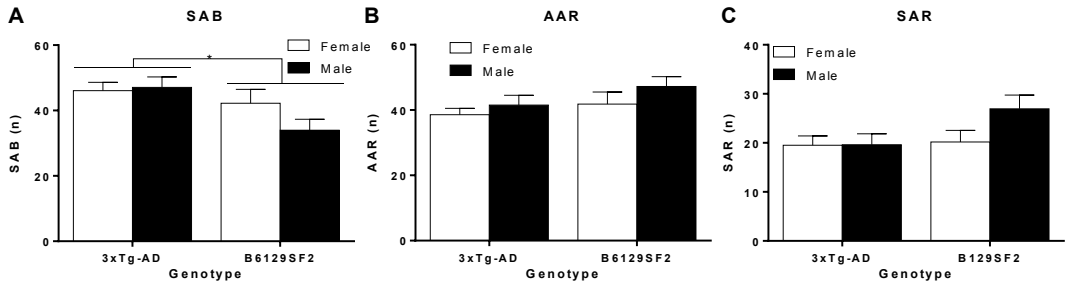

Figure 7.1 Mean (± SEM) percentage of spontaneous alternation behaviours (A), alternate arm returns (B), and same arm returns (C) for male and female 3xTg-AD and B6129SF2 mice in the Y-maze test of spontaneous alternation. * = a difference between groups at a 95% confidence interval.

7.4.2 NOVEL OBJECT RECOGNITION TASK

During the test phase of the NORT task, discrimination scores for the amount of time spent interacting with each object and for the number of interaction bouts were calculated. There was a slight preference for the novel object (Figure 7.2A and 7.2B),

however the best model for each measure was the null model (Supplemental Tables 2.1 and 2.2), which indicated that neither genotype nor sex affected scores for the NORT task. All groups had scores near 50%, which indicates they spent roughly equal time with each object.

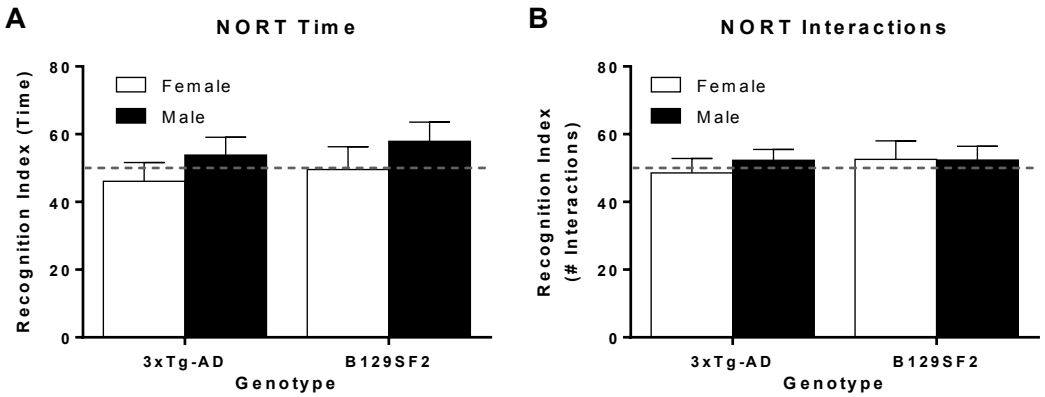

Figure 7.2 Mean (± SEM) discrimination score for the amount of time (A) and number of bouts (B) interacting with the novel object compared to the familiar object for male and female 3xTg-AD and B6129SF2 mice in the novel object recognition task. The dotted line represents no preference between the novel and familiar object.

7.4.3 BARNES MAZE

For the latency to find the escape hole during the acquisition phase of the Barnes maze task (Figure 7.3A) the linear mixed effects model with day and sex (AICc=14131.662, weight=0.300, Supplemental Table 7.3.1), was the best and differed significantly from the null model ($\chi 2(15, N=85)=539.62$, $p<0.001$). Confidence intervals indicated females had shorter latencies to escape than males (CI_{95}= -2.033 – 43.134s), and that the latency to escape decreased over acquisition trials (Day 1 to 15: CI_{95}= -110.168 – -76.998s).

For the distance travelled during acquisition (Figure 7.3B) the best model had only day (AICc=18374.018, weight=0.341, supplemental Table 7.3.2), which differed

significantly from the null model ($\chi 2(14, N=85)=183.6$, $p<0.001$), indicating that neither genotype nor sex predicted the distance travelled. Confidence intervals showed that the distance travelled decreased over days (Day 1 to 15: CI_{95}= -488.380 -303.865cm).

The number of errors (head dips into incorrect escape holes) during acquisition was best explained by a model with genotype, day, sex, and a genotype by sex interaction (AICc=8810.488, weight=0.469, Supplemental Table 7.3.3, Figure 7.3D), which differed significantly from the null model ($\chi 2(17, N=85)=136.39$, $p<0.0001$). Confidence intervals showed that 3xTg-AD mice made more errors than B6129SF2 wildtype mice (CI_{95}= -0.397 – 3.619 errors), and that the number of errors decreased throughout acquisition (CI_{95}= -6.343 – -2.071). The genotype by sex interaction occurred because the male 3xTg-AD mice made more errors than the females (CI_{95}= 0.121 – 5.835 errors), while in the male B6129SF2 mice made equal or slightly fewer errors than females (CI_{95}= -1.067 – 4.519 errors).

The best model for the average speed during the acquisition trials had day, genotype, and sex, and both day by sex and day by genotype interactions (AICc=7082.355, weight=0.677, Supplemental Table 7.3.4, Figure 7.3C), which differed significantly from the null model ($\chi 2(44,N=85)= 428.66$, $p<0.0001$). Confidence intervals indicated that 3xTg-AD mice moved faster than B6129SF2 wildtype mice (CI_{95}= 0.517 – 3.381 cm/s), that females moved faster than males (CI_{95}= 1.167 –3.998 cm/s), and that the average speed increased over days (Day 1 to 15: CI_{95}= 6.900 – 9.001 cm/s). The genotype by day interaction occurred because there was no difference between genotypes on day 1 (CI_{95}= -2.067 – 2.054 cm/s), but by day 15 3xTg-AD mice moved faster than B6129SF2 wildtype mice (CI_{95}= 1.630 – 5.672 cm/s). The day by sex interaction was due

to by a similar pattern: on day 1 there was little evidence for a sex difference (CI_{95}= -1.997 – 2.117 cm/s), but by day 15 females were moving faster than males (CI_{95}= 1.459 – 5.575 cm/s).

