LOCOMOTOR BEHAVIOUR, EMOTIONALITY, AND COGNITION IN THE 3XTG-AD MOUSE MODEL OF ALZHEIMER'S DISEASE: A CROSS-SECTIONAL STUDY

by

Leanne M. Fraser

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The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "LOCOMOTOR BEHAVIOUR, EMOTIONALITY, AND COGNITION IN THE 3XTG-AD MOUSE MODEL OF ALZHEIMER'S DISEASE: A CROSS-SECTIONAL STUDY" by Leanne M. Fraser in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Dated: May 13, 2013

External Examiner: _____

Research Supervisor: _____

Examining Committee: _____

_____

Departmental Representative: _____

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Abstract

The triple transgenic (3xTg-AD) mouse model of Alzheimer's disease (AD) possesses three transgenes that lead to the development of amyloid-beta (Aβ) plaques (APP_{swe}, $PS1_{M146V}$) and neurofibrillary tangles (and tau_{P301L}) (Oddo et al., 2003b). Although the neuropathology of these mice has been extensively studied (Sy et al., 2011), less research has been done to investigate their working memory, emotionality, and locomotor-related behaviour. Using a cross-sectional design, male and female 3xTg-AD mice were compared to control mice (B6129SF2/J) at five ages (2-, 6-, 9-, 12-, and 15-months of age) on a battery of five tests designed to measure: anxiety- and locomotor-related behaviours (open field [OF], elevated plus maze [EPM]); depression (forced swim test [FST]); motor coordination and motor learning (rotarod); and working and reference memory (8-arm radial maze [RAM]). Additionally, the brain tissue of male and female 3xTg-AD and control mice at 2- and 15-months of age was analyzed for the presence of Aβ plaques and human tau_{P301L}. 3xTg-AD mice were found to travel less and freeze more in both the OF and the EPM, engage in fewer bouts of immobility in the FST, have a longer latency to fall on the rotarod, and make more working and reference memory errors in the RAM than controls. There was no effect of age on performance in any of the tests. Intracellular Aβ plaques and limited human tau were present in the brain tissue of 2-month old 3xTg-AD mice. At 15-months of age, the brain tissue of 3xTg-AD mice showed extensive intra- and extracellular Aβ plaques as well as tau_{P301L} staining. The presence of intracellular Aβ at 2-months of age supports the behavioural differences observed in the 3xTg-AD mice at 2-months of age. However, the lack of progressive behavioural change does not match the increase in neuropathology seen in the brains of the 15-month old 3xTg-AD mice. The results of the present study suggest that while the 3xTg-AD mice display similar neuropathology and some of the behavioural differences seen in individuals with AD, they also exhibit contradictory behaviours; findings that should be taken into consideration for future researchers using 3xTg-AD mice.

List of Abbreviations Used

3xTg-AD	Triple transgenic mouse model of Alzheimer's disease
Aβ	Amyloid-beta
AD	Alzheimer's disease
ANOVA	Analysis of variance
APP	Amyloid precursor protein
ApoE4	ApolipoproteinE4
EPM	Elevated plus maze
FAD	Familial Alzheimer's disease
FST	Forced swim test
MCI	Mild cognitive impairment
MWM	Morris water maze
NFT	Neurofibrillary tangles
OF	Open field
PB	Phosphate buffer
PBS	Phosphate buffer with saline
RAM	Radial arm maze
SAP	Stretch attend postures

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Chapter 1: Introduction

1.0 Alzheimer's Disease in Humans

Alzheimer's disease (AD) is the most common form of dementia (Sy et al., 2011), affecting six in every 100 people over the age of 60 (Abbott, 2011), with the number of individuals living with AD expected to triple by 2050 (Herbert et al., 2013). The disease is characterized by progressive deterioration of cognitive functions, most notably memory, followed by changes in behaviour and sleep patterns as well as deficits in motor and language abilities (McDonald & Overmier, 1998).

This pattern of cognitive decline is thought to be the result of neuronal lesions that have been caused by amyloid-beta (Aβ) plaques, neurofibrillary tangles (NFTs), synapse loss, and cell death (Götz et al., 2004). The combined presence of Aβ plaques and NFTs is considered to be the hallmark of AD (Duyckaerts et al., 2008) and the lesions tend to be most severe in the hippocampus, amygdala, and temporal pole (Duyckaerts et al., 2003).

1.1 Neuropathology of AD

Amyloid plaques are extracellular aggregations of the amyloid beta (Aβ40 and Aβ42) peptide, derived from the amyloid precursor protein (APP; Gomez-Isla et al., 2008). Plaque deposition normally begins in the basal portions of the neocortex, and eventually spreads to more sub-cortical areas over time (Braak & Braak, 1991). The second hallmark of AD, the presence of neurofibrillary tangles (NFTs), is caused by irregular phosphorylation of the protein tau. Similar to Aβ plaques, tangle deposition also follows a pattern, beginning first between the entorhinal region and

temporal isocortex, progressing into the limbic areas of the brain, and finally affecting the cortex (Braak & Braak, 1991). The density of Aβ plaque deposition in the brain is not always related to the severity of AD or the number of NFTs; however a high number of NFTs is generally associated with a high plaque deposition (Braak & Braak, 1991). Additionally, unlike plaque deposition, widespread NFTs are correlated with the severity of AD symptoms (Nelson et al., 2012).

1.2 Genetic Underpinnings

Although specific neuropathic lesions characterize AD, there are at least two sub-types of the disease: sporadic onset AD and familial AD (FAD). Ninety-five to 99% of patients diagnosed with AD are afflicted with the sporadic form of the disease, while only 1-5% of patients have FAD (Delacourt et al., 2002). The two sub-types of AD have been associated with separate genetic mechanisms (Selkoe, 2011). The genetic influence on sporadic AD is not well understood, but it has been linked to the apolipoproteinE4 (ApoE4) gene, which has been shown to increase susceptibility for developing the disease (Saunders et al., 1993). However, although the ApoE4 gene increases susceptibility to AD, some individuals with the gene do not develop AD, while other individuals without ApoE4 develop AD (Selkoe, 2011).

In contrast, the genes involved with FAD have been identified and isolated. Specific genetic mutations were identified in three genes: the APP gene, and the genes encoding presenilins 1 and 2 (Sherrington et al., 1995; Gomez-Isla et al., 2008). Mutations in the APP gene increase Aβ plaque deposition, while mutations in the presenilins increase the production of the more fibrillogenic Aβ42 over Aβ40, thus increasing the overall amount of Aβ plaques (Sinha & Lieberburg, 1999).

Although one of the hallmarks of AD, neurofibrillary tangles, develops due to the accumulation and aggregation of tau proteins, there is no evidence linking the mutation of the tau gene to AD (Selkoe, 2011). In fact, disorders involving a mutation in the tau gene have shown that the development of neurofibrillary tangles does not lead to the development of Aβ plaques. Individuals afflicted with tau mutations develop extensive neurofibrillary tangles but do not develop Aβ plaques. Therefore, it has been concluded that Aβ plaque deposition may lead to the development of neurofibrillary tangles, but tangles do not lead to the development of plaques (Selkoe, 2011).

Despite extensive research efforts over the past few decades, there is still much to learn about the development and progression of AD. When mutations in the APP and presenilin genes were linked to the development of Aβ aggregation, and inserting those mutated genes into mice caused a similar development of Aβ plaques, it was thought that the cause of AD had been discovered (Schnabel, 2011). The idea that Aβ plaque deposition led to the cognitive decline seen in humans with AD, was known as the "Aβ plaque hypothesis" (Schnabel, 2011). However, it was soon discovered that mouse models with mutant APP and presenilins did not develop the same level of neuronal death or cognitive decline seen in humans with AD. Additionally, autopsies began revealing the presence of Aβ plaques in the brains of normal, symptom-free individuals (Schnabel, 2011). Since its introduction in the 1990's, the Aβ hypothesis has been widely debated and a number of alternative explanations have been put forward in an attempt to explain the underlying mechanisms responsible for the development of the disease (e.g., tau-axis

hypothesis (Ittner & Götz, 2011)). However, although many questions remain, Aβ plaques and neurofibrillary tangles are still considered to be the neuropathological hallmarks of the disease.

1.3 Development of Mouse Models of AD

Studying the development and progression of the neuropathology of Alzheimer's disease in humans presents a myriad of challenges. For this reason considerable time and money has been invested in the development of rodent models of the disease. Despite accounting for only a very small portion of AD patients, identifying the genes involved with FAD greatly influenced AD research (Selkoe, 2011). Researchers were able to create transgenic mice that possessed mutant forms of APP and presenilins, and discovered that these genetic manipulations led to the expression of Aβ plaques and cognitive deficits, similar to those seen in human AD patients (Götz et al., 2004; Schnabel, 2011). However, although mice with these mutations successfully express one of the neuropathological hallmarks of the disease (Aβ plaques) they do not develop neurofibrillary tangles (NFTs) (Sy et al., 2011), and are therefore considered to be an "incomplete" model of AD pathology.

1.4 Triple Transgenic Mouse Model of AD

In an attempt to develop a mouse model of AD that exhibited NFTs in addition to Aβ plaques, Oddo et al. (2003b) developed the triple transgenic (3xTg-AD) mouse. The 3xTg-AD mice possess the Swedish mutation of APP (APP_{SWE}), in addition to a mutation (P301L point) of the tau gene (tau_{P301L}), which were both microinjected into a germline of $PS1_{M146V}$ knock-in mouse embryo. The result was a

transgenic mouse that expressed both plaque deposition and neurofibrillary tangles that increased with age (Oddo et al., 2003b). The 3xTg-AD mice express Aβ plaques and NFTs in similar brain regions to human AD patients and the progression of the neuropathology also follows that seen in humans: Aβ plaque deposition begins first, followed by the development of NFTs (Sy et al., 2011).

1.4.1 Neuropathology of 3xTg-AD Mice

Triple transgenic mice were designed to express AD-like neuropathology in an age-related manner, with plaque deposits and NFTs increasing in severity over the lifetime of the animal (Sy et al., 2011). At two months of age, 3xTg-AD mice show no expression of Aβ plaques or NFTs (Oddo et al., 2003b); however, they have been shown to have some deterioration of the myelin sheath on the axons of the Schaffer collateral pathway which projects from the hippocampus (Desai et al., 2009). Aβ pathology begins to appear in the neocortex at around three to four months of age. At this stage, elevated levels of Aβ are present, but are in soluble form and have not yet begun to accumulate into plaques. Aβ levels continue to increase as the mice age; by six months of age intraneuronal Aβ plaques are detectable in the hippocampus and the amygdala of the 3xTg-AD mice (Oddo et al., 2006). Around nine months of age, intraneuronal Aβ decreases and extracellular Aβ plaques begins to appear in the cortex. The size and number of plaques increases with age, and by 12 months of age Aβ plaques are present in the hippocampus. By 15 months of age Aβ plaques are rampant throughout the hippocampus and cortex (Sy et al., 2011).

Tau pathology in the 3xTg-AD mice also progresses with age. At six months of age small amounts of the tau protein can be detected in the pyramidal neurons of the

CA1 region in the hippocampus (Oddo et al., 2003a). By nine months of age increased levels of phosphorylated tau are present in the hippocampus, and are also detectable in the cortex. Between 10-15 months of age, hyperphosphorylated tau aggregates (NFTs) become detectable in both the hippocampus and cortex and continue to increase in density as the mice age (Oddo et al., 2003b).

1.4.2 Behavioural Phenotype of 3xTg-AD Mice

Although extensive research has examined the neuropathology of the 3xTg-AD mice, less has been done to investigate their behavioural phenotype. Of the studies that have been conducted, most are in agreement that the 3xTg-AD mice behave similarly to their background control strain (B6129SF2/J) early in life (Billings et al., 2005; Billings et al., 2007; Giménez-Llort et al., 2007). However, there is much disagreement regarding the behavioural phenotype of the 3xTg-AD mice at older ages. Reports vary about what behavioural deficits exist, when those deficits first appear, and how they progress over time (Billings et al., 2005; Billings et al., 2007; Clinton et al., 2007; Giménez-Llort et al., 2007; Pietropaolo et al., 2008; Sterniczuk et al., 2010). For example, Sterniczuk et al. (2010) found no differences between 3xTg-AD mice and controls in motor coordination on the rotarod, while Filali et al. (2012) found 3xTg-AD mice had superior performance compared to controls. In the Morris water maze, a test of spatial learning and memory, Billings et al. (2005) and Clinton et al. (2007) found deficits in memory but not learning at six months of age, while Giménez-Llort et al. (2007) found deficits in both learning and memory at six months of age. In the open field, Pietropaolo et al. (2008) found no differences in anxiety (measured by time spent in the centre of the apparatus)

between 3xTg-AD and controls, while Sterniczuk et al. (2010) found 3xTg-AD mice had lower levels of anxiety and Giménez-Llort et al. (2007) found 3xTg-AD mice to have higher levels of anxiety, compared to controls in the open field.

To test new AD treatments using the 3xTg-AD mouse model of AD, it is crucial to have a thorough characterization of the age-related changes in their behavioural phenotype. Because previous studies have conflicting results, additional research must be conducted to determine the age-related changes in both the cognitive and non-cognitive behaviours of 3xTg-AD mice, at multiple ages, using both sexes, and proper control animals (same background strain), to increase our understanding about the neural and behavioural deficits of the 3xTg-AD mouse model.