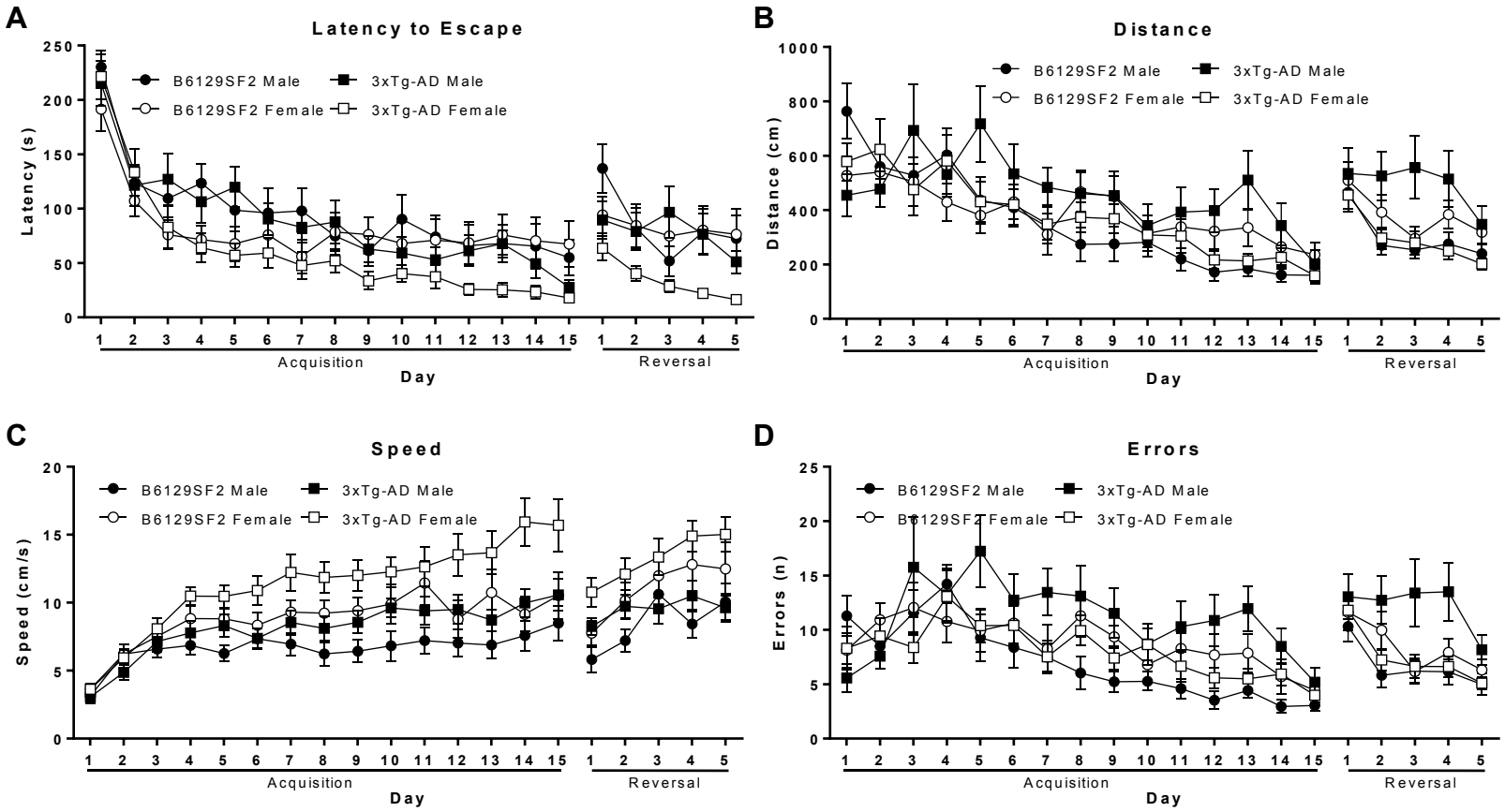

Figure 7.3 Mean (± SEM) latency (s) to escape (A), distance travelled (B), number of incorrect head dips (Errors, C), and moving speed (D) for male and female 3xTg-AD and B6129SF2 mice during acquisition and reversal learning in the Barnes maze

On the acquisition probe trial the data for one 3xTg-AD male mouse was not collected due to an equipment error. The best linear regression model for the duration of time spent in the correct zone (Figure 7.4A) had only genotype (AICc=896.423, weight=0.419, Supplemental Table 7.3.5), which was significantly different from the null model ($F(1,82)=4.766$, $p=0.032$). Confidence intervals indicated that 3xTg-AD mice spent less time in the correct zone than B6129SF2 wildtype mice (CI_{95}= -44.63 – -3.38 s).

For the frequency of entries into the correct zone during the acquisition probe trial (Figure 7.4B) the best model had only sex (AICc=529.332, weight=0.382, Supplemental Table 7.3.6) as there was a trend for males to enter the correct zone less frequently than females (CI_{95}= -4.318 – 0.415 entries), however the model did not differ significantly from the null model ($F(1,82)=82.012$, $p=0.104$).

During the probe trial with a curtain blocking the spatial cues the null model was the best model for both time spent in the correct zone (AICc=635.461, weight=0.439, Figure 7.4C, Supplemental Table 7.3.7) and the frequency of entries into the correct zone (AICc=401.454, weight=0.375, Figure 7.4D, Supplemental Table 7.3.8), indicating that neither genotype nor sex predicted the amount of time spent in the correct quadrant during the curtain probe.

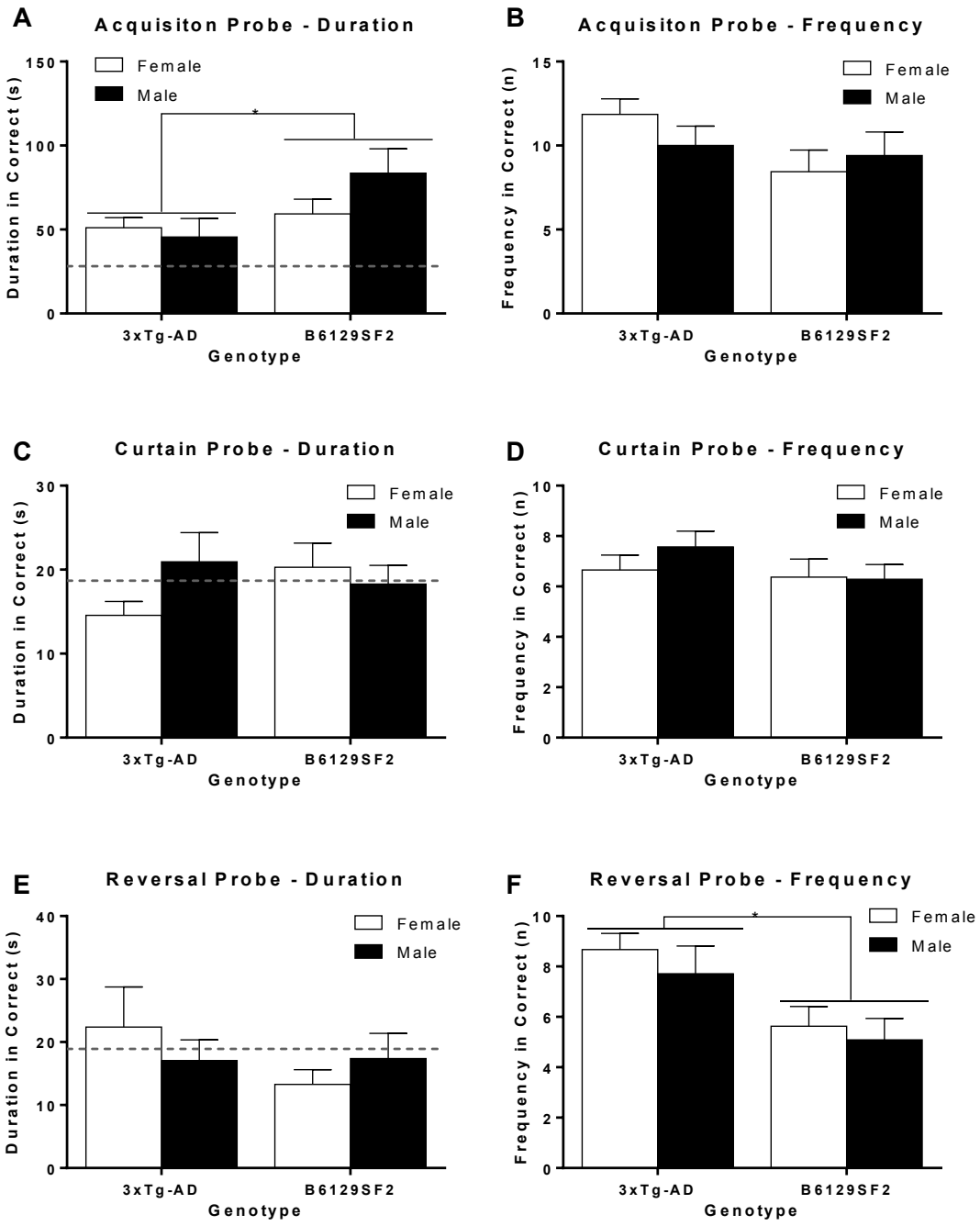

Figure 7.4 Mean (± SEM) duration of time spent in the correct zone during the acquisition (A), curtain (C) and reversal (E) probe trials in the Barnes maze. The dotted line represents chance performance. Mean (± SEM) frequency of entries into the correct zone during the acquisition (B), curtain (D), and reversal (F) probe trial in the Barnes maze for male and female 3xTg-AD and B6129SF2 mice. * = a difference between groups at a 95% confidence interval.

For the latency to escape during reversal training (Figure 7.3A) the best linear mixed effects model had genotype, sex, and day (AICc=4522.949, weight=0.09, Supplemental Table 7.3.9), which was significantly different from the null model ($\chi 2(6, N=85)=57.527$, $p<0.0001$). The confidence intervals indicated that 3xTg-AD mice escaped faster than B6129SF2 wildtype mice (CI_{95}= -2.643 – 60.568 s), that females escaped faster than males (CI_{95}= -5.734 – 56.82 s), and that latency to escape decreased over days (Day 1 to 5: CI_{95}= -53.847 – -30.405 s).

The best linear mixed effects model for average distance travelled during reversal learning (Figure 7.3B) had day, genotype, sex, and a genotype by sex interaction (AICc=5801.704, weight=0.657, Supplemental Table 7.3.10), which was significantly different from the null model ($\chi 2(7, N=85)=55.867$, $p<0.0001$). Confidence intervals indicated that there was a significant decrease in distanced travelled over days during reversal (Day 1 to 5: CI_{95}= -282.336 – -153.0194), and the genotype by sex interaction showed that male 3xTg-AD mice travelled a longer distance than females (CI_{95}= 71.363 – 323.625 cm), but within B6129SF2 wildtypes there was no sex difference (CI_{95}= -201.098 – 46.962 cm).

The number of errors during reversal learning (Figure 7.3C) was best explained by a model with day, genotype, sex, and a genotype by sex interaction (AICc=2778.690, weight=0.747, Supplemental Table 7.3.11), which was significantly different from the null model ($\chi 2(7,N=85)=55.061$, $p<0.0001$). Confidence intervals showed that 3xTg-AD mice made more errors than B6129SF2 wildtypes (CI_{95}= 0.185 – 4.364 errors), that males made more errors than females (CI_{95}= -0.600 – 3.544 errors), and that the number of errors decreased over days (Day 1 to 5: -7.333 -3.814 errors). There was a genotype by

sex interaction as there was no difference between male and female B6129SF2 wildtype mice (CI_{95}= -1.218 – 4.738 errors), but the 3xTg-AD males made more errors than 3xTg-AD females (CI_{95}= 1.804 – 7.702 errors).

For average speed during the reversal trials (Figure 7.3D) the best model had day, genotype, and sex (AICc=2393.387, weight=0.299, Supplemental Table 7.3.12), which was significantly different from the null model ($\chi 2(6, N=85)=79.194$, $p<0.0001$). Confidence intervals indicated that 3xTg-AD mice moved faster than B6129SF2 wildtype mice (CI_{95}= -0.200 – 3.812 cm/s), that females moved faster than males (CI_{95}= 1.217 – 5.300 cm/s), and that speed increased over days (CI_{95}= 2.622 – 4.623 cm/s).

During the reversal phase probe trial the data of two mice (both female B6129SF2 wildtypes) was not collected due to an equipment error. The best model for the duration of time spent in the correct zone (Figure 7.4E) was the null model (AICc=732.973, weight=0.454, Supplemental Table 7.3.13). The best model for the frequency of entries into the correct zone (Figure 7.4F) had only genotype (AICc=466.261, weight=0.597, Supplemental Table 7.3.14), which was significantly different from the null model ($F(1,81)=11.003$, $p=0.001$). The confidence intervals indicated that 3xTg-AD mice performed better on the reversal probe as they made more entries into the correct zone than B6129SF2 wildtype mice (CI_{95}= 1.047 – 4.543 entries).

7.4.4 CONTEXTUAL AND CUED FEAR MEMORY

During the fear memory tests the total time spent freezing was analyzed. The data from 2 mice were lost due to an equipment error (one 3xTg-AD female and one B6129SF2 wildtype female). During the contextual fear memory trial the best linear regression model for amount of time spent freezing (Figure 7.5A) had both genotype and

sex (AICc=919.399, weight=0.625, Supplemental Table 7.4.1), which was significantly different from the null model ($\chi 2(7, N=83)=7.068$, $p<0.001$). Confidence intervals indicated that the 3xTg-AD mice spent more time freezing than B6129SF2 wildtype mice (CI_{95}= 8.66 – 59.40 s), and that females spent more time freezing than males (CI_{95}= 12.78 – 63.33 s).

The data of five mice were lost due to an equipment error for the cued trials (one 3xTg-AD male, one 3xTg-AD female, and three B6129SF2 wildtype females). For the amount of time spent freezing (Figure 7.5B) the best model had test phase, sex, and a test phase by sex interaction (AICc=1587.764, weight=.0245, Supplemental Table 7.4.2), which was significantly different from the null model ($\chi 2(3, N=80)=195.42$, $p<0.0001$). Confidence intervals indicated that mice spent more time freezing during the cue phase (CI_{95}= 90.927 – 109.581 s), and that females spent more time freezing than males (CI_{95}= 5.911– 30.0275 s). The test phase by sex interaction occurred because there was no sex difference during the no-cue phase (CI_{95}= -23.055 – 7.706 s), but females spent more time freezing than males during the cue phase (CI_{95}= 12.683 – 43.545 s).

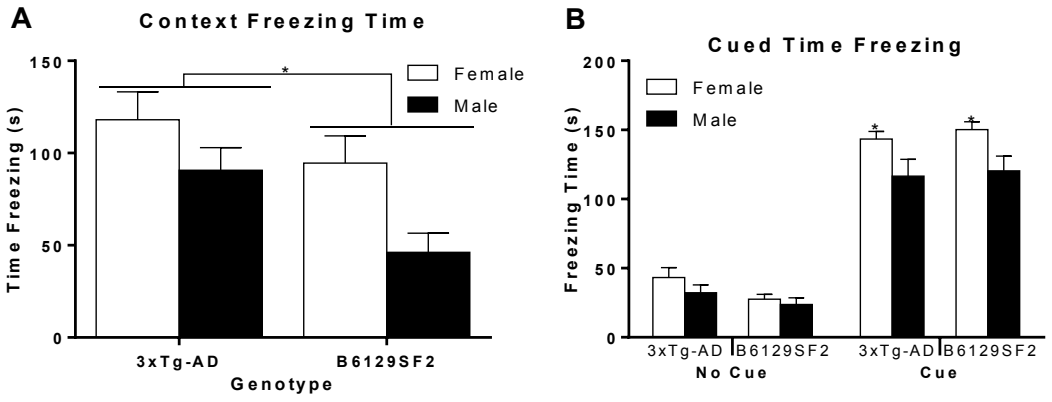

Figure 7.5 Mean (± SEM) time spent freezing during the context (A), and cued (B)

memory tests for male and female 3xTg-AD and B6129SF2 mice. * = a difference between groups at a 95% confidence interval.