1.5 Objective of the Present Study

Given the diverse number of behaviours affected by AD it is important to investigate whether the behavioural phenotype of the 3xTg-AD mice are analogous to that seen in AD patients. To date, relatively few studies have focused primarily on the behaviour of the 3xTg-AD mouse model and fewer still have investigated multiple facets of their behaviour (Pietropaolo et al., 2008). The aim of the present study was to thoroughly characterize the age-related changes in both the cognitive and non-cognitive behaviours in 3xTg-AD mice. These behaviours include motor coordination and learning, exploratory behaviour, locomotion, anxiety, depression, and working and reference memory.

1.6 Experimental Design

The present study involved a 30-day test battery designed to measure both cognitive and non-cognitive behaviours. The battery involved five tests used to

measure six different behaviours: Open field (OF; exploratory behaviour, locomotion, and anxiety); Elevated plus maze (EPM; locomotion and anxiety); Accelerating rotarod (motor coordination and learning); Forced swim test (FS; depression); and the 8-arm radial maze (RAM; working and reference memory). The battery was administered in the following order with corresponding durations: OF (1 day); EPM (1 day); FS (1 day); Rotarod (5 days); RAM (22 days). All tests were administered on separate days. See Figure 1.1 for apparatus designs.

A cross-sectional design was used to investigate cohorts of mice aged 2-3, 6-7, 9-10, 12-13, and 15-16 months of age. The age span refers to the age (in months) of the mice at the beginning of testing and at the end of testing. For simplicity, throughout the report mice are referred to by their age at testing onset (e.g., 15-16 month-old mice are referred to as 15 month old mice).

Control mice used in the present study were derived from the background strain (B6129SF2/J) of the 3xTg-AD mice. Use of this strain as a control minimizes the possibility of behavioural differences caused by the background strain in the 3xTg-AD mice. Several other studies have also used a strain with the same genetic background (B6129SF2/J) as the 3xTg-AD mice (e.g., Billings et al., 2005 & 2007; Clinton et al., 2007; Pietropaolo et al., 2008), which should allow us to directly compare our results to previous studies. Some researchers have used the C57BL/6J mouse strain (e.g., Sterniczuk et al., 2010) as a control for comparison with the 3xTg-AD mice. However, using a different mouse strain from the background strain in the experimental animal could lead to an incorrect interpretation of behavioural differences between the two groups of mice. Inherent behavioural differences exist

between different mouse strains (O'Leary et al., 2013); therefore it is important to select the correct strain to ensure the best control comparison.

Finally, both male and female 3xTg-AD and control mice were tested at all ages. Many of the studies that have investigated the behaviour of the 3xTg-AD mice have only used one sex (Pietropaolo et al., 2008; Filali et al., 2012) or have not examined differences between the sexes (Billings et al., 2005, 2007; Giménez-Llort et al., 2007). Therefore, it is important not only to include both male and female mice in the study, but to also examine whether any meaningful sex differences exist at any age.

Testing took place over a 1.5-year period. The groups of mice tested during each of the month-long test batteries included mice from varied ages, sexes and genotypes. The experimenter was blind to the age and genotype of the mice at the time of testing. All data were analyzed using Microsoft Excel 2011 and SPSS v.20 for Mac.

1.7 Animals

A total of 158 mice, 69 3xTg-AD and 89 B6129SF2/J, were used in this study (see Table 1.1 for a breakdown by age and sex). The protocol for this experiment was approved by the Dalhousie University Committee on Animal Care (protocol #11-042). Mice were tested using a cross-sectional design at five different ages: 2-3, 6-7, 9-10, 12-13, and 15-16 months of age, creating a 2 x 2 x 5 (genotype x sex x age) design. Previous studies (Billings et al., 2005; Pietropaolo et al., 2008; Sterniczuk et al., 2010) have used between 3 and 15 mice per group and found significant effects;

therefore, with 3-12 mice per genotype per sex per age, differences between groups were expected to be detectable.

1.8 Housing

Mice were born in the lab to parents purchased from The Jackson (Jax) Laboratory (Bar Harbor, Maine), weaned at 21 days of age and housed in same-sex, age-matched groups of 2 to 4. Housing cages were clear Plexiglas, measuring 18.75 x 28 x 12.5 cm, covered with a wire cage-top, and contained wood-chip bedding, one PVC tube, and half of a compressed cotton square (Nestlet) for nest construction. To prevent transmission of possible pathogens cages were also equipped with a plastic cover that contained a Hepa-filter. Mice were fed Purina 5001 rodent chow and water *ad libitum*, unless stated otherwise. Mice were housed on a reversed 12:12 light:dark cycle, with lights off at 9:45 am. The housing room was kept at 22±2°C. All mice were tested during the dark phase of their light:dark cycle. The experimenter was blind to the age and genotype of the mice until the completion of testing.

1.9 Histology

Twenty-four mice were sacrificed for neuropathological analyses: 12 2-month old mice (3 male 3xTg-AD, 3 female 3xTg-AD, 3 male B6129SF2/J, and 3 female B6129SF2/J) and 12 15-month old mice (3 male 3xTg-AD, 3 female 3xTg-AD, 3 male B6129SF2/J, and 3 female B6129SF2/J). Mice were anaesthetized with pentobarbital sodium and perfused through the heart with phosphate buffer followed by paraformaldehyde. The brains were extracted and placed into paraformaldehyde solution for 24 hours and then transferred to a phosphate buffer with saline (PBS) plus azide until further processing. Brains were then placed into a

sucrose and azide solution for 24-72 hours, then sliced using a microtome and stained for Aβ and phosphorylated tau.

1.10 Summary

The details of the research from this project are presented in four chapters, each written as a manuscript. Chapter 2 presents the background, procedure, and results from the experiment on motor coordination and learning ability on the rotarod. Chapter 3 presents the research on locomotion, anxiety and depression in the 3xTg-AD mice. Chapter 4 focuses on the visuo-spatial working and reference memory in the 8-arm radial maze, and Chapter 5 outlines the qualitative analysis of the brain tissue for Aβ and human tau in 2- and 15-month 3xTg and control mice. Chapters 6 examines the meaning and implications of the findings from the present study, and finally, chapter 7 briefly summarizes the conclusions of the project.

Chapter 2: Motor Coordination and Learning

2.0 Motor Coordination in Alzheimer's Patients

Although memory impairment is often considered the hallmark symptom of AD, as the disease progresses other cognitive functions and physical abilities also deteriorate. Patients with AD often experience mild motor-related slowing early in the disease that gradually progresses to gross motor deficits as the disease advances (Goldman et al., 1999; Pettersson et al., 2005). These deficits are thought to be associated with progression of amyloid plaque deposits to the motor cortex of the brain (Olazarán et al., 2013).

2.1 Motor Coordination and Learning in 3xTg-AD mice

Little research has been conducted to investigate the non-cognitive abilities of the 3xTg-AD mouse model (Pietropaolo et al., 2008; Filali et al., 2012). Historically, mouse models expressing the P301L mutation of human tau (the mutation present in the 3xTg-AD mouse model) have been shown to display motor deficits (e.g., Lewis et al., 2000; Götz et al., 2001; Ramsden et al., 2005). Because most learning and memory tests involve movement of some form, it is crucial to test the motor performance of the 3xTg-AD mice to ensure that any cognitive deficits found are not due to motor behaviour confounds.

Pietropaolo et al. (2008) compared male 3xTg-AD and control (B6129SF2/J) mice and found the 3xTg-AD mice were significantly more active in the open field test at 12 months of age, but not at 6 months of age. Sterniczuk et al. (2010) and Filali et al. (2012) compared female 3xTg-AD mice to controls (C57BL/6J) on the rotarod test using a single trial. When comparing 7-11 month old 3xTg-AD mice to 5-

7 month old C57BL/6J mice, Sterniczuk et al. (2010) found no differences in rotarod performance. However, Filali et al. (2012) found that female 3xTg-AD mice at 12-14 months of age performed significantly better (longer latency to fall) compared to age-matched C57BL/6J mice. Finally, using the same rotarod protocol as used in the present study (six trials over five days), Stover (2012; 2013, personal communication) found both male and female 3xTg-AD mice performed significantly better than age-matched B6129SF2/J mice at 2, 6, 12 and 18 months of age.

The accelerating rotarod test, used in the present study, is commonly used to assess motor coordination and motor learning in rodents (Hamm et al., 1994; LeMarec & Lalonde; 1997; Hyde et al., 2001). Over single or multiple trials, subjects are placed on top of a stationary rod and the latency to fall from the rod, as it increases in rate of rotation over time, is recorded. The rotarod is considered a powerful tool for measuring both motor coordination and motor learning in a wide variety of genetically altered, brain damaged, or drug-treated mice (Paylor et al., 1999; Rustay et al., 2003; Shiotsuki et al., 2010). However, it is important to note that differences in rotarod apparatus and procedures may lead to different results (Bohlen et al., 2009; Rustay et al., 2003). Therefore, the present study employed a standardized apparatus and procedure that was identical to that used in multiple studies examining motor-related differences among mouse strains (Brown & Wong, 2007; Gunn et al., 2011a; Gunn et al., 2011b; Stover, 2012). The procedure involved multiple trials (6) over multiple days (5) since it has been shown that repeated trials on the accelerating rotarod are essential for measuring motor learning (Buitrago et al., 2004; Luft & Buitrago, 2005).

2.2 Hypotheses for Motor Ability and Learning in 3xTg-AD mice

Based on the results of Filali et al. (2012) and Stover (2012; 2013, personal communication), it is hypothesized that 3xTg-AD mice will display superior motor coordination and motor learning performance (measured by latency to fall) compared to sex- and age-matched B6129SF2/J control mice.

Although Pietropaolo et al. (2008; male), Sterniczuk et al. (2010; female), and Filali et al. (2012; female) included only one sex of mice in their studies, Stover (2012; 2013, personal communication) tested both male and female mice on the same apparatus and protocol used in the present study, and found no sex difference. Additionally, over extensive testing Bohlen et al. (2009), found no sex differences on rotarod performance, thus no sex difference is expected.

It is difficult to predict the possible effect of age on rotarod performance. Neither Sterniczuk et al. (2010) nor Filali et al. (2010) compared mice at multiple ages, and Stover (2012) only investigated mice at 2 and 6 months of age (and found no effect of age). In general, older mice have been found to show impairment on the accelerating rotarod (Kennard & Woodruff-Pak, 2011). However, the results cannot be generalized across strains. Specifically, the two strains (129S1/SvImJ and C57BL/6J) that make up the background strain for the 3xTg-AD mice (and were used to create the control strain in the present study) have different patterns of age-related performance on the Rotarod. C57BL/6J mice show an age-related decrease in motor learning beginning at 12-13 months of age (Thouvarecq et al. 2001), although it has been shown to occur as early as 7 months of age (Serradj & Jamon, 2007); while 129/Sv mice show no change in rotarod performance between 1 and

13 months of age (Serradj & Jamon, 2007). Therefore, using the information available, it is expected that there may be an effect of age, with older mice having a lower latency to fall compared to younger mice.

Finally, because body weight may influence performance on the rotarod (Brown & Wong, 2007), we recorded the weight of the mice on each day of testing and hypothesize that heavier mice may fall from the rotarod sooner than lighter mice.

2.3 Animals

As outlined in section 1.7, a total of 158 mice (69 3xTg-AD and 89 B6129SF2/J) were used in this study (see Table 1.1 for a breakdown by age and sex). Mice were tested using a cross-sectional design at five different ages: 2-3, 6-7, 9-10, 12-13, and 15-16 months of age, creating a 2 x 2 x 5 (genotype x sex x age) design. Previous studies (Billings et al., 2005; Pietropaolo et al., 2008; Sterniczuk et al., 2010) have used between 3 and 15 mice per group and found significant effects; therefore, with 3-12 mice per genotype per sex per age, differences between groups were expected to be detectable.

Mice were born in the lab to parents purchased from The Jackson (Jax) Laboratory (Bar Harbor, Maine), weaned at 21 days of age and housed in same-sex, age-matched groups of 2 to 4. Housing cages were clear Plexiglas, measuring 18.75 x 28 x 12.5 cm and contained wood-chip bedding, one PVC tube, and half of a compressed cotton square (Nestlet) for nest construction. Cages were covered by a wire cage-top and a plastic cover that contained a Hepa-filter, to prevent transmission of possible pathogens. Mice were fed Purina 5001 rodent chow and

water *ad libitum*, unless stated otherwise. Mice were housed on a reversed 12:12 light:dark cycle, with lights off at 9:45 am. The housing room was kept at 22±2°C. All mice were tested during the dark phase of their light:dark cycle. The experimenter was blind to the age and genotype of the mice until the completion of testing.

2.4 Rotarod Apparatus

The rotarod is an apparatus (Figure 1.1d) used to measure motor ability, learning, and fatigue (La Marec & Lalonde, 1997). The AccuRotor Rotarod (Accuscan Instruments Inc., Columbus, Ohio), used in the present study, was designed to accelerate over time, making it a better measure of learning than a constant speed rotarod (Bogo et al., 1981). It consisted of a rotating rod (3 cm diameter) divided by Plexiglas dividers (15 cm radius) into four separate sections (each 11 cm wide). Four automatic timers were built into the apparatus that began counting the moment the rotarod was started. Each section of the rotarod had a holding chamber equipped with motion sensors (infrared photobeams) that stopped the timers when the mouse fell (39 cm drop) into the chamber. The rotarod rotated with a constant acceleration (6 rpm/min) beginning at 0.0 rpm and increasing to a maximum of 48.0 rpm over the length of the trial (360 seconds). The floors of the holding chambers were removable and cleaned with a damp cloth and mild detergent after each squad of mice was tested. The rotarod was located in a room measuring 112 cm x 260 cm and was illuminated by a 60W red light bulb.

2.5 Procedure

The mice were tested for six trials per day for five consecutive days, for a total of 30 trials. They were weighed prior to testing each day and then transported

in their home cages to the testing room. All testing was done during the dark phase of their light/dark cycle.

To begin a trial, mice were placed onto the rod, one mouse per section, facing the opposite direction of the rotation. Upon starting, the rotarod began with a rotation speed of 0.0 rpm and increased to 48.0 rpm over the 360-second trial at a rate of 6 rpm/min, and the automatic timers started. When a mouse fell from the rotating rod it dropped into the holding compartment below, triggering the motion sensors and stopping the automatic timer. Latency to fall was then recorded by the experimenter. After the last mouse had fallen into its holding compartment, the experimenter waited 60 seconds before placing all of the mice back on the motionless rod and began the next trial. If a mouse succeeded in remaining on the rod for the full 360 seconds, the rotation was stopped and the mouse was placed into the holding chamber for the 60-second inter-trial-interval. After the mice had completed six trials, they were placed back into their home cages and transported back to the colony room.

Prior to the start of the experiment it was determined that mice that used a "passive rotation" (holding onto the rod and rotating passively) strategy for more than one rotation would be excluded from analyses. However, none of the mice engaged in passive rotation for more than one rotation and therefore no data were excluded. Additionally, any mouse that consistently jumped from the apparatus and therefore failed to complete a trial was also excluded. One mouse was excluded due to excessive jumping.

Mean latency to fall for each day and learning scores [LS = (100*(Day 5 Performance – Day 1 Performance)/(Day 1 Performance)] were analyzed using SPSS 20.0 for MacIntosh. The data were first analyzed using a between-within (split-plot) analysis of variance (ANOVA) with day as the within factor, and genotype, sex, and age as the between factors. For repeated-measures analyses, if Mauchley's test of sphericity was significant (assumption of sphericity violated) the Greenhouse-Geisser correction was employed and the corresponding adjusted degrees of freedom. Tukey HSD post hoc analyses were performed when applicable and for independent samples t-tests, equality of variance was tested using Levene's test. If equal variances could not be assumed ($p>0.05$) an adjusted t-statistic (using the pooled estimate of the error term) and degrees of freedom (using the Welch-Satterthwaite method) were used to determine significance.

2.6 Results

2.6.1 ANOVA

The data were first analyzed using a between-within (split-plot) analysis of variance (ANOVA) with day as the within factor and genotype, sex, and age as the between factor (see Figure 2.1).

There was a significant overall effect of genotype on latency to fall ($F(1,137)=60.54$, $p<0.001$) with 3xTg-AD mice remaining on the rotating rod for longer than controls (Figure 2.2a). A significant overall main effect of sex was also found ($F(1,137)=4.49$, $p<0.05$) with females having a longer latency to fall than males at all ages (Figure 2.2b). Age, however, did not have a significant effect on performance (Figure 2.2c).

A significant age x genotype interaction was found ($F(4,137)=3.64$, $p<0.01$) (Figure 2.2c). Post hoc independent samples t-tests revealed that at 2, 6, 12, and 15 months of age (all $p<0.01$) 3xTg-AD mice had a significantly longer latency to fall than controls; however at 9 months of age there was no effect of genotype on latency to fall.

As expected, a significant main effect of day was found with all mice showing an increase in latency to fall over the five days of testing ($F(2.43,332.83)=261.47$, $p<0.001$) (see Figure 2.1).

A significant genotype x day interaction was found ($F(2.43,332.83)=45.87$, $p< 0.001$), with post hoc independent t-tests revealing that 3xTg-AD mice had a longer latency to fall than controls on each of the five days of testing (all $p<0.001$), with the difference increasing over days (see Figure 2.2a).

A genotype x age x day interaction was also found ($F(9.72,332.8)=2.41$, $p<0.01$) (Figure 2.3). Independent samples t-tests revealed that: at 2-months of age 3xTg-AD mice outperformed controls on all 5 days of testing (all $p<0.05$); at 6-months of age 3xTg-AD mice outperformed controls on all days (all $p<0.01$); at 9 months of age 3xTg-AD mice had a longer latency to fall than controls, on day 5 only ($p<0.05$); at 12 months of age 3xTg-AD mice had longer latency than controls on all five days (all $p<0.001$); and at 15 months of age 3xTg-AD mice outperformed controls on all days (all $p<0.01$) except day 1.

2.6.2 Body Weight

Following finding of a significant effect of sex on rotarod performance, it was decided to investigate the influence of weight on performance. Given that males tend

to weigh more than females, if weight had a significant effect on performance, it could explain the sex effect. Therefore, an ANOVA investigating the relationship of weight to age, genotype, and sex was conducted. Additionally, a correlation was conducted between latency to fall and the corresponding day's weight.

There was no effect of genotype on mean body weight (see Figure 2.4a), but there was a significant sex difference ($F(1,137)=20.10$, $p<0.001$), with males weighing more than females (Figure 2.4b).

There was a significant effect of age ($F(4,137)=40.42$, $p<0.001$) on mean body weight, with Tukey HSD post hoc analyses revealing that weight generally increased with age, though not all increases were significant (see Figure 2.4a). Two-month old mice weighed significantly less than all other ages (all $p<0.001$); 6-month old mice weighed significantly less than 12- and 15-month old mice (both $p<0.001$), but did not differ from 9 month old mice; and 9-, 12-, and 15-month old mice did not significantly differ from each other (Figure 5a).

A significant genotype x sex interaction was found ($F(1,137)=26.02$, $p<0.001$) (Figure 5b), with independent samples t-tests revealing no significant differences between control and 3xTg-AD female mice, but male controls weighed significantly more than male 3xTg-AD mice ($p<0.001$). There was no significant difference in weight between male and female 3xTg-AD mice, but male control mice weighed more than female control mice ($p<0.001$).

An genotype x sex x age interaction on body weight was also found ($F(4,137)=4.69$, $p<0.001$). Independent samples t-tests showed that: at 2-months of age female 3xTg-AD mice weighed less than 2-month old female control mice

($p<0.05$); at 12-months of age female 3xTg-AD mice weighed more than 12-month female controls ($P<0.01$); and at 15-months of age female 3xTg-AD mice weighed more than female control mice, but 15-month old male 3xTg-AD mice weighed less than male controls (see Figure 2.4a).

Finally, when comparing weight with rotarod performance on each of the five days, significant correlations were found for days 1-4, but not for day 5 (see Table 2.1). Correlations were highest on Day 1 and decreased each day. When a correlation was conducted to investigate mean weight with mean latency to fall (over all 5 test days) for both genotype and sex (Table 2.2), only the weight of the male control mice was significantly correlated with their performance ($r=-0.406$, $p<0.01$). When age considered, none of the correlations were significant. It was possible that this lack of significant correlation may have been due to the small sample size (e.g., n=3) in some groups. Therefore, ages were binned into age categories based upon previous reports of behavioural deficits and neuropathology: Young: 2-3 months of age; Adult: 6-10 months of age; and Old: 12-16 months of age. The correlation for age, genotype, and sex (Table 2.3) revealed a significant relationship between weight and performance for Old male ($r=.730$, $p<0.05$) and Old female 3xTg-AD mice ($r=-.550$, $p<0.05$). Based upon the results of these correlations, and the finding that weight is related to performance for some groups, it was decided to re-run the analyses of rotarod performance using weight as a covariate.

2.6.3 ANCOVA

An ANCOVA was conducted, with mean weight (average weight for five days of testing) as a covariate (see Figures 2.5). All significant main effects and

interactions from the original ANOVA were still present when weight was used a covariate, however the main effect of sex was no longer significant ($F(1,136)=1.45$, $p>0.05$). Thus, the better performance of females was accounted for by their lighter weight.

2.6.4 Learning Score

An ANCOVA, with weight as a covariate, was conducted to investigate the effects of genotype, sex and age on the learning score (percent improvement from day 1 to day 5) (see Figure 2.6a). A significant main effect of genotype ($F(1,136)=17.11$, $p<0.001$) was found, with transgenic mice having a higher learning score than controls (Figure 2.6a). No other significant main effects were found; however, a significant interaction between genotype and sex was found ($F(,1361)=8.65$, $p<0.01$) (Figure 2.6b). Post hoc independent samples t-tests showed that male 3xTg-AD mice outperformed male controls ($p<0.01$), but no there was no significant difference between female 3xTg-AD and female control mice. There was also a significant difference between male and female 3xTg-AD mice ($p<0.05$), but not between male and female controls (Figure 2.6b).

2.7 Discussion

The hypothesis that 3xTg-AD mice would out-perform controls was supported. A significant main effect of genotype was found, with 3xTg-AD mice remaining on the rotarod longer than control mice. These results support the findings of Filali et al. (2012) and Stover (2012; 2013 personal communication), but differ from those reported by Sterniczuk et al. (2010). Although Filali et al. (2012) used a different procedure (using data from only a single trial), they used control

mice with the same background strain as the 3xTg-AD mice, and compared control and 3xTg-AD mice of the same age. However, Sterniczuk et al. (2010) used C57BL/6J mice as their control and compared 5-7 month old controls with 7-11 month old 3xTg-AD mice, which could explain the difference in findings. Our procedure and results replicated those of Stover (2012; 2013, personal communication), which lends support to the finding that 3xTg-AD mice have superior motor coordination compared to their age-matched controls.

Although a sex difference was initially found, when weight was used as a covariate, the effect no longer remained. This finding is in line with that of Stover (2012; 2013, personal communication) who found no sex differences on motor coordination or learning. Previous research has often found females perform better than males on the rotarod due to their lighter weight (Brown & Wong, 2007).

Finally, the hypothesis that older mice would have poorer performance than younger mice was not supported. A main effect of age was not found, however an age x genotype interaction revealed that while 3xTg-AD mice outperformed controls at 2, 6, 12, and 15 months of age, there was no difference in performance at 9 months of age.

Motor learning on the rotarod was examined using the Learning Score [LS = (100*(Day 5 Performance – Day 1 Performance)/(Day 1 Performance)] that reflected the percent improvement in performance from Day 1 to Day 5 of testing. Once again, a main effect of genotype was found with 3xTg-AD mice having a higher learning score (significantly more improvement over days of testing) than controls. A sex by genotype interaction was also found that showed male 3xTg-AD mice had a

higher learning score than female 3xTg-AD mice, while no difference existed between control males and females. Therefore, 3xTg-AD mice were not only found to have significantly better motor coordination on the rotarod, but at least for male 3xTg-AD mice, they also had significantly better motor learning ability.

The meaning and implications of these results are discussed in Chapter 6: Meaning and implications.

Chapter 3: Locomotion, Anxiety, and Depression

3.0 Anxiety and Depressive Symptoms in Alzheimer's Patients

Patients with AD are commonly diagnosed with changes in behaviour and emotionality, including depression and anxiety (Lee & Lyketsos, 2012). These emotional disturbances occur in AD patients at rates three to four times higher than in age-and sex-matched controls (Lyketsos & Olin, 2002; Hwang et al., 2004), with depression affecting as many as 50% (Lyketsos & Olin, 2002), and symptoms of anxiety affecting upwards of 70% (Lyketsos et al., 2000), of patients with AD. These symptoms can be just as debilitating to the patient as memory loss and may increase the rate of cognitive decline (Stern et al., 1997). It is therefore important to investigate symptoms of anxiety- and depressive-like behaviours in mouse models of AD.

3.1 Locomotion, Anxiety- and Depressive-like Symptoms in 3xTg-AD mice

The tests most commonly used to measure anxiety and depression in rodents are the elevated plus maze (EPM), the open field (OF), and the forced swim test (FST). The OF consists of a large, open arena designed to allow the animals to explore and move about freely. Behaviours can be scored to measure locomotor behaviour and anxiety (Walsh & Cummins, 1976). The EPM is an apparatus in the shape of a "plus", with four arms radiating from a central square. Two of the arms have sides (closed arms) while two of the arms do not. Time spent in the arms without sides (open arms) is considered to be indicative of lower levels of anxiety (Lister, 1990). The FST is a simple test used to measure depression-related behaviour. Animals are placed into a container of water from which there is no

escape. Their behaviour is monitored for a set period and time spent not swimming or trying to escape (immobility) is recorded. Higher frequency and duration of time spent immobile are considered to be related to higher levels of depression (Cryan & Mombereau, 2004).