7.5.5 EFFECT SIZE COMPARISON

For all of the measures with significant genotype or sex differences, the effect sizes were compared using Cohen's d with a Hedges correction for an unbiased measure of effect size (dunb) (Tables 1 and 2). In Table 7.1 positive effect sizes indicate higher scores in 3xTg-AD mice relative to B6129SF2 wildtypes and negative scores indicate higher scores in B6129SF2 wildtypes. The largest deficit in cognitive function in 3xTg-AD mice compared to B6129SF2 wildtypes was the decreased amount of time that 3xTg-AD mice spent in the correct zone during the acquisition probe trial in the Barnes maze relative to B6129SF2 wildtype mice (dunb=-0.472, CI95= -0.918 – -0.026) and the next largest deficit with a CI that does not include 0 was the number of errors during the reversal phase in the Barnes maze (d_{unb}= 0.300 CI_{95}= 0.107 – 0.494). This indicates that the Barnes maze was the most sensitive test for detecting cognitive deficits in the 3xTg-AD mice at 6.5 months of age.

Table 7.1 Genotype difference effect size estimates were calculated with a pooled SD and Hedges correction for all models including genotype. Positive values indicate 3xTg-AD mice had higher scores than B6129SF2 wildtype mice and negative scores indicate B6129SF2 wildtype mice had higher scores than 3xTg-AD mice. A '#' indicates that the confidence interval includes zero.

Measure	d_{unb}	95% Confidence Interval	
3xTg-AD higher than Wildtype		Lower	Upper
Barnes Maze – Reversal Probe Frequency Correct	0.721	0.265	1.178
Fear Conditioning – Context Freezing Time	0.554	0.103	1.004
Y-Maze – SAB	0.538	0.094	0.983
Barnes Maze – Acquisition Speed (Day 15)	0.490	0.046	0.933
Barnes Maze – Reversal Errors	0.300	0.107	0.494
Barnes Maze – Reversal Speed	0.286	0.093	0.480
Barnes Maze – Reversal Distance	0.185	0.008	0.378
Wildtype higher than 3xTg-AD			
Barnes Maze – Reversal Latency (Day 5)	-0.526	-0.970	-0.082

Barnes Maze – Acquisition Probe Duration Correct	-0.472	-0.918	-0.026
Barnes Maze – Acquisition Errors (Day 1)	-0.353	-0.794	0.086 #

In Table 7.2 positive effect sizes indicate higher scores in male mice relative to female mice and negative scores indicate higher scores in female mice. Interestingly in all measures females had better cognitive performance than males, though there are some genotype by sex interactions which indicated there were sex differences in the 3xTg-AD but not the B6129SF3 mice. Overall it appears that female mice had better cognitive performance than male mice.

Table 7.2 Sex defence effect size estimates were calculated with a pooled SD and Hedges correction for all models including sex. Positive values indicate male mice had higher scores than female mice and negative scores indicate female mice had higher scores than male mice. A '#' indicates that the confidence interval includes zero**.**

Measure	d_{unb}	95% Confidence Interval	
Males higher than females		Lower	Upper
Barnes Maze – Reversal Latency	0.278	0.086	0.472
Barnes Maze – Acquisition Latency	0.260	0.015	0.370
Barnes Maze – Reversal Distance	0.194	0.001	0.387
Barnes Maze – Reversal Errors	0.190	-0.383	0.002 #
Barnes Maze – Acquisition Errors	0.064	-0.046	0.174 #
Females higher than males			
Fear Conditioning – Cued Freezing Time (cue)	-0.676	-1.14	-0.211
Fear Conditioning – Context Freezing Time	-0.617	-1.07	-0.164
Barnes Maze – Reversal Speed	-0.550	-0.746	-0.353
Barnes Maze – Acquisition Speed	-0.502	-0.615	-0.391
Barnes Maze –Acquisition Probe Frequency Correct	-0.363	-0.805	0.080 #

7.5 DISCUSSION

In this study, the Barnes maze was the most sensitive test for detecting cognitive deficits in 3xTg-AD mice. We found evidence for a deficit in spatial learning and memory in the Barnes maze in both male and female 3xTg-AD mice at 6.5 months of age, as 3xTg-AD mice made more errors during acquisition than B6129SF2 wildtype mice (Figure 7.3D), and spent less time in the correct zone during the probe trial (Figure 7.4A). The 3xTg-AD mice moved faster than B6129SF2 wildtype mice by the end of acquisition, which may be related to their enhanced motor abilities (Stover et al., 2015a), and females moved faster than males, which may explain their faster latency to escape (Figures 3A and C). Mice were using spatial cues to navigate the maze, as they performed at chance in the curtain probe trial where the spatial cues were blocked. There was some evidence that 3xTg-AD mice had a deficit in memory during reversal (Garthe et al., 2009), as they made more errors than B6129SF2 wildtype mice (Figure 7.3D),

which could also be an indication that they have decreased cognitive flexibility. However, there was no evidence of a memory deficit in the reversal probe trial (Figures 4E and F). These findings are similar to those of Clinton et al. (2010), who found that 3xTg-AD mice made more errors and had a longer latency to escape than B6129SF2 wildtype mice at six months of age in their Barnes maze protocol. Our analysis of effect sizes indicated that the Barnes maze was the best task of those we used for detecting memory deficits in the 3xTg-AD mice, but the apparatus and procedure used must be carefully considered. O'Leary and Brown (2012, 2013) demonstrated that a curtain probe trial where the spatial cues are blocked must be used to indicate whether or not the mice are using a spatial strategy and determined the optimal apparatus design to ensure the mice are able to use spatial search strategies, and we used these parameters in this study. This Barnes maze paradigm has also been used to detect cognitive deficits in the APPswe/PS1dE9 mouse model of AD at 16 months of age (O'Leary and Brown, 2009).

In contextual fear conditioning we found no evidence for a memory deficit in 3xTg-AD mice at 6.5 months of age. In fact the 3xTg-AD mice spent a greater amount of time freezing than the B6129SF2 wildtype mice (Figure 7.5A), so it is possible that the 3xTg-AD mice have better memory on this task or found the stimulus more anxiety-inducing than the B6129SF2 wildtype mice. Females spent a greater amount of time freezing than males in both genotypes. There are conflicting reports about when the 3xTg-AD develop a deficit in this task, with some reporting a deficit by six months of age and others reporting no difference between genotypes by that age or even at 13 months of age (Billings et al., 2005; Pietropaolo et al., 2008; España et al., 2010; Chu et al., 2012). Our results showed an increase in the amount of time spent freezing during the

cue, indicating that all mice learned the task (Figure 7.5B). During the cued phase female mice spent more time freezing than male mice, which may indicate that males had a small memory deficit. In support of our findings with 6.5 month old mice Chu et al. (2012) found no difference between male or female 3xTg-AD and B6129SF2 mice in cued fear conditioning at 13-14 months of age.

In the Barnes maze, we found sex differences in memory as male 3xTg-AD performed worse than female 3xTg-AD mice, but there was no sex effect in B6129SF2 mice. This finding is supported by the results of Stevens and Brown (2014), who found that male 3xTg-AD mice had worse working and reference memory performance than females on the radial arm maze at 2, 6, 12, and 15 months of age in a cross-sectional study, but no difference in B6129SF2 mice. There are other reports of sex differences in spatial memory in this strain. Clinton et al. (2007) found that on the reference memory task in the Morris water maze (MWM) female mice performed worse than male mice at six and nine months of age, but there were no sex differences at two, four, twelve of fifteen months of age. Blázquez et al. (Blázquez et al., 2014) also found that female 3xTg-AD had poorer learning than males in the MWM at 12 and 15 months of age. In fear conditioning we found that female mice of both genotypes had better performance than males in both cued and contextual fear memory. However Clinton et al. (2007) found that female 3xTg-AD mice performed worse than male mice in an inhibitory avoidance task.