Previous studies that have investigated locomotion in the 3xTg-AD mice report conflicting results. In the OF, Giménez-Llort et al. (2007) found that 3xTg-AD mice at both 2.5 and 6 months of age reared significantly less than controls, while Stover (2012) found no differences in rearing at 2 months of age but found controls reared significantly more than 3xTg-AD mice at 6 months of age. Both Sterniczuk et al. (2010) and Filali et al. (2012) found 3xTg-AD mice (7.5-11 months of age, 12-14 month of age, respectively) less active (travelled less distance) than controls; Stover (2012) found no differences in activity levels at 2 and 6 months of age, while Pietropaolo et al. (2008) found no differences at 6 months of age but found 3xTg-AD mice were more active than controls at 12 months of age. In the EPM, Pietropaolo et al. (2008) found no differences in locomotion at 6 months of age, but at 12 months of age 3xTg-AD were more active than controls. Giménez-Llort et al. (2007) found no differences in locomotion at 2.5 or 6 months of age, while Stover (2012) found 3xTg-AD mice less active at 2 and 6 months of age. Although the findings are somewhat inconsistent, overall it appears as though 3xTg-AD mice are less active in both the OF and the EPM compared to controls at both young and older ages.

Some consistency is found for measures of anxiety (freezing and immobility) in the OF, with Sterniczuk et al. (2010), Filali et al. (2012), and Giménez-Llort et al. (2007) all reporting that 3xTg-AD mice have higher frequency or duration of

freezing/immobility, and although Stover (2012) found no differences in frequency and duration of freezing, he did find 3xTg-AD mice had higher frequencies of stretch attend postures (SAPs), all behaviours that may suggest higher levels of anxiety in the 3xTg-AD mice (Blanchard et al., 2001). In the EPM Filali et al. (2012), Pietropaolo et al. (2008), Giménez-Llort et al. (2007), and Stover (2012) all found no differences in the amount of time spent in the open arms between 3xTg-AD and control mice. However, Stover (2012) found conflicting results for other anxiety-related behaviours in the EPM, with 3xTg-AD mice freezing more often than control mice (indicative of higher levels of anxiety), but also engaging in more head dips than controls (indicative of lower levels of anxiety). Taken together, these results suggest no differences in anxiety levels in the elevated plus maze.

Finally, to date, no studies have investigated depression-related behaviours in the 3xTg-AD mice using the FST or any other measure of depression.

3.2 Hypotheses For Locomotion, Anxiety And Depression In 3xTg-AD Mice

3.2.1 Locomotion

Although previous studies have reported varying results, 3xTg-AD mice appear to be less active in both the open field and the elevated plus maze. Therefore, it was hypothesized that 3xTg-AD mice would engage in fewer rearing behaviours and travel significantly less distance compared to controls in both the OF and the EPM.

The effect of sex on locomotion in the OF and EPM was difficult to predict based on previous research: Sterniczuk et al. (2010; female), Filali et al. (2012; female), and Pietropaolo et al. (2008; male) only included one sex in their

experiments, and although Giménez-Llort et al. (2007) used both males and females for a variety of measures, they did not compare them on the same tests. Stover (2012) compared locomotor behaviours in the OF and EPM for both male and female mice and found no effect of sex, while Giménez-Llort et al. (2010) found males to be more active than females. Due to the similarity of testing procedures between the present study and those used by Stover (2012), it was hypothesized that for measures of locomotion there would be no effect of sex.

Few studies have investigated locomotor behaviour in the 3xTg-AD mice at multiple ages, and fewer still have examined age-related effects. Although Giménez-Llort et al. (2007) and Stover (2012) studied mice at different ages they did not explore age-related effects. Pietropaolo et al. (2008) compared mice at 6- and 12-months of age for distance travelled in the OF and found no age-related change for 3xTg-AD mice, although control mice did show a decrease in distance travelled. Given that the present study investigated mice at five different ages, from 2- to 15-months of age, it was difficult to predict age-related differences based upon previous research; however, it was hypothesized that there would be an age-related decline in locomotor-related behaviours in the OF and the EPM.

3.2.2 Anxiety-related behaviours

Previous studies have found 3xTg-AD mice to exhibit more anxiety-related behaviours (e.g., freezing, SAPs) than controls in the OF, but have found no differences in anxiety-like behaviours in the EPM (no differences in time spent in open arms). Therefore, it was hypothesized that 3xTg-AD mice would engage in more anxiety-related behaviours than control mice, at least in the OF.

As outlined in section 5.2.1, few studies have investigated the possible effects of sex on anxiety-related behaviours. Stover (2012) found no sex differences for any of the anxiety-related behaviours, nor did Giménez-Llort et al. (2010); therefore, it was hypothesized sex would not have an effect on anxiety-related behaviours in the OF and EPM.

The effect of age on anxiety-related behaviours, similar to that of locomotor-related behaviours, has not been thoroughly studied. Pietropaolo et al. (2008) found no effect of age on time spent freezing in the OF or time spent in the open arms in the EPM. Therefore, it was hypothesized that there would be no effect of age on anxiety-related behaviours.

3.2.3 Depressive-related behaviour

No studies have directly investigated depression in the 3xTg-AD mice, however the increased incidence rate of depression in AD patients compared to age-matched healthy controls (Lyketsos & Olin, 2002) led to the hypothesis that the 3xTg-AD mice would display more depressive-like symptoms than control mice. Since Oddo et al. (2003b) found no sex difference in the neuropathology of the 3xTg-AD mice, it was hypothesized that there would be no effect of sex on bouts or duration of immobility in the FST. Finally, it has been suggested that depression in patients with AD is related to the underlying neuropathology of AD (Lyketsos & Olin, 2002), suggesting that 3xTg-AD mice may develop age-related depression-related symptoms as brain pathology worsens. Therefore, it was hypothesized that 3xTg-AD mice would not differ from controls in the FST at 2 months of age, but would display

age-related increases in bouts of immobility and time spent immobile at from 6-15 months of age.

3.3 Animals

As outlined in section 3.2, a total of 158 mice (69 3xTg-AD and 89 B6129SF2/J) were used in this study (see Table 1.1 for a breakdown by age and sex). Data from a total of seven mice were excluded from the OF analyses: data from one mouse were excluded due to a software malfunction, and data from six mice were excluded due to video and tracking device failures. Data from four mice were excluded from EPM analyses due to video and tracking device failures. Data from one mouse were excluded from FST analyses due to experimenter error.

Mice were tested using a cross-sectional design at five different ages: 2-3, 6-7, 9-10, 12-13, and 15-16 months of age, creating a 2 x 2 x 5 (genotype x sex x age) design. Previous studies (Billings et al., 2005; Pietropaolo et al., 2008; Sterniczuk et al., 2010) used between 3 and 15 mice per group and found significant effects; therefore, with 3-12 mice per genotype per sex per age, differences between groups were expected to be detectable.

3.4 Apparatus and Procedure

As outlined in section 1.7, mice were born in the lab to parents purchased from The Jackson (Jax) Laboratory (Bar Harbor, Maine), weaned at 21 days of age and housed in same-sex, age-matched groups of 2 to 4. Housing cages were clear Plexiglas, measuring 18.75 x 28 x 12.5 cm and contained wood-chip bedding, one PVC tube, and half of a compressed cotton square (Nestlet) for nest construction. Cages were covered by a wire cage-top and a plastic cover that contained a Hepa-

filter, to prevent transmission of possible pathogens. Mice were fed Purina 5001 rodent chow and water *ad libitum*, unless stated otherwise. Mice were housed on a reversed 12:12 light:dark cycle, with lights off at 9:45 am. The housing room was kept at 22±2°C. All mice were tested during the dark phase of their light:dark cycle. The experimenter was blind to the age and genotype of the mice until the completion of testing.

Three tests were used to measure locomotion, anxiety- and depression-related behaviours: the open field (OF; locomotion and anxiety), elevated plus maze (EPM; locomotion and anxiety), and forced swim test (FST; depression). One test was conducted per day for three consecutive days, in the order presented in this report. Data were analyzed using SPSS 20.0 for MacIntosh. Multivariate analyses of variance (ANOVA) were conducted on the data with Tukey HSD post hoc analyses performed when applicable. For independent samples t-tests, equality of variance was tested using Levene's test. If equal variances could not be assumed ($p>0.05$) an adjusted t-statistic (using the pooled estimate of the error term) and degrees of freedom (using the Welch-Satterthwaite method) were used to determine significance.

3.4.1 Open Field

The open field (OF) test was used to measure anxiety-, exploratory, and locomotor-related behaviours. The apparatus consisted of a plywood box measuring 72 x 72 cm with 36 cm high walls. One wall of the box was constructed of clear Plexiglas rather than plywood to allow for easier observation of behaviours. The wooden walls and floor were painted white with blue lines drawn on the floor. The

lines divided the floor into 16, 18 x 18 cm squares. A central square (also measuring 18 x 18 cm) was drawn in the middle of the open field, and the floor was covered with a clear piece of Plexiglas. The apparatus was located in a testing room measuring 1.8 x 4.6 m and was lit by a single 60-watt soft white light bulb. Trials were recorded via a video camera (fixed 2.1 m above the apparatus) that was connected to a computerized tracking system (Limelight, Actimetrics Inc., Wilmette IL). All behaviours were scored using the Limelight software at the time of testing.

The procedure used for the OF replicated that used by Brown et al. (1999). Mice were transported in their home cage to an unlit holding area outside of the testing room. For testing, mice were placed into a separate cage and transported into the testing room one at a time. Mice were placed into a corner of the OF and allowed to explore the apparatus for five minutes. Following the completion of the trial, mice were returned to their home cage and the apparatus was cleaned with a 70% ethanol solution.

The Limelight tracking software measured the total distance travelled, while nine other behaviours were scored manually: Centre rearing (standing on hind legs without support); Wall rearing (standing on hind legs with forepaws touching wall); Stretch attend postures (SAPs; extending head without moving hind legs, then returning to original position); frequency and duration of bouts of grooming; frequency and duration of bouts of freezing (no movement except for breathing); time spent in the centre square; defecations and urine spots. Distance travelled, centre rears and wall rears were used as measures of locomotion (Brown et al., 1999); while time spent in the central square (Brown et al., 2013), SAPs (Rodgers et

al., 1997), freezing (Lister, 1990), grooming (Estanislau, 2012), and defecation and urine spots (Hall, 1934), were used to measure anxiety-like behaviour.

3.4.2 Elevated Plus Maze

The elevated plus maze (EPM) was used to measure anxiety and exploratory behaviours. The maze was shaped like a "plus" with two open arms measuring 30 x 5 cm and two closed arms measuring 30 x 5 cm with 15 cm high walls. The arms extended from a 5 x 5 cm central square. The entire apparatus was constructed of Plexiglas, with gray Plexiglas floors and clear Plexiglas walls. The open arms had a slightly raised edge (4 mm) to prevent mice from slipping and falling off while exploring. All arms were marked with a white line at the centre-point (15 cm), which was used for scoring locomotor behaviour. The apparatus was located in a testing room measuring 1.8 x 4.6 m and was lit by two 60-watt soft white light bulbs. Trials were recorded via a video camera fixed 2.1 m above the apparatus, which was connected to a computerized tracking system (Limelight, Actimetrics Inc., Wilmette IL). All behaviours were scored using the Limelight software at the time of testing.

The procedure used for the EPM replicated that used by Brown et al. (1999). Mice were transported in their home cage to an unlit holding area outside of the testing room. For testing, mice were placed into a separate cage and transported into the testing room one at a time. Mice were placed into the central square and observed for five minutes. Following the completion of the trial, mice were returned to their home cage and the apparatus was cleaned with a 70% ethanol solution.

Tracking software (Limelight) measured the total distance travelled and time spent in the open arms, while eight other behaviours were scored manually: line

crosses (all four paws cross white line), rearing, SAPs, head dips (moving its head over the edge of the open arm and pointing it downward), freezing (as described in section 5.3.1), grooming, defecations and urine spots. Distance travelled, line crosses, and rearing were used as measures of locomotion, while SAPs, percentage of time spent in the open arms, head dips, freezing, grooming, defecation and urine spots were used as measures of anxiety (Brown et al., 1999; Brown et al., 2013).

3.4.3 Forced Swim Test

The forced-swim test (FST) was used to measure depressive-like behaviour. The test consisted of a 2 litre glass cylinder 80% filled (1600 mL) with luke-warm water (25 ± 2°C). The water level in the cylinder was high enough to ensure that the tails of the mice would not touch the bottom, but not high enough that the mice could climb out and escape from the cylinder. Frequency and duration of bouts of immobility were manually recorded using the Hindsight software program for MS-DOS version 1.5. The test was conducted under a 60-watt soft white light bulb.

Mice were transported in their home cage to a room next to the testing room and then placed individually into holding cages and transported to the testing room. Using a plastic cup, mice were gently dropped into the cylinder of water, ensuring their head was pointed upwards so that it did not immediately go under the water. Mice were continuously observed for immobility (no movement; floating) for six minutes and were then removed from the water and placed back into the holding cage. Bouts and duration of immobility in the FST are used to measure depressive-like behaviour (Porsolt et al., 1977). An animal is considered to be more “depressed”

if it engages in more bouts of immobility or remains immobile for longer periods of time.

3.5 Results

3.5.1 Open Field

3.5.1.1 Locomotor-Related Behaviours

Distance travelled. A main effect of genotype was found for distance travelled ($F(1,131)=25.95$, $p<0.00$), with 3xTg-AD mice travelling significantly less than control mice. There were no main effects of age or sex on distance travelled (see Figure 3.1a).

A significant genotype x age interaction ($F(4,131)=5.22$, $p<0.001$) was found for distance travelled, with an independent samples t-test revealing that at 2-, 9-, and 15-months of age 3xTg-AD mice travelled significantly less than control mice, but at 6- and 12- months of age there was no significant difference between genotypes (see Figure 3.1b).

Finally, there was a significant sex x age interaction ($F(4,131)=2.81$, $p<0.05$) for distance travelled, with an independent t-test revealing that males travelled significantly farther than females ($p<0.05$) only at 15-months of age (see Figure 3.1c).

Rearing.

Centre rearing. There was no effect of genotype on the frequency of centre rears (see Figure 3.2a), however male mice engaged in significantly more centre rearing than female mice ($F(1,131)=6.79$, $p<0.01$). Age also had a significant effect on centre rearing ($F(4,131)=4.83$, $p<0.001$), with Tukey HSD post hoc analyses

revealing that 12-month old mice engaged in significantly more centre rearing than both 2-month old ($p<0.001$) and 15-month old mice ($p<0.01$).

A genotype x sex x age interaction was found for centre rears ($F(4,131)=2.48$, $p<0.05$; Figure 3.2a). A post hoc independent samples t-test revealed no differences for female 3xTg-AD and control mice at any age. However, male 3xTg-AD mice at 9-months of age engaged in significantly more centre rears than male control mice at 9-months of age ($p<0.01$). No other significant differences were found.

Wall rearing. Genotype had a significant effect on wall rears ($F(1,131)=54.62$, $p<0.00$; see Figure 3.2b), with 3xTg-AD mice engaging in significantly fewer wall rears than control mice. Age was also found to have a significant effect on wall rearing ($F(4,131)=4.80$, $p<0.001$). Interestingly, the Tukey HSD post hoc analyses showed that 12-month old mice engaged in significantly fewer wall rears compared to all other ages (all $p<0.01$) (Figure 3.2b). There was no sex difference in wall rearing.

A genotype x age interaction was found for frequency of wall rears ($F(4,131)=3.31$, $p<0.05$), with an independent samples t-test revealing that at 2-, 6-, 9-, and 15-months of age 3xTg-AD mice engaged in significantly fewer wall rears than control mice (all $p<0.05$), however there was no significant difference between genotypes at 12-months of age (Figure 3.2d).

3.5.1.2 Anxiety-Related Behaviours.

Entries and time in the centre square.

Entries into the centre square. There were no main effects of genotype, sex, or age on number of entries into the central square (Figure 3.3a). However, a

significant genotype x age interaction was found for centre square entries ($F(4,131)=3.63$, $p<0.01$), with an independent samples t-test revealing that at 12-months of age 3xTg-AD mice entered the central square significantly more often than control mice ($p<0.01$) (Figure 3.3b).

A genotype x sex interaction was also found for centre square entries ($F(1,131)=4.80$, $p<0.05$; Figure 3.3c). Independent samples t-tests revealed no difference between male and female 3xTg-AD mice, but showed that male control mice engaged in more centre square entries than female control mice ($p<0.01$).

Time in the centre square. There were no main effects of genotype, sex, or age on time spent in the centre square (Figure 3.3d).

A genotype x sex interaction was found for time spent in the central square ($F(1,131)=5.67$, $p<0.05$; Figure 3.3e). Independent samples t-tests revealed no difference between male and female 3xTg-AD mice, but showed that male control mice spent more time in the central square ($p<0.01$) than female control mice. As well, while no difference was found between male 3xTg-AD and male control mice, female 3xTg-AD mice were found to spend significantly more time in the centre square than female control mice ($p<0.05$).

Stretch attend postures. There were no main effects of either genotype or sex on SAPs. However, there was a main effect of age ($F(4,131)=2.53$, $p<0.05$), with a Tukey HSD post hoc analysis revealing that mice at 6-months of age engaged in significantly more SAPs than 9- and 15-month old mice (both $p<0.05$), and 12-month old mice engaged in more SAPs than 15-month old mice ($p<0.05$) (Figure 3.4a).

A genotype x age interaction ($F(4,131)=3.74$, $p<0.01$) was also found for SAPs. A one-way ANOVA found a significant effect of age for control mice ($F(4,83)=5.72$, $p<0.001$), but no effect of age for 3xTg-AD mice. The Tukey HSD post hoc analysis revealed 12-month old control mice engaged in significantly more SAPs than 9- and 15-month old mice (both $p<0.01$) (Figure 3.4b).

Freezing.

Frequency. Genotype was found to have a significant effect on the frequency of bouts of freezing ($F(1,131)=9.59$, $p<0.01$; Figure 3.5a) with 3xTg-AD mice freezing more often than control mice. A significant effect of age was also found for frequency of freezing ($F(4,131)=3.38$, $p<0.05$; Figure 3.5a). A Tukey HSD post hoc test revealed that 15-month old mice froze significantly fewer times compared to 6- and 12-month old mice (both $p<0.05$), and 12-month old mice froze less often than 9-month old mice ($p<0.05$). There was no effect of sex on the frequency of freezing.

A sex x age interaction was found for the frequency of freezing ($F(4,131)=2.83$, $p<0.05$; Figure 3.5b). A one-way ANOVA revealed a significant effect of age for female mice only ($F(4,78)=4.52$, $p<0.01$), with a Tukey HSD post hoc analysis revealing that 12-month old female mice froze more often than 2-, 9-, and 15-month old female mice (all $p<0.05$) (Figure 3.5b).

Finally, a genotype x sex x age interaction was found for the frequency of freezing ($F(4,131)=3.86$, $p<0.01$; Figure 3.5a) with independent samples t-tests revealing no significant differences between female 3xTg-AD and control mice at any age. However, male 2-month old 3xTg-AD mice froze significantly more often than male 2-month control mice ($p<0.05$). No other significant effects were found.

Duration. Genotype was found to have a significant effect on time spent freezing ($F(1,131)=8.43$, $p<0.01$; Figure 3.5c), with 3xTg-AD mice spending more time freezing than control mice.

A sex x age interaction was also found for time spent freezing ($F(4,131)=3.18$, $p<0.05$; Figure 3.5d). A one-way ANOVA revealed a significant effect of age for female mice only ($F(4,78)=2.60$, $p<0.05$), with a Tukey HSD revealing that 12-month old female mice froze for significantly longer periods than 2-month old female mice ($p<0.05$) (Figure 3.5d).

Finally, a genotype x sex x age interaction was found for time spent freezing ($F(4,131)=3.43$, $p<0.01$; Figure 3.5b); however independent samples t-tests revealed no significant differences between male 3xTg-AD and male control mice at any age, nor any differences between female 3xTg-AD and female control mice at any age.

Grooming. No main effects or interactions were found for frequency or duration of grooming (Figure 3.6a & b).

Defecation and urine spots. There was no difference in defecations between 3xTg-AD and control mice. However, a significant effect of sex was found for defecations ($F(1,131)=4.04$, $p<0.05$), with male mice defecating significantly less than female mice (Figure 3.7a). A significant effect of age was also found for number of defecations ($F(4,131)=2.52$, $p<0.05$). A Tukey HSD post hoc revealed that 6-month old mice had more defecations than 9-month old mice ($p<0.05$) (Figure 3.7a).

There were no main effects of genotype, sex, or age, nor any interactions found for number of urine spots (Figure 3.7b).

3.5.2 Elevated Plus Maze

3.5.2.1 Locomotor-Related Behaviours

Distance travelled. A significant effect of genotype was found for distance travelled in the EPM ($F(1,134)=32.39$, $p<0.001$), with 3xTg-AD mice travelling significantly less than control mice. Neither sex nor age had a significant effect on distance travelled (Figure 3.8).

Line crosses. A significant effect of genotype was found ($F(1,134)=30.11$, $p<0.00$) for number of line crosses in the EPM with 3xTg-AD mice engaging in significantly fewer line crosses than control mice. No other significant effects were found (Figure 3.9).

Rearing. A significant effect of genotype was found ($F(1,134)=16.95$, $p<0.001$) for rearing in the EPM with 3xTg-AD mice engaging in less rearing behaviour than control mice (Figure 3.10a). Sex was also found to have a significant effect ($F(1,134)=4.60$, $p<0.05$) with males rearing significantly less often than females (Figure 3.10a). A significant effect of age on rearing was found ($F(4,134)=3.02$, $p<0.05$) with a Tukey HSD post hoc analysis revealing that 6-month old mice engaged in significantly more rearing than 12-month old mice ($p<0.05$) (Figure 3.10b).

3.5.2.2 Anxiety-Related Behaviours

Open arm entries and percent time.

Entries. Genotype had a significant effect on entries into the open arms ($F(1,134)=5.32$, $p<0.05$; Figure 3.11a), with 3xTg-AD mice entering the open arms less often than control mice. There was no effect of sex on entries into the open

arms; however, age had a significant effect on open arm entries ($F(4,134)=4.09$, $p<0.01$), with a Tukey HSD post hoc test revealing that 2-month old mice entered the open arms significantly more than both 6- and 15-month old mice (both $p<0.05$) (Figure 3.11a).

Percent time. Genotype had a significant effect on the percentage of time spent in the open arms ($F(1,134)=57.47$, $p<0.001$; Figure 3.11b), with 3xTg-AD mice spending a higher percentage of time in the open arms than control mice. There were no effects of sex or age on the percentage of time spent in the open arms.

A genotype x sex x age interaction was found for percentage of time spent in the open arms in the EPM ($F(4,134)=2.64$, $p<0.05$). Independent samples t-tests revealed that at 2-, 6-, 12-, and 15-months of age, 3xTg-AD females spent a higher percentage of time in the open arms than control females at the same age (all $p<0.05$). While, at 6-, 9-, and 12-months of age, male 3xTg-AD mice spent a higher percentage of time in the open arms compared to male controls at the same age (all $p<0.01$) (Figure 3.11b).

Stretch attend postures. No significant effects of genotype or sex were found for SAPs. However, a significant effect of age was found ($F(4,134)=9.25$, $p<0.001$), with a Tukey HSD post hoc analysis revealing that 6-month old mice engaged in significantly more SAPs than mice of all other ages (all $p<0.001$). (See Figure 3.12a)

A genotype x age interaction was found for SAPs ($F(4,134)=4.81$, $p<0.001$). An independent samples t-test revealed that at 6-months of age, 3xTg-AD mice engaged in significantly fewer SAPs than control mice ($p<0.05$) (Figure 3.12b).

A sex x age interaction was also found for SAPs ($F(4,134)=3.97$, $p<0.01$). One-way ANOVAs found a significant age effect for female mice ($F(4,78)=10.30$, $p<0.001$) but not for male mice. A Tukey HSD post hoc analysis found that 6-month old female mice engaged in significantly more SAPs than female mice at all other ages (all $p<0.001$) (Figure 3.12c).

Head dips. There were no significant main effects or interactions for head dips in the EPM. See Figure 3.13.

Freezing.

Frequency. A significant effect of genotype was found for the frequency of freezing ($F(1,134)=38.18$, $p<0.001$; Figure 3.14a), with 3xTg-AD engaging in freezing significantly more often than controls. There were no effects of sex or age on frequency of freezing.

Duration. A significant effect of genotype was found for time spent freezing ($F(1,134)=13.39$, $p<0.001$; Figure 3.14b), with 3xTg-AD mice spending significantly more time freezing than controls. There were no effects of sex or age on time spent freezing.

Grooming. There were no significant main effects or interactions for frequency or duration of grooming. See Figure 3.15a & b.

Defecations and urine spots. There were no significant main effects or interactions for number of defecations or urine spots. See Figure 3.16a & b.

3.5.3 Forced Swim Test

3.5.3.1 Frequency of Immobility

A main effect of genotype was found for frequency ($F(1,136)=64.15$, $p<0.001$; Figure 3.17a) of immobility, with 3xTg-AD mice being immobile less often than controls. Sex was also found to have an effect ($F(1,136)=5.52$, $p<0.05$; Figure 25a), with male mice engaging in fewer periods of immobilization than females.

Age was found to have a significant effect on frequency of immobilization ($F(4,136)=5.76$, $p<0.001$; Figure 3.17a), with a Tukey HSD post hoc analysis revealing that 2-month mice engaged in significantly fewer bouts of immobility than both 6- and 12-month old mice (both $p<0.01$).

A genotype x age interaction was found for frequency of immobilization ($F(4,136)=5.20$, $p<0.001$; Figure 3.17b). Independent samples t-tests revealed that 3xTg-AD mice at 2-, 6-, 9-, and 15-months of age engaged in significantly fewer bouts of immobilization than 3xTg-AD mice at the same age (all $p<0.05$) (Figure 3.17b).

Finally, a genotype x sex interaction was found for frequency of immobilization ($F(1,136)=4.06$, $p<0.05$; Figure 3.17c). Independent samples t-tests revealed that male 3xTg-AD mice engaged in significantly fewer bouts of immobilization than male control mice ($p<0.001$), and female 3xTg-AD mice engaged in significantly fewer bouts of immobilization than female 3xTg-AD mice ($p<0.001$). An additional independent samples t-test revealed that female 3xTg-AD mice engaged in significantly more bouts of immobilization than male 3xTg-AD mice ($p<0.05$), but no difference was found between male and female control mice (Figure 3.17c).