Male 3xTg-AD mice have a greater immune dysfunction and a higher mortality rate than female 3xTg-AD mice (Giménez-Llort et al., 2008). Immune function appears to be impaired in 3xTg-AD mice at two months of age, and by 12 months of age there are

severe immunological abnormalities, including splenomegaly, which may be the result of autoimmune disease in transgenic mice, though this has only been studied in male 3xTg-AD mice (Marchese et al., 2014). We have found that male 3xTg-AD mice died significantly earlier than females (450 vs 744 days), which may explain why males performed worse than females on some tasks (Rae and Brown, Unpublished Results).

In the Y-maze spatial alternation test we found that 6.5 month old 3xTg-AD mice performed more spontaneous alternations than the B6129SF2 wildtype mice, indicating they have no deficit in this task at this age (Figure 7.1A and 7.1C). We found similar levels of spontaneous alternations in our 3xTg-AD mice (~50%, Figure 7.1A), but lower levels (~35%, Figure 7.1A) in our B6129SF2 wildtype mice compared to other reports (Rosario et al., 2006; Carroll et al., 2007; Zhang et al., 2010). In the novel object recognition task we found no difference between genotypes or sexes, no mice appeared to have a preference for the novel object, indicating that the mice did not perform the task as expected (Figure 7.2A and 7.2B). There are conflicting reports in the literature about when the 3xTg-AD mice develop a deficit on this task. There are reports of a deficit in a 15 minute delay short-term memory version of this task in female 3xTg-AD at six months of age (Blanchard et al., 2010; Chen et al., 2013), and reports of no differences until 9 months of age in 1.5 and 24 hour memory versions of this task (Clinton et al., 2007; Martinez-Coria et al., 2010). It is possible that the 3xTg-AD mice have only a deficit in short term memory at six months of age, which is why we were unable to detect a difference in our 24 hour test. Another issue may be differences in our control mice; we found little preference for the novel object (~50%) in our B6129SF2 wildtype mice, while other studies typically find a preference score above 70%.

Both the Y-maze and the novel object recognition task are often used as behavioural tests when assessing novel drug treatments for AD (Rosario et al., 2006; Carroll et al., 2007; Ma et al., 2009; Blanchard et al., 2010; Corona et al., 2010; García-Mesa et al., 2011). However we found no difference between 3xTg-AD and B6129SF2 mice using these tasks at 6.5 months of age. In addition to the Y-maze or novel object recognition task a test of spatial memory, such as the Barnes maze, should be used to increase the chances of detecting a deficit, and any reduction in that deficit.

One issue that arises when attempting to compare the results of behavioural tests of 3xTg-AD mice across studies is the variety of control strains that have been used, each of which may have a distinct behavioural phenotype. Some studies have used the original background strain provided by Dr. Frank LaFerla (C7BL/6;129X1/SvJ;129S1/Sv)(Billings et al., 2007), while others, including us, used B6129SF2 mice (the second generation offspring of mice created by a cross between C57BL/6J females and 129S1/SvImJ males), which approximates the control strain and are recommend by the supplier of 3xTg-AD mice (http://jaxmice.jax.org/strain/004807.html), and one study used C57BL/6J as a control group (Sterniczuk et al., 2010b).

Another issue is the age of the control mice used: two studies used only one age of B6129SF2 wildtype mice when comparing across several ages of 3xTg-AD mice, while others used C57BL/6J rather than B6129SF2 mice as a control strain (Rosario et al., 2006; Carroll et al., 2007; Sterniczuk et al., 2010b). A third problem is the source of the mice: some researchers received the mice from the creator of the strain (Dr. Frank

LaFerla), while others purchased the mice from the Jackson Laboratories (Rosario et al., 2006; Carroll et al., 2007).

Differences in motor performance between 3xTg-AD and B6129SF3 mice are an important consideration when evaluating behavioural tasks. We have previously found enhanced motor performance on the Rotarod in the 3xTg-AD relative to B6129SF2 mice (Oore et al., 2013; Stover et al., 2015a), and in the present study we found that the 3xTg-AD mice travelled faster than the B6129SF2 mice during the acquisition phase of the Barnes maze, which can confound the latency measure.

After comparing the effect sizes for significant genotype differences (Table 7.1), the largest cognitive deficit in 3xTg-AD mice relative to B6129SF2 wildtype mice was the amount of time spent in the correct quadrant during the acquisition probe of the Barnes maze (d_{unb}= -0.472, CI_{95}= -0.026 – 0.918). The Barnes maze, specifically the acquisition phase and probe, appears to be the most sensitive task to detect a cognitive deficit in the 3xTg-AD mice. The reversal phase of the Barnes maze showed little difference between the genotypes, possibly due to the amount of training, and so could be omitted in future testing. We also found that females generally had better cognitive performance than males. Overall at six months of age the 3xTg-AD mice have some mild cognitive deficits compared to B6129SF2 mice and so could be suitable for testing interventions that are expected to have a large effect, for more subtle effects the 3xTg-AD may need to be used at an older age when the cognitive deficits are more pronounced. However the sex difference in mortality suggests that male and female 3xTg-AD mice differ in rates of ageing, or that males die at middle age, possibly due to immune complications, which may confound the results of neuro-behavioural studies.

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7.8 SUPPLEMENTAL TABLES

Description of supplemental tables.

The supplemental tables summarize the statistics used to choose the best model for each behaviour. The top five models for each measure are included. The model column describes the factors analyzed, terms separated by a '*' indicate both individual main effects and interactions between those two terms, terms separated by a '+' are both simple main effects, and terms separated by a ':' indicate an interaction alone. The 'AICc' column is the second order Akaike information criterion, which is a measure used to evaluate the models based on the complexity and how well the model fits the data; lower values are better. The "Δ AICc" column provides the difference between the given model's AICc and the model with the lowest AICc. The 'Wt' column is the Akaike weight, a measure of relative likelihood that the fit is the best, ranging from 0 (unlikely) – 1(likely). The 'ER' column is the evidence ratio which provides the likelihood that the model with the lowest AICc is better than the model in question.

Supplemental Table 7.1.1 Y-Maze – SAB.

Model	AICc	ΔAICc	Wt	ER
Genotype	714.038	0	0.429	1
Genotype+Sex	715.037	0.999	0.261	1.648
Genotype*Sex	715.393	1.355	0.218	1.969
Null	718.081	4.043	0.057	7.548
Sex	719.050	5.011	0.035	12.252

Supplemental Table 7.1.2 Y-Maze – AAR.

Model	AICc	ΔAICc	Wt	ER
Genotype	691.275	0	0.250	1
Genotype+Sex	691.449	0.174	0.229	1.091
Null	691.461	0.186	0.227	1.098
Sex	691.587	0.312	0.214	1.169
Genotype*Sex	693.528	2.253	0.081	3.085

Supplemental Table 7.1.3 Y-Maze – SAR.

Model	AICc	ΔAICc	Wt	ER
Genotype	654.141	0	0.235	1
Genotype+Sex	654.166	0.025	0.232	1.013
Genotype*Sex	654.388	0.247	0.208	1.132
Sex	654.879	0.738	0.163	1.446
Null	654.891	0.750	0.162	1.455

Supplemental Table 7.2.1 NORT – Interaction Number Decimation Score

Model	AICc	ΔAICc	Wt	ER
Null	622.789	0	0.518	1
Genotype	624.731	1.942	0.196	2.641
Sex	624.785	1.996	0.191	2.713
Genotype+Sex	626.802	4.014	0.070	7.440
Genotype*Sex	628.889	6.101	0.025	21.123

Supplemental Table 7.2.3 NORT –Interaction Time Discrimination Score.