3.5.3.2 Duration of Immobility

A main effect of genotype was found for duration of immobilization ($F(1,136)=65.20$, $p<0.001$; Figure 3.17d), with 3xTg-AD mice remaining immobile for shorter periods of time than controls (Figure 3.17e). Sex was also found to have an effect ($F(1,136)=17.33$, $p<0.001$; Figure 3.17f), with male mice remaining immobile for more less than females. There was no effect of age on the duration of immobility.

3.6 Discussion

3.6.1 Locomotor-Related Behaviours

The hypothesis that 3xTg-AD mice would be less active in both the OF and the EPM, travelling and rearing less than controls, was supported. In both the open field and the elevated plus maze, 3xTg-AD mice travelled significantly less and reared less often than control mice (see Table 3.1). These findings support those of Giménez-Llort et al. (2007), Sterniczuk et al. (2010), and Filali et al. (2012) that all showed 3xTg-AD mice to engage in less activity than control mice.

The hypothesis that there would be no effect of sex on locomotor behaviours in the OF and EPM was also supported. Male and female mice did not differ in the distances they travelled in either the OF or the EPM, and although males engaged in more centre rears in the OF, females engaged in more rearing in the EPM (see Table 3.1). As previously mentioned, few studies have compared the behaviour of male and female 3xTg-AD mice, as most experiments used only one sex. However, our findings support those of Stover (2012) who found no differences in locomotor behaviour between male and female 3xTg-AD mice in a longitudinal study.

The hypothesis that there would be an age-related decline in locomotor behaviours was not supported. There was no effect of age on distance travelled in either the OF or the EPM, nor was there any difference in line crossings in the EPM. In the OF, 12-month old mice engaged in significantly more centre rears than mice of all other ages, but engaged in significantly less wall rears than all other ages. These findings suggest that 12-month mice may not have engaged in more rearing overall, but simply favoured one type of rearing over the other. As well, in the EPM, the only difference in locomotion was that 6-month old mice engaged in more rearing than 12-month old mice (but not more than any of the other ages) (see Table 3.1). Previous studies have found mixed results when comparing 3xTg-AD mice at different ages; however, of the studies that have investigated 3xTg-AD mice at more than one age, most have found no age differences in locomotor behaviour. The findings from the present study, in combination with previous reports, suggest that at least for 3xTg-AD mice aged 2- to 15-months of age, age does not appear to influence locomotor behaviour in the OF and EPM.

3.6.2 Anxiety-Related Behaviours

The hypothesis that 3xTg-AD mice would engage in more anxiety-related behaviours in the OF was supported. 3xTg-AD mice froze more often and spent more time freezing than control mice in both the OF and the EPM, in the present study (see Table 3.2). These findings are in line with those of Sterniczuk et al. (2010), Filali et al. (2012), and Giménez-Llort et al. (2007) that all reported 3xTg-AD mice to have higher levels of freezing, and although Stover (2012) found no differences in levels

of freezing, he did find that 3xTg-AD mice had higher frequencies of stretch attend postures (SAPs).

The 3xTg-AD mice also showed more anxiety-related measures in the EPM than controls. The 3xTg-AD mice engaged in significantly more freezing behaviours (frequency and duration) compared to controls, but spent significantly more time in the open arms of the EPM (see Table 3.2). These findings are seemingly contradictory given that more freezing behaviour and decreased time in the open arms are associated with higher levels of anxiety. Despite the contradictory nature of the present findings, they support the results reported by Stover (2012) who found that although 3xTg-AD mice froze more often than control mice, they also engaged in more head dips and showed no difference in the amount of time spent in the open arms. The results of the present study, combined with similar results from Stover (2012) and the lack of differences presented by Giménez-Llort et al. (2007), Pietropaolo et al. (2008), and Filali et al. (2012), suggest that either time in the open arms of the EPM is not an appropriate measure of anxiety in the 3xTg-AD mice, or that the difference between 3xTg-AD mice and controls is complex and may depend upon testing procedures and other factors.

The hypothesis that sex would not have an effect on anxiety-related behaviours was supported. The present study found almost no differences between males and females for anxiety-related behaviours (see Table 3.2). The only significant difference was that females were found to defecate more often than males in the OF test. Although defecation has been considered an anxiety-related behaviour (Hall, 1934), the lack of any other differences on anxiety-related

measures between males and females, suggests the increased number of defecations may not indicate that females had higher levels of anxiety than males. Supporting this conclusion, a study by O'Leary et al. (2013) examining 15 different strains of mice, reported no correlation between defecations and other traditional measures of anxiety (percent time in the open arms in the EPM, time in the centre in the OF). The general lack of differences in anxiety-related behaviours between male and female mice on both the OF and EPM, supports the previous findings of Giménez-Llort et al. (2010) and Stover (2012) who also found no sex differences in either test.

The hypothesis that there would be an age-related change in anxiety-related behaviours in the OF and the EPM, was partially supported (see Table 3.2). The effect of age on the anxiety-related behaviours in the present study was challenging to interpret. No age differences were found for the traditional measures of anxiety – the amount of time spent in the central square of the OF or the percent of time spent in the open arms of the EPM. Additionally, there were some significant age effects on freezing and defecations in the OF, but none in the EPM. The only trend to present itself was a greater number of anxiety-related behaviours in 6-month old mice. In both the OF and the EPM, 6-month old mice engaged in more SAPs than mice from some, but not all, other ages. Six-month old mice also froze more often than 15-month old mice and had a higher number of defecations than 9-month old mice. However, this trend is not consistent and does not apply to most of the anxiety-related measures. The effect of age on anxiety-related behaviours has not been extensively studied in the 3xTg-AD mice and therefore it is impossible to determine where the findings of the present study fit with previous research.

3.6.3 Depression-Related Behaviours

The hypothesis that 3xTg-AD mice would display more depressive-related behaviours than controls was not supported. Although, a significant effect of genotype was found, the 3xTg-AD mice engaged in significantly fewer bouts of immobility and spent significantly less time immobile than control mice. As mentioned in section 5.2.3, no studies have investigated depression-like behaviour in 3xTg-AD mice, and the prediction that 3xTg-AD mice might have higher levels of depression was based upon the correlation between depression and AD in human patients. The greater number of depressive-like behaviours in control mice is an intriguing finding that should be investigated further.

The hypothesis that male and female mice would not differ on measures of depression was also not supported. Females engaged in more bouts of immobilization and spent more time immobile than males in both genotypes. It was hypothesized that there would be no sex difference in depressive behaviour based upon the report by Oddo et al. (2003b) that 3xTg-AD males and females did not differ in terms of neuropathology. Therefore, the difference between males and females on the FST is likely caused by a factor other than 3xTg-AD neuropathology.

Finally, the hypothesis that 3xTg-AD mice would not differ from controls in the FST at 2-months of age, but would display age-related increases in bouts of immobility and time spent immobile from 6-15 months of age, was not supported. Two-month old mice did engage in fewer bouts of immobilization, but only compared to 6- and 12-month old mice. There was no age-related increase in depression-related behaviour and the genotype x age interaction did not reveal an

increase in depression with age in 3xTg-AD mice. The control mice engaged in more depressive-related behaviours than the 3xTg-AD mice at all ages. The hypothesis that depression-related symptoms would increase with age in the 3xTg-AD mice was based upon the theory that depression in patients with AD is likely related to the underlying neuropathology of AD (Lyketsos & Olin, 2002). This suggested that 3xTg-AD mice might develop depression-related symptoms as brain pathology worsened, however the findings of the present study do not support this theory.

The meaning and implications of the locomotor-, anxiety-, and depression-related behaviours from the present study are discussed in further detail in Chapter 6: Meaning and implications.

Chapter 4: Visuo-spatial Working and Reference Memory

4.0 Memory Deficits and Alzheimer's Disease

Memory impairment is the hallmark of Alzheimer's disease. Individuals affected by AD often begin with deficits in episodic memory, followed by working memory and eventually long-term memory (Morris & Baddeley, 1988; Albert, 2011). These deficits in memory have been linked to the presence of Aβ plaques, neurofibrillary tangles and neuronal death in the hippocampus (Braak & Braak, 1991; Huntley & Howard, 2009). The progression from normal functioning to the level of memory impairment associated with AD is classified as mild cognitive impairment (MCI). MCI is characterized by subtle memory impairments that can be useful in identifying which individuals will later develop AD. To better understand the progression of memory impairment in individuals with AD, it is important to explore different types of memory in AD mouse models across different ages in an attempt to identify whether similar subtle changes in memory functioning occurs.

4.1 Working and Reference Memory in 3xTg-AD Mice

To investigate working and reference memory in the 3xTg-AD mice, the present study employed the 8-arm radial maze, a classic test used to investigate hippocampal-dependent learning and memory in rodents (Crusio et al., 1993). Mimicking the neuropathology of human AD patients, 3xTg-AD mice have been shown to first express amyloid-beta plaques, and later neurofibrillary tangles, in the CA1 region of the hippocampus. It is therefore important to measure hippocampal-dependent behaviour in the 3xTg-AD mice to ensure the model not only expresses

similar neuropathology, but also a similar behavioural phenotype to humans with the disease.

To date, only one study has examined working and reference memory of 3xTg-AD mice using the radial arm maze (Gabbita et al., 2012). In their study, Gabbita at al. investigated neuroinflammation and possible novel drug interventions; however, the comparison of 6-month control mice (B6129SF2/J) to 6-month non-treated 3xTg-AD mice was relevant to the present study. Using similar protocols and operational definitions of errors as the present study (Win-shift paradigm), Gabbita et al. found that 6-month old 3xTg-AD mice made significantly more working memory errors than controls, but did not differ in number of reference memory errors.

Other studies have investigated spatial/reference memory in the 3xTg-AD mice using the Morris water maze (MWM). In the MWM, mice must find a hidden platform in a pool of water using extra-maze cues to guide them to the correct location. Reports of 3xTg-AD behaviour in the MWM have been mixed. Most studies seem to be in agreement that reference memory deficits are not yet present in 2-month old 3xTg-AD mice (Billings et al., 2005; Billings et al., 2007; Clinton et al., 2007; Giménez-Llort et al., 2007; McKee et al., 2008), although Stover (2012) reported learning deficits in 3xTg-AD mice at 2-months of age. At 4-months of age, Billings et al. (2005) found reference memory deficits in 3xTg-AD mice and multiple studies (Billings at al., 2007; Clinton et al., 2007; Giménez-Llort et al., 2007; McKee et al., 2008; Stover, 2012) found deficits in reference memory in 3xTg-AD mice by 6-

months of age. In contrast, neither Pietropaolo et al. (2008) nor Giménez-Llort et al., (2010) found differences in reference memory between 3xTg-AD and control mice.

4.2 Hypotheses for Working and Reference Memory in 3xTg-AD Mice

Based on the findings of Gabbita et al. (2012) it was hypothesized that 3xTg-AD mice would make significantly more working memory errors in the RAM than controls. Additionally, although the findings are mixed, the majority of studies using the MWM suggest that 3xTg-AD mice have impaired reference memory compared to controls, therefore we hypothesized that 3xTg-AD mice will make more reference memory errors in the RAM compared to controls.

The two studies that investigated the effect of sex on reference memory found no difference between male and female 3xTg-AD mice (Giménez-Llort et al., 2010; Stover, 2012). Therefore, it was hypothesized that there would be no difference in reference memory between male and female 3xTg-AD mice. Although no studies have investigated sex differences in working memory in the 3xTg-AD mice, it was hypothesized that similar to reference memory, there would be no difference in working memory between male and female 3xTg-AD mice in the RAM.

Based upon previous findings, it was hypothesized that there would be no differences in working and reference memory errors between 3xTg-AD and control mice at 2-months of age, but by 6-months of age it was expected that 3xTg-AD mice would have both working memory and reference memory deficits, and that the deficits would increase with age.

4.3 Animals

As outlined in section 1.7, a total of 158 mice (69 3xTg-AD and 89 B6129SF2/J) were used in this study (see Table 1.1 for a breakdown by age and sex). Mice were tested using a cross-sectional design at five different ages: 2-3, 6-7, 9-10, 12-13, and 15-16 months of age, creating a 2 x 2 x 5 (genotype x sex x age) design. Previous studies (Billings et al., 2005; Pietropaolo et al., 2008; Sterniczuk et al., 2010) have used between 3 and 15 mice per group and found significant effects; therefore, with 3-12 mice per genotype per sex per age, differences between groups were expected to be detectable.

Mice were born in the lab, weaned at 21 days of age and housed in same-sex, age-matched groups of 2 to 4 in clear Plexiglas cages, measuring 18.75 x 28 x 12.5 cm, that contained wood-chip bedding, one PVC tube, and half of a compressed cotton square (Nestlet) for nest construction. Cages had a wire tops that were covered by a plastic lid that contained a Hepa-filter, to prevent transmission of possible pathogens. Mice were fed Purina 5001 rodent chow and water *ad libitum*, unless stated otherwise. The housing room was on a reversed 12:12 light:dark cycle, with lights off at 9:45 am and kept at a temperature of 22±2°C. All mice were tested during the dark phase of their light:dark cycle. The experimenter was blind to the age and genotype of the mice until the completion of testing.