Model	AICc	ΔAICc	Wt	ER
Null	667.958	0	0.349	1
Sex	668.092	0.134	0.326	1.069
Genotype	669.645	1.686	0.150	2.324
Genotype+Sex	669.884	1.926	0.133	2.619
Genotype*Sex	672.193	4.235	0.042	8.310

Supplemental Table 7.3.1 Barnes Maze – Acquisition Latency.

Model	AICc	ΔAICc	Wt	ER
Day+Sex	14131.662	0	0.300	1
Day+Genotype+Sex	14132.372	0.710	0.210	1.427
Day	14132.822	1.161	0.168	1.787
Day+Genotype	14133.532	1.871	0.118	2.548
(Day+Genotype+Sex)+(Genotype:Sex)	14134.103	2.442	0.088	3.390

Supplemental Table 7.3.2 Barnes Maze – Acquisition Distance.

Model	AICc	ΔAICc	Wt	ER
Day	18374.018	0	0.341	1
Day+Genotype	18374.671	0.653	0.246	1.386
Day+Sex	18375.574	1.556	0.157	2.177
(Day+Genotype+Sex)+(Genotype:Sex)	18375.792	1.773	0.141	2.427
Day+Genotype+Sex	18376.202	2.184	0.115	2.980

Supplemental Table 7.3.3 Barnes Maze – Acquisition Errors.

Model	AICc	ΔAICc	Wt	ER
(Day+Genotype+Sex)+(Genotype:Sex)	8810.488	0	0.469	1
Day+Genotype	8812.127	1.639	0.207	2.269
Day	8812.604	2.115	0.163	2.880
Day+Genotype+Sex	8813.871	3.382	0.087	5.426
Day+Sex	8814.374	3.885	0.067	6.977

Supplemental Table 7.3.4 Barnes Maze – Acquisition Speed.

Model	AICc	ΔAICc	Wt	ER
(Day+Genotype+Sex)+(Day:Genotype)+(Day:Sex)	7082.355	0	0.677	1
(Day+Genotype+Sex)+(Day:Genotype)+(Day:Sex)+(Genotype:Sex)	7083.890	1.535	0.314	2.154
(Day+Genotype+Sex)+(Day:Genotype)	7091.823	9.468	0.006	113
(Day+Genotype+Sex)+(Day:Genotype)+(Genotype:Sex)	7093.310	10.955	0.003	239
Day*Genotype*Sex	7101.321	18.966	0.000	13132

Supplemental Table 7.3.5 Barnes Maze –Acquisition Probe Duration Correct.

Model	AICc	ΔAICc	Wt	ER
Genotype	896.423	0	0.419	1
Genotype+Sex	897.777	1.354	0.213	1.968
Genotype*Sex	897.987	1.564	0.192	2.186
Null	899.016	2.594	0.115	3.657
Sex	900.268	3.845	0.061	6.840

Supplemental Table 7.3.6 Barnes Maze –Acquisition Probe Frequency Correct.

Model	AICc	ΔAICc	Wt	ER
Sex	529.332	0	0.382	0.382
Null	529.904	0.572	0.287	0.668
Genotype+Sex	531.395	2.062	0.136	0.804
Genotype	531.886	2.554	0.106	0.911
Genotype*Sex	532.239	2.907	0.089	1

Supplemental Table 7.3.7 Barnes Maze – Cur*t*ain Probe Duration Correct.

Model	AICc	ΔAICc	Wt	ER
Null	635.461	0	0.439	1
Sex	636.890	1.429	0.215	2.043
Genotype	637.339	1.878	0.171	2.558
Genotype*Sex	638.543	3.082	0.094	4.670
Genotype+Sex	638.834	3.373	0.081	5.401

Supplemental Table 7.3.8 Barnes Maze – Curtain Probe Frequency Correct.

Model	AICc	ΔAICc	Wt	ER
Null	401.454	0	0.375	1
Genotype	401.967	0.514	0.290	1.293
Sex	403.168	1.714	0.159	2.356
Genotype+Sex	403.707	2.253	0.122	3.085
Genotype*Sex	405.316	3.862	0.054	6.897

Supplemental Table 7.3.9 Barnes Maze – Reversal Latency.

Model	AICc	ΔAICc	Wt	ER
Day+Genotype+Sex	4522.949	0	0.095	1
Day+Genotype	4523.517	0.567	0.072	1.328
Day*Genotype*Sex	4523.609	0.659	0.069	1.391
Day+Genotype+Sex)+(Genotype:Sex	4523.635	0.686	0.068	1.409
Day+Sex	4524.360	1.411	0.047	2.025

Supplemental Table 7.3.10 Barnes Maze – Reversal Distance.

Model	AICc	ΔAICc	Wt	ER
(Day+Genotype+Sex)+(Genotype:Sex)	5801.704	0	0.657	1
(Day+Genotype+Sex)+(Day:Genotype)+ (Genotype:Sex)	5804.104	2.400	0.198	3.320
Day	5807.860	6.156	0.030	21.718
(Day+Genotype+Sex)+(Day:Sex)+ (Genotype:Sex)	5808.170	6.466	0.026	25.351
Day+Sex	5808.546	6.841	0.021	30.591

Supplemental Table 7.3.11 Barnes Maze – Reversal Errors.

Model	AICc	ΔAICc	Wt	ER
(Day+Genotype+Sex)+(Genotype:Sex)	2778.690	0	0.747	1
(Day+Genotype+Sex)+(Day:Sex)+ (Genotype:Sex)	2782.780	4.091	0.097	7.731
(Day+Genotype+Sex)+(Day:Genotype)+ (Genotype:Sex)	2783.922	5.232	0.055	13.682
Day+Genotype	2785.168	6.479	0.029	25.515
Day+Genotype+Sex	2785.590	6.900	0.024	31.504

Supplemental Table 7.3.12 Barnes Maze – Reversal Speed.

Model	AICc	ΔAICc	Wt	ER
Day+Genotype+Sex	2393.387	0	0.299	1
Day+Sex	2394.174	0.787	0.202	1.482
(Day+Genotype+Sex)+(Genotype:Sex)	2395.218	1.831	0.120	2.498
(Day+Genotype+Sex)+(Day:Genotype)	2395.621	2.233	0.098	3.055
(Day+Genotype+Sex)+(Day:Sex)	2395.826	2.439	0.088	3.385

Supplemental Table 7.3.13 Barnes Maze – Reversal Probe Duration Correct.

Model	AICc	ΔAICc	Wt	ER
Null	732.973	0	0.454	1
Genotype	734.148	1.174	0.253	1.799
Sex	735.090	2.116	0.158	2.881
Genotype+Sex	736.332	3.358	0.085	5.361
Genotype*Sex	737.369	4.395	0.050	9.004

Supplemental Table 7.3.14 Barnes Maze – Reversal Probe Frequency Correct.

Model	AICc	ΔAICc	Wt	ER
Genotype	466.261	0	0.597	1
Genotype+Sex	467.688	1.427	0.292	2.041
Genotype*Sex	469.895	3.634	0.097	6.153
Null	474.680	8.418	0.009	67.294
Sex	475.940	9.679	0.005	126.378

Supplemental Table 7.4.1 Fear Conditioning – Context Freezing Time.

Model	AICc	ΔAICc	Wt	ER
Genotype+Sex	919.399	0	0.625	1
Genotype*Sex	920.996	1.597	0.281	2.222
Sex	924.063	4.664	0.061	10.297
Genotype	925.511	6.112	0.029	21.241
Null	929.754	10.355	0.004	177.262

Supplemental Table 7.4.2 Fear Conditioning – Cued Freezing Time.

Model	AICc	ΔAICc	Wt	ER
Status*Sex	1587.764	0	0.245	1
(Status+Genotype+Sex)+(Status:Genotype)+ (Status:Sex)	1588.267	0.503	0.190	1.286
(Status+Genotype+Sex)+(Status:Sex)	1589.604	1.841	0.097	2.510
Status+Sex	1590.099	2.335	0.076	3.214
(Status+Genotype+Sex)+(Status:Genotype)+ (Status:Sex)+(Genotype:Sex)	1590.494	2.730	0.062	3.917

CHAPTER 8 GENERAL DISCUSSION

8.1 SUMMARY OF FINDINGS

8.1.1 GENOTYPE DIFFERENCES

In each of the four chapters we report a number of behavioural differences between the 3xTg-AD and B6129SF2 control mice. In chapter 2 we found that the 3xTg-AD have decreased anxiety-like behaviour on both the EPM and OF, and enhanced motor ability on the Rotarod. These findings were relatively stable from 2 to 18 months of age, though there was some evidence of a shift in behaviours at 18 months of age. In chapter 3 we found that the 3xTg-AD had an increased acoustic startle response relative to the B6129SF2 mice and no deficit at PPI at any age. In chapter 4 in the MWM we found that the 3xTg-AD mice have a deficit in spatial learning and an age-dependant deficit in spatial memory. By 18 months of age the performance of all mice decreased in the MWM, indicating there was an age-related cognitive decline. There was no genotype difference olfactory-dependant memory, even when testing 12 month long term memory. In chapter 5 we found some evidence that the 3xTg-AD mice have decreased social behaviour relative to B6129SF2 controls in the SNSP task, but no consistent changes with age.

When assessing the motor phenotype of the 3xTg-AD at six months of age in chapter 6 we found that the 3xTg-AD mice had enhanced performance on the Rotarod, which supports our findings in chapter 2. The enhanced motor performance may be explained by their longer gait that we found in gait analysis, and their better ability to recover from foot-slips we found on the balance beam. Interestingly the 3xTg-AD mice

had poorer grip strength on the grid suspension task. The 3xTg-AD mice also had a disrupted circadian rhythm in voluntary wheel running.

In chapter 7 we ran the 3xTg-AD on several commonly used cognitive tasks to determine which was the most sensitive at detecting the cognitive deficits present at 6.5 months of age and found that the Barnes maze was the most sensitive. In the Barnes maze the 3xTg-AD mice made more errors during acquisition than B6129SF2 wildtype mice and spent less time in the correct zone during the acquisition probe, and this difference had the largest effect size of the cognitive tasks. There was some evidence that the 3xTg-AD mice had enhanced performance on contextual fear conditioning compared to B6129SF2 wildtype mice, and no difference between genotypes in cued fear conditioning. The 3xTg-AD also performed better than the B1269SF2 control mice in the Y-maze test of spatial alternation test. Neither the 3xTg-AD nor the B6129SF2 mice had a preference for the novel or familiar object, indicating that the mice did not perform the task as expected.

Overall the 3xTg-AD mice have decreased anxiety-like behaviour, an enhanced motor phenotype, and deficits in spatial learning and memory in the MWM and Barnes mazes. These genotype differences are present at 2 months of age and generally continue until at least 18 months of age.

8.1.2 SEX DIFFERENCES

In each of the chapters we found several differences between sexes and some interactions between sex and genotype, though the sex differences were generally smaller than the genotype differences. In chapter two when assessing mice longitudinally from 2 to 18 months of age we found that female mice had a larger acoustic startle response and

better performance on the Rotarod than male mice, but little evidence of a sex difference in anxiety-like behaviour. In chapter five we found that male mice were more aggressive than female mice in home cage observations. Males generally had better olfactory memory than females, and there was no consistent sex difference in spatial memory.

In our analysis of motor function at six months of age in chapter six the only sex difference we found was that females had a narrower stride width than males. There were several other differences in motor behaviour that we initially attributed to sex but were better explained by body weight. In our assessment of cognitive tasks in 6.5 month old 3xTg-AD mice we found that female mice moved faster than males in the Barnes maze and performed better than males in cued and contextual fear conditioning. In the Barnes maze there was also a genotype by sex interaction, as the 3xTg-AD males performed worse than females, but there was a smaller sex difference in B6129SF2 mice.

8.1.3 AGE DIFFERENCES

In chapter 2, 3, 4, and 5 we tested mice on a longitudinal study from 2 to 18 months of age. In the two measures of anxiety, the elevated plus maze and open field, we found that the measures of locomotion tended to decrease with age, possibly due to habituation, but the measures of anxiety were relatively stable with age. The enhanced performance of the 3xTg-AD relative to the B6129SF2 mice was stable from 2 to 18 months of age. The amount of acoustic startle increased from 2 to 6 months of age then decreased with age. The increase from 2 to 6 months of age could be the result of an increase in body weight, and the decrease from 6 to 18 months of age could be the result of habituation or hearing loss. The amount of prepulse inhibition increased from 2 to 18 months of age. In the Morris water maze performance generally increased with age, as

the latency and distance to reach the platform decreased from 2 to 18 months of age. In the conditioned odour preference task there was no difference in short term memory with age. Generally mice had better memory for the more recent odours than more distant odours. Overall the mice habituated to the mazes with age, the anxiety, motor, and learning phenotypes were stable with age.

8.1.4 MATERNAL GENOTYPE DIFFERENCES

In chapters 2, 3, 4, and 5 we analyzed the effect of maternal genotype on behaviour and neuropathology in the 3xTg-AD mice in a longitudinal study from 2 to 18 months of age. Overall we found few consistent, lasting differences between mice reared by 3xTg-AD and B6129SF2 mothers. In Morris water maze and the conditioned odour preference task we found some evidence that the mice reared by B6129SF2 mothers had deficits in spatial and olfactory memory, though the results were not consistent across ages. The only consistent difference was that mice reared by B6129SF2 mothers had better motor performance on the Rotarod than mice reared by 3xTg-AD mothers, though the effect size was fairly small. This contrasts with our findings in these mice during a neurodevelopmental test battery, where maternal genotype affected the development of several reflexes, pups reared by 3xTg-AD mothers weighed more than those reared by B6129SF2 mothers, and mice reared by B6129SF2 mice reared more in an automated open field (Blaney et al., 2013). In our assessment of the levels of Aβ and tau neuropathology we found an increased density of tau positive neurons in the amygdala of mice reared by 3xTg-AD mothers, but no difference in tau in the hippocampus or in Aβ levels between maternal genotypes.

Blaney et al. (2013) found no differences in maternal care between the 3xTg-AD and B6129SF2 mothers, so it is possible the causes of the maternal effect are small and difficult to detect, and could be a factor other than behaviour, for example the quality of milk. Many studies have shown that maternal care and early life environment can have a lasting effect on behaviour (Priebe et al., 2005; Szyf et al., 2007), so the similar levels of care of 3xTg-AD and B6129SF2 mothers may explain why there were few lasting effects of maternal genotype. The relatively large differences between genotypes may have also masked any subtle differences in behaviour caused by maternal genotype.