4.4 Apparatus

The 8-arm radial maze (RAM) used in the present study (see Figure 1.1e) was based upon the design of Schwegler et al. (1990), and consisted of eight arms (23 cm long, 7 cm wide, 31.5 cm high) radiating from a fixed circular cylinder (20 cm

diameter, 31.5 cm high). The walls of the arms and central cylinder were constructed of clear Plexiglas, while the floor was black Plexiglas. Evenly spaced openings (6 x 6 cm) were cut into the central cylinder to allow access into each of the arms. Within the fixed cylinder was a removable clear Plexiglas barrel (18.5 cm diameter, 33.5 cm high) with a single 4.5 x 6 cm opening. The opening of the barrel was covered by a guillotine door made from thin acrylic sheeting (8 cm wide, 36cm high), which slid between two brackets fixed to the inside of the barrel. The experimenter was able to control access to the arms of the maze by moving the barrel up and down within the fixed cylinder and sliding the guillotine open and closed.

A circular recess (1.4 cm diameter) was cut into the floor at the end of each of the arms and contained a removable stainless steel food cup (1.8 cm diameter). A Plexiglas tube (40 mm long, 7 mm inner diameter) penetrated the end of each arm and was used to place the food reward (45 mg sucrose Noyes food pellets, #PJFSC-0045, PJ Noyes, New Brunswick, NJ) into the food cup from outside the maze. This set-up allowed food to be placed into the cup without the food being visible from the arm entrance.

The maze was placed upon an octagonal gray wooden platform (90 cm across and 38 cm on each side), with each arm numbered 1 through 8. To minimize anxiety, the platform was not elevated, but placed directly on the floor of the testing room (2.6 x 5.1 m).

Visual cues were positioned on the platform between the arms of the maze in a fixed configuration for all mice. Larger, extra-maze room cues included the

experimenter who was present during testing and remained in the same position for all trials, and fixed furniture within the room. The testing room was lit by four ceiling mounted light fixtures, each containing two CFL 23-watt bulbs.

4.5 Procedure

4.5.1 General Radial Arm Maze Procedure

The radial-arm test procedure took 22 days: beginning with two days of food deprivation, followed by two days of habituation, 14 days of training, a three day break, and a single-day probe-trial. Four days prior to the start of training, mice were weighed and placed on food deprivation (all food was removed from the home cages; *ad libitum* water remained). During habituation and training, the body weight of the mice was kept between 85-90% of pre-test weight. Mice were transported in their home cages from the colony room to a holding room next door and were then placed in a holding cage and taken to the test room where they were placed into, and removed from, the apparatus by hand. A trial ended after 15 minutes had elapsed or the mouse had visited all four baited arms, whichever came first. Four measures were scored in the RAM: the number of correct arm entries out of the first four arms entered; working memory errors; reference memory errors; and total arm entries. Entrance into an arm was recorded if all four paws had entered the arm. Based on the operational definitions used by Yan et al. (2004), working memory errors were defined as re-entry into a previously baited arm, while reference memory errors were defined as entry or re-entry into a non-baited arm.

The apparatus was cleaned with paper towel and 5% Sparkleen solution after each trial. After all mice had completed the trials for the day the apparatus was

rotated 45° to ensure that mice were not using intra-maze cues during training and were relying on the extra-maze cues to locate the food reward. At the end of each day, the food cups were also removed and washed and allowed to dry overnight.

4.5.2 Habituation

Two habituation trials were conducted prior to training the RAM. The first habituation trial was conducted 48 hours prior to test Day 1 and involved placing a mouse into the centre of the RAM, removing the inner barrel, and allowing the mouse to freely explore the apparatus for 10 minutes, without food reward in any arms. The second habituation trial occurred 24 hours prior to training Day 1 and was the same as the first trial, but this time four assigned arms (specific to each mouse) were baited. After the first and second habituation trials, 1-2 sugar pellets, per mouse, were sprinkled into the home cages of the mice. This allowed the mice to become familiar with the food used as a reward.

4.5.3 Training

A modified Win-Shift protocol, similar to that described by Crusio et al. (1993), was used in the present study. In the modified win-shift paradigm, only four of the eight arms were baited with a sucrose pellet. Each mouse was assigned four baited arms that remained constant throughout testing, with no more than two consecutive arms (e.g., 1-2-4-6, but not 1-2-5-6) baited for each mouse.

A trial began after the mouse had been placed into the central cylinder and the inner barrel was lifted, allowing access to all eight arms. After the mouse entered an arm, the inner barrel was lowered, ensuring the opening lined up with the opening in the fixed cylinder and the guillotine door was lifted. When the mouse

returned to the centre the guillotine door was lowered and the inner barrel was rotated to prevent the door on the inner barrel from lining up with the door on the fixed cylinder (and avoid influencing the mouse's next door selection). The experimenter then waited 5 s before lifting the inner barrel and allowing the mouse to select another arm. A trial ended after the animal had entered all four baited arms, or 15 minutes had passed, whichever came first. After 14 days of training, mice were returned to *ad libitum* food access for two days.

4.5.4 Probe Trial

A probe trial was conducted to investigate long-term memory retention. On the third day post-training, mice were placed back on food deprivation. On the fourth day mice were weighed and then re-tested in the radial arm apparatus. The same procedure was used for the probe-trial as was used during training, however after a mouse had entered four arms the trial ended. The number of correct entries out of the four total entries was recorded.

4.5.5 Statistical Analyses

Data were analyzed using SPSS 20.0 for MacIntosh. The data were first analyzed using a between-within (split-plot) analysis of variance (ANOVA) with day as the within factor, and genotype, sex, and age as the between factors. For repeated-measures analyses, if Mauchley's test of sphericity was significant (assumption of sphericity violated) the Greenhouse-Geisser correction was employed and the corresponding adjusted degrees of freedom.

To increase reliability and investigate possible trends in the data, the data were "binned" into three, four-day groups: Days 1-4 (1); Days 6-9 (2); and Days 11-

14 (3). Days 5 and 10 were omitted to ensure an equal number of days in each bin. Multivariate analyses of variance (ANOVA) were conducted on the binned data, with Tukey HSD post hoc analyses performed when applicable. For independent samples t-tests, equality of variance was tested using Levene's test. If equal variances could not be assumed ($p>0.05$) an adjusted t-statistic (using the pooled estimate of the error term) and degrees of freedom (using the Welch-Satterthwaite method) were used to determine significance.

4.6 Results

4.6.1 Working Memory Errors

4.6.1.1 Entire 14 Day Training Period

There was a significant effect of genotype on working memory errors ($F(1,138)=50.40$, $p<0.001$), with 3xTg-AD mice making significantly more working memory errors than controls (see Figure 4.1). There were no main effects of sex or age on working memory.

A significant genotype x sex interaction was also found ($F(1,138)=4.52$, $p<0.05$) with independent samples t-tests revealing that female 3xTg-AD mice made significantly more working memory errors than female control mice ($p<0.001$), and male 3xTg-AD mice made significantly more working memory errors than male control mice ($p<0.001$). Additionally, male 3xTg-AD mice made more working memory errors than female 3xTg-AD mice ($p<0.05$), but no differences were found between male and female control mice (Figure 4.2).

A significant effect of day was found for working memory errors ($F(13,126)=19.93$, $p<0.001$), with the total number of errors decreasing over the 14 days of training.

Finally, a day x sex x age interaction was found for working memory errors ($F(52,516)=1.70$, $p<0.01$) with independent samples t-tests revealing the following: at 2-months of age, males made more errors than females on Day 1 only ($p<0.05$); at 6-months of age, males and females did not differ; at 9-months of age, males made significantly fewer errors than females on Day 1 only ($p<0.05$); at 12-months of age, males made more errors than females on Day 3 only ($p<0.05$); and at 15-months of age, males made more errors than females on Day 1 only ($p<0.05$).

4.6.1.2 Binned Errors

A significant effect of genotype was found for each of the three bins of days, with 3xTg-AD mice making significantly more working memory errors than controls in bin 1 ($F(1,138)=34.76$, $p<0.001$), bin 2 ($F(1,138)=33.04$, $p<0.001$), and bin 3 ($F(1,138)=11.88$, $p<0.001$) (see Figure 4.3a-c).

A significant sex effect in working memory errors was found for bin 1 ($F(1,138)=5.61$, $p<0.05$) and bin 2 ($F(1,138)=5.22$, $p<0.05$), but not bin 3. For both bin 1 and bin 2, males made significantly more working memory errors than females.

A significant genotype x sex interaction was also found for both bin 1 ($F(1,138)=9.43$) and bin 2 ($F(1,138)=5.76$), but not for bin 3. Independent samples t-tests revealed that female 3xTg-AD mice made significantly more working memory errors in all three bins of days than control females, and male 3xTg-AD mice made

significantly more errors than male control mice in all three bins of days (all $p<0.05$). Additionally, male 3xTg-AD mice made more errors during Days 1-4 and 6-9 of training than female 3xTg-AD mice (both $p<0.01$), but no difference was found for the in bin 3, nor were any differences found between male and female controls (see Figure 4.3d-f).

4.6.2 Reference Memory Errors

4.6.2.1 Entire 14 Day Training Period

A significant effect of genotype was found ($F(1,138)=30.56$, $p<0.001$) with 3xTg-AD mice making significantly more reference memory errors than controls. A sex effect was also found ($F(1,138)=4.10$, $p<0.05$) as males made more reference memory errors than females. However, age did not have an effect on reference memory errors. (See Figure 4.4)

The number of reference memory errors decreased significantly over the 14 days of training ($F(10.15,1400.28)=12.51$, $p<0.001$). There was also a significant day x genotype interaction ($F(10.15,1400.28)=2.34$, $p<0.01$), with independent t-tests revealing that 3xTg-AD mice made more reference memory errors than controls on Days 1, 2, 4, 5, 6, 7, 12, and 13 (all $p<0.05$). Finally, a significant day x sex interaction was found ($F(10.15,1400.28)=2.22$, $p<0.05$), with independent samples t-tests revealing that male mice made more reference memory errors than females on Days 6 and 9 only (both $p<0.05$).

4.6.2.2 Binned Errors

A significant genotype effect was found for all three bins of days, with 3xTg-AD mice making more reference memory errors than controls: bin 1

($F(1,138)=18.56$, $p<0.001$); bin 2 ($F(1,138)=13.53$, $p<0.001$); bin 3 ($F(1,138)=5.77$, $p<0.05$). (Figure 4.5a-c)

The only significant effect of sex was for bin 2 ($F(1,138)=9.21$, $p<0.01$), with males making more reference memory errors during days 6-9 than females.

There was a significant interaction between genotype and sex in bin 2 ($F(1,138)=6.15$, $p<0.05$) with an independent samples t-test revealing that 3xTg-AD males made more reference memory errors during days 6-9 than control males ($p<0.001$), but there was no difference between 3xTg-AD and control females. Male 3xTg-AD mice made more reference memory errors in bin 2 than 3xTg-AD female mice ($p<0.01$), but no differences were found between male and female control mice (see Figure 4.5d-f).

4.6.3 Correct Entries in the First Four Arms Entered

A significant genotype effect ($F(1,138)=8.20$, $p<0.01$) found that 3xTg-AD mice made fewer correct entries than control mice, in the first four arm entries. (Figure 4.6)

A significant day effect ($F(13,126)=6.54$, $p<0.001$) was found for the number of correct entries made by mice in the first four arm entries. As expected, the number of correct entries increased over the 14 days of training. (Figure 4.6)

A day x genotype interaction was found ($F(13,126)=1.74$, $p<0.05$) with independent samples t-tests revealing that 3xTg-AD mice made fewer correct entries in the first four arm entries than controls on Days 3, 6, 7, and 14 (all $p<0.05$).

There were no main effects of sex or age on the number of correct entries in the first four arm entries, nor were any other interactions significant.

4.6.4 Total Arm Entries

Genotype had a significant effect on the total number of arm entries ($F(1,138)=33.58$, $p<0.001$) with 3xTg-AD mice entering more arms than controls. Sex was also found to have a significant effect on total arm entries ($F(1,138)=6.03$, $p<0.05$) with males making more entries than females. Additionally, a genotype x sex interaction was found ($F(1,138)=5.51$, $p<0.05$), with independent samples t-test revealing that 3xTg-AD males made more entries than control males and 3xTg-AD females made more entries than control females (both $p<0.01$). Additionally, 3xTg-AD males made more entries than 3xTg-AD females ($p<0.05$), but there was no difference between control male and control female mice. Age did not have any effect on total entries (see Figure 4.7)

A significant effect of day was found for total entries ($F(10.06,1388.04)=17.03$, $p<0.001$), with entries decreasing over days. A day x genotype interaction was found ($F(10.06, 1388.04)=2.18$, $p<0.05$), with independent samples t-tests revealing that 3xTg-AD mice made significantly more entries than control mice on all days except 8, 10, 11, and 14 ($p<0.05$). A day x sex interaction was found ($F(10.06, 1388.04)=2.48$, $p< 0.01$), with independent samples t-tests revealing that male mice made more entries than female mice on Days 6 and 9 only (both $p<0.05$). Finally, a day x sex x age interaction was found ($F(40.23, 1388.04)=1.62$, $p<0.01$), with independent samples t-tests revealing the following: at 2-months of age, male mice made more entries than female mice on Days 1 and 9 (both $p<0.05$), but female mice made more entries than males on Day 2 ($p<0.05$); at 6-months of age, male mice made more entries than female mice on Day 10 only; at

9-months of age, males made more entries than females on Day 6 only; at 12-months of age, there were no differences between male and female mice on any of the days of training; and at 15-months of age, females made more entries than males on Day 12 only ($p<0.05$).

4.6.5 Probe Trial

There were no significant main effects, nor were there any significant interactions for the number of correct arm entries during the probe trial.

4.7 Discussion

The hypotheses that 3xTg-AD mice would make more working and reference memory errors were supported. Triple transgenic mice made significantly more working and reference memory errors over all 14 days of training, as well as when performance was binned into three groups of days. Additionally, 3xTg-AD mice entered fewer correct arms in the first four arm entries during each trial. These findings support the results of Gabbita et al. (2012) indicating that 3xTg-AD mice had working memory deficits in the radial arm maze, and the findings showing 3xTg-AD mice had reference memory deficits in the MWM (Billings at al., 2005 & 2007; Clinton et al., 2007; Giménez-Llort et al., 2007; McKee et al., 2008; Stover, 2012).

The hypothesis that there would be no sex difference in working or reference memory between 3xTg-AD males and females was not supported. Triple transgenic male mice made more working and reference memory errors than females. Over the entire 14 days of training, male 3xTg-AD made significantly more working memory errors and significantly more reference memory errors than 3xTg-AD females (this

sex difference was not shown by control mice). Similarly, when the days of training were binned, 3xTg-AD male mice made more working memory errors than females during days 1-4 and days 6-9, and more reference memory errors during days 6-9 only. These results indicate that the sex difference between the 3xTg-AD mice was no longer present by the end of the training. Not surprisingly, males were also found to make more overall entries than females over the 14 days of training. These findings contradict the only two studies that investigated the effect of sex on reference memory (Giménez-Llort et al., 2010; Stover, 2012); however, no studies have previously compared working memory in male and female 3xTg-AD mice.

The hypothesis that there would be no memory differences between 3xTg-AD mice and controls at 2-months of age, but that 3xTg-AD mice would develop memory deficits by 6-months of age, was not supported. There was no effect of age found in the present study, with the genotype differences already present at 2-months of age. The difference between 3xTg-AD and control mice did not increase or decrease at different ages, but 3xTg-AD mice at all ages showed both working and reference memory deficits. The findings that 3xTg-AD mice at 2-months of age made more working and memory errors compared to 2-month old control mice, contradicts the findings of multiple studies (Billings et al., 2005; Billings et al., 2007; Clinton et al., 2007; Giménez-Llort et al., 2007; McKee et al., 2008), although it does support the findings reported by Stover (2012) who found learning deficits in 3xTg-AD mice at 2-months of age.

The meaning and implications of the radial arm maze findings are discussed in further detail in Chapter 6: Meaning and implications.

Chapter 5: Brain pathology

5.0 Brain pathology in humans with AD

As outlined in section 1.2, Aβ plaques and neurofibrillary tangles are the most common neuropathological indicators of AD in humans (Nelson, 2012). Despite some controversy, it is generally accepted that Aβ plaque deposition is directly related to the cause of AD in humans and the development of NFTs typically follows the accumulation of Aβ (Oddo et al., 2006). Aβ plaque deposition normally begins intraneuronally in humans, and is followed by extracellular Aβ deposits (LaFerla, 2010). Plaque deposition normally begins in the basal portions of the neocortex, and eventually spreads to more sub-cortical areas over time (Braak & Braak, 1991); while NFTs are often first detected between the entorhinal region and temporal isocortex, and eventually progress into the limbic areas of the brain, and eventually the cortex (Braak & Braak, 1991).

5.1 Reported brain pathology in 3xTg-AD mice

As outlined in section 1.4.1, the 3xTg-AD mouse model was designed to exhibit similar neuropathology to that seen in humans with AD. The mice develop Aβ plaque deposits and NFTs in an age-related manner, with plaque deposits and NFTs increasing in severity over the lifetime of the animal (Sy et al., 2011).

In the initial report of the 3xTg-AD mouse model, Oddo et al. (2003b) found no Aβ or tau pathology at 2-months of age. Intracellular Aβ was reported to be detectable by 3 to 4 months of age in the neocortex, and present in the CA1 subfield of the hippocampus by 6 months of age (Oddo et al., 2003b). Extracellular plaques were detected in the frontal cortex by 6-months of age and present in the

hippocampus by 12-months of age. Human tau immunoreactivity was only present after extensive Aβ deposition, first appearing in the CA1 region of the hippocampus at 12-months of age and later progressing to other cortical areas (similar to what is reported in humans (Mesulam, 2000)).

Clinton et al. (2007) examined sex differences between male and female 3xTg-AD mice, and although they found behavioural sex differences (e.g., females more impaired than males in the MWM), they found no differences in the amount or location of Aβ plaques or tau pathology.

Finally, Mastrangelo and Bowers (2008) conducted an in-depth, age-related analysis of the age-related progression of Aβ plaque formation and tau pathology in male 3xTg-AD mice. They examined a number of brain regions (including the primary motor cortex (M1) and the CA1 subfield of the hippocampus) in 3xTg-AD mice at 2, 3, 6, 9, 12, 15, 18, and 26 months of age and detected the presence (although limited) of intracellular Aβ deposition at 2-months of age in the M1 and CA1. They did not however, detect extracellular Aβ plaques until 15-months of age (in the hippocampus). Interestingly, the human tau transgene was detected in the CA1 region of the hippocampus (in very limited quantities) in mice as young as 2-months of age, followed by consistent staining in the CA1 region by 6-months of age. The results presented by Mastrangelo and Bowers (2008) indicated the presence of intracellular Aβ plaques and human tau transgenes at earlier ages, and the development of extracellular plaques at a much later age, than those reported by Oddo et al. (2003b).

5.2 Hypothesis for brain pathology at 2 and 15 months of age

The present study compared the brain pathology of 2- and 15-month old 3xTg-AD mice with B6129SF2/J controls. The results from previous research into the presence of Aβ plaques at 2-months of age have been mixed. Oddo et al. (2003b) reported no neuropathological differences between 3xTg-AD mice and age-matched controls at 2-months of age. However, Mastrangelo and Bowers (2008) detected a limited number of intracellular plaques and human tau transgenes in 2-month old 3xTg-AD mice. Therefore, it was hypothesized that mice at both 2- and 15-months of age would differ from age-matched controls.

Neither Oddo et al. (2003b) nor Clinton et al. (2007) found differences between male and female 3xTg-AD mice for the presence of Aβ deposits and NFTs; therefore, it was hypothesized that male and female 3xTg-AD mice would not differ in densities of plaque deposition and tau pathology at both of the measured ages.

Finally, based upon the results of Mastrangelo and Bowers (2008), it was hypothesized that intracellular Aβ plaques would be detected in both the primary motor cortex and the CA1 region of the hippocampus of 2-month old 3xTg-AD mice, but there would be no extracellular Aβ plaques present. It was expected that at 15-months of age both brain regions would show extensive intra- and extracellular plaque deposition. Additionally, it was hypothesized that at 2-months of age 3xTg-AD may exhibit a very limited amount of human tau in both the primary motor cortex and the CA1 region of the hippocampus, and that by 15-months of age 3xTg-AD mice would have consistent staining for human tau in both the M1 and the hippocampus.

5.3 Procedure

This work was performed in the lab of Dr. Sultan Darvesh by Mark Bartolacci as part of an individual research project under Leanne Fraser's supervision.

5.3.1 Perfusion

Mice were anaesthetized with an intraperitoneal injection (0.1 mL/25 g) of pentobarbital sodium (Euthanyl, 65 mg/mL; Bimeda-MTC, Cambridge, ON) and perfused through the heart with 0.1 M phosphate buffer (PB; pH 7.4; Sigma-Aldrich, Oakville, Ontario, Canada) followed by 4% paraformaldehyde (Sigma-Aldrich) in 0.1 M PB. Mice were then decapitated and their brains were extracted. Brains were placed into 4% paraformaldehyde at 4°C for 24 hours and then transferred to a phosphate buffer with saline (PBS) plus Azide (0.01%) until further processing.

5.3.2 Sectioning

To cryoprotect the brain tissue, brains were transferred to a sucrose and sodium azide (0.01%) solution 24 to 72 hours prior to sectioning. A Leica SM2000R microtome (Leica Microsystems Inc., Nussloch, Germany) with Physitemp freezing stage and BFS-30TC controller (Physitemp Instruments, Inc., Clifton, NJ) was used to cut the brains into 40 µm serial sections in a coronal plane. The stage of the microtome was covered with 0.1 M phosphate buffer (PB) and the temperature was set to -42.1°C, which permitted freezing of the PB. The brain was then adhered to the stage of the microtome using Optimal Cutting Temperature (OCT) compound (VWR International LLC, Radnor, PA), and was oriented so that the olfactory bulbs were pointing downwards. The brain tissue was frozen by surrounding it with dry ice, and 40 micrometre sections were cut and placed using a paintbrush into a 24-well tray

containing 0.5% sodium azide in PB. Following cutting, the tray was covered with a layer of Parafilm (Pechiney Plastic Packaging Inc., Chicago, IL) and placed in the refrigerator until staining.

5.3.3 Immunohistochemistry

5.3.3.1 Staining for Aβ and NFTs

Sections were removed with a paintbrush from the 24-well tray (containing 0.5% sodium azide in PB) and were transferred to a 12-well tray that contained transferring wells that were immersed in 0.1 M PB pH 7.4. Sections were rinsed three times for ten minutes each on a shaker. For each rinse the wells were transferred to a new tray containing 0.1 M PB pH 7.4.

Aβ Staining. Sections stained for Aβ (full-length 1-43) were rinsed for five minutes in 0.05 M PB (prepared by mixing equal amounts of 0.1 M PB pH 7.4 and dH_2O). The sections were then rinsed in dH_2O three times for five minutes each time. Following the final rinse, the sections were transferred under a fume hood to trays containing 90% formic acid. The sections remained in the formic acid for two minutes, during which time the trays were agitated gently by hand. The formic acid rinse was done to improve immunohistochemical staining. After the formic acid rinse, the sections were rinsed five times, for one minute each time, in dH_2O. The sections were then removed from the fume hood and rinsed on the shaker in 0.1M PB pH 7.4 three times (for 10 minutes each time). The sections were then transferred to a tray containing 0.3% hydrogen peroxide (H_2O_2; made by diluting 30% H_2O_2 with PB) in order to quench endogenous peroxidase activity. Following an additional three rinses in 0.1M PB pH 7.4, the sections were removed from the

transferring wells using a paintbrush and placed into a tray containing the primary antibody (polyclonal rabbit anti β-Amyloid; 1:400; 71-5800, Invitrogen, Camarillo, CA) specific for the 4- to 5-kDa amyloid peptide derived from cleavage of the amyloid precursor protein, PB and 0.1% Triton X-100 (PBT; Sigma- Aldrich), and normal goat serum (NGS; 1:100; Vector Laboratories, Burlingame, CA). The solution was made following a brief (10-15 second) period of centrifugation of the antibody and NGS. The sections were left in the primary antibody overnight (approximately 16 hours) at room temperature.

Tau Staining. Following the initial three rinses (for 10 minutes each) in 0.1M PB pH 7.4, sections stained for tau (recombinant human tau protein 243-441) were rinsed in a 0.3% solution of H_2O_2 for 30 minutes to quench endogenous peroxidase activity. After the rinse in H_2O_2, the sections were rinsed three times (for 10 minutes each) in 0.1M PB pH 7.4, and were then removed from the transferring wells and placed into a tray containing the primary antibody solution. The solution was composed of PBT, (polyclonal rabbit anti-human tau; 1:16000; A0024, Dako, Burlington, Ontario, Canada) and NGS (1:100). The sections were left in the primary antibody solution overnight (approximately 16 hours) at room temperature.

Post-primary antibody staining for Aβ and tau. Following the overnight incubation period in the primary antibody, the tray containing the sections was removed from the shaker and the sections were transferred into PB. The sections were rinsed in PB three times for 10 minutes each. Next, the sections were removed from the transferring wells with a paintbrush and placed into a tray with the secondary antibody solution, which was composed of PBT, Biogar (biotinylated goat

anti-rabbit; 1:100; BA-1000, Vector Laboratories, Burlingame, CA), and NGS (1:1000). The sections were left to incubate on the shaker in the secondary antibody for a period of one hour at room temperature.

Following incubation in the secondary antibody, the sections were transferred to PB and rinsed three times. Next, the sections were transferred to a tray containing Vectastain Elite ABC Kit (1:182, PK-6100, Vector Laboratories, Burlingame, CA) and PBT. Following the period in ABC peroxidase, the sections were rinsed three times for ten minutes each in PB. After the final rinse, the sections were placed in a solution containing of PB and 1.39 mmol/L 3,3'- diaminobenzidine tetrahydrochloride (DAB; 5637, Sigma-Aldrich) for a period of five minutes. Following the five-minute period in DAB solution, 50 µL of 0.3% H_2O_2 per milliliter of DAB solution was added to each well. The tissue was examined periodically after the addition of the H_2O_2 in order to ensure that it was not incubated for too long in the solution, which could lead to excessive background staining. Following the period in the DAB/H_2O_2 solution, the sections were rinsed three times (for 10 minutes each) in 0.01M acetate buffer pH 3.3 in order to stop the reaction. The sections were then transferred to a tray containing 0.01M acetate buffer pH 3.3, covered in Parafilm, and placed in the refrigerator