Overall it appears that maternal genotype may have affected pup behaviour early in development, but there were few lasting effects of maternal genotype on behaviour at later ages in the 3xTg-AD. Moreover, the differences in cognitive behaviours between genotypes were not affected by maternal genotype, so maternal genotype does not appear to be an important consideration when using this strain as a mouse model of AD.

8.2 ASSESSMENT OF THE 3XTG-AD AS A MOUSE MODEL OF AD

The 3xTg-AD mouse develops neuropathology and behavioural deficits that are analogous to some aspects of AD. The introduction described three criteria for a mouse model of AD: face validity (are the symptoms in the animal model the same as in human AD), construct validity (does the model have the same mechanisms underlying the disease process as in human AD), and predictive validity (will treatments that work in the animal model translate to humans) (Willner, 1984).

The predictive validity of the 3xTg-AD mice has yet to be determined. To date none of the treatments assessed in the 3xTg-AD mice have translated into treatments for AD, though there were some successes in clinical trials for immunization therapy had

been previously tested in the 3xTg-AD mice, and research intro treatments using the 3xTg-AD mouse is ongoing (Giménez-Llort et al., 2013; Lemere, 2013). The only animal models of AD with any predictive validity thus far have been the cholinergic models, as cholinesterase drugs first tested in those models have been approved for use in AD (Scarpini et al., 2003; LaFerla and Green, 2012). However, the cholinergic models had little face validity, as their neuropathology was very different from AD, with no development of Aβ plaques or NFTs. The cholinergic models also had very little construct validity, as the underlying process was completely different.

The 3xTg-AD mouse model has a fairly high level of face validity in terms of neuropathology compared to other models of AD. The 3xTg-AD mouse develops the two hallmarks of AD neuropathology, Aβ plaques and NFTs; to date no other mouse model of AD develops both Aβ plaques and NFTs. The 3xTg-AD also develops synaptic dysfunction, similarly to human AD (Oddo et al., 2003). This neuropathology also increases with age, as in AD. However the face validity of the behavioural symptoms is much lower. While the 3xTg-AD mice develop a deficit in learning and memory, we found that the deficits in the MWM were relatively stable from 2 to 18 months of age, while in AD the memory deficits increase with age (Becker et al., 1988). The memory deficits that are present also appear to be relevantly mild and restricted to spatial memory until at least 18 months of age, while in AD the memory deficits become severe and spread to virtually all types of memory as the disease progresses. Another study on the 3xTg-AD in our lab found that the 3xTg-AD had a deficit in working and reference memory in the radial arm maze from 2 to 15 months in a cross-sectional study (Stevens and Brown, 2014). This supports our finding and suggests that the deficits can be

detected even with our longitude design. The 3xTg-AD also do not have increased anxiety or any of the other associated neuropsychiatric symptoms that would be expected in a model of AD.

Like all mouse models of AD, the construct validity of the 3xTg-AD is relatively low. While the 3xTg-AD do have two transgenes associated with AD (APP_{swe} and $PS1_{M146V}$), these genes are associated with familial AD. Familial AD a fairly rare (< 5% of AD cases) subtype of AD with an earlier onset and different genetic risk factors than sporadic late-onset AD (Campion et al., 1999). Due to the sporadic nature of AD using genes associated with familial AD is currently the best method for recapitulating AD-like symptoms in transgenic mice. The tau gene in the 3xTg-AD, tau_{P301L}, is associated with the development of frontotemporal dementia, and thus the development of tau pathology in the 3xTg-AD likely has different underlying mechanisms than the development of tau pathology in AD (Hutton et al., 1998). In humans mutations in the APP or PS1 genes alone are sufficient to cause familial AD, which involves the development of both Aβ and tau neuropathology (Campion et al., 1999), while in mice mutations in APP or PS1 alone can cause the development of Aβ pathology but not NFTs (Garcia-Alloza et al., 2006; Kanno et al., 2014). This discrepancy points to a difference between human and mouse physiology which will necessarily limit the face validity of the neuropathology and resulting behavioural changes of any mouse model of AD.

Another factor to consider when assessing the face and construct validity of the 3xTg-AD is the timing of gene expression in development. The transgenes are all expressed from an early stage of development and the first signs of neuropathology are present in the 3xTg-AD at two to three months of age (Mastrangelo and Bowers, 2008).

The development of neuropathology fairly early in the lifespan of this model provides relatively poor construct and face validity, as AD develops at an advanced age, and FAD develops in late adulthood. Additionally the presence of these transgenes from conception and the resulting neuropathology could interfere with normal developmental processes and cause behavioural and other deficits not directly related to the neuropathology in the adult brain.

While the 3xTg-AD mouse model may not perfectly recapitulate all the behavioural and neuropathological changes of AD there is still value in animal models which only partially model AD. The cholinergic models of AD had far worse face and construct validity but were still useful for assessing therapies based on the cholinergic system for AD. Similarly mouse models of AD which replicate the neuropathology of AD still be useful for assessing therapies to treat the neuropathology, even if the underlying mechanism and behaviour symptoms differ (Radde et al., 2008).

8.3 GENERAL CONCLUSIONS

Overall we found little evidence of a lasting effect of maternal genotype on the behaviour or neuropathology of the 3xTg-AD mice from 2 to 18 months of age. There were also few sex differences. Male mice had somewhat better olfactory-dependant memory than female mice and female mice performed better on fear conditioning. Female mice performed better on the Rotarod though this was better explained by body weight, were faster in the Barnes maze, and male mice had a wider stride length than female mice, likely due to a larger body size. The most reliable behavioural difference between the 3xTg-AD and B6129SF2 control mice was the enhanced motor phenotype of the 3xTg-AD on the Rotarod. Another mouse model with the tau_{P301L} mutation, JNPL3

mice, develops enhanced motor behaviour relative to controls on the Rotarod (Morgan et al., 2008), and so this is likely the cause of the enhanced motor behaviors of the 3xTg-AD relative to the B6129SF2 mice. We also found that the 3xTg-AD have decreased anxiety-like behaviour, which was stable from 2 to 18 months of age. The only cognitive deficits we detected in the 3xTg-AD relative to B6129SF2 mice were in spatial learning and memory, in the MWM from 2 to 18 months of age and in the Barnes maze at 6 months of age. The 3xTg-AD had enhanced performance in fear conditioning and no deficits in the novel object recognition task or the Y-maze relative to B6129SF2 mice. The tau_{P301L} gene may again be responsible for this phenotype. Transgenic mice with the tau_{P301L} transgene alone display enhanced cognitive abilities early in life (Boekhoorn et al., 2006; Morgan et al., 2008), this may be masking the effect of the other two transgenes (APP_{swe} and $PS1_{M146V}$), which generally produce cognitive deficits in other strains. The relativity late onset of tau pathology in the 3xTg-AD mouse may mean that the cognitive enhancing properties of the tau_{P301L}gene may last until the development of tau pathology, around 12-18 months of age, as the development of cognitive deficits in tau_{P301L}transgenic mice follow the development of tau pathology (Ramsden et al., 2005; Boekhoorn et al., 2006). We saw some changes in behaviour in our longitudinal study at 18 months of age, which may reflect the beginnings of these changes, however due to relatively high mortality ageing mice past 18 months of age is impractical.

The relatively minor cognitive deficits in the 3xTg-AD relative to wildtype mice may be difficult to reliability detect, thus assessing if any potential therapy is improving cognition in the 3xTg-AD may be difficult. However development of both Aβ and tau neuropathology is unique to the 3xTg-AD, which allows for the reliable assessment of

treatments targeted at reducing Aβ or tau neuropathology. Overall the 3xTg-AD is an advancement in modeling the neuropathology of AD but offers few advantages in modeling behavioural symptoms compared to other mouse models of AD.

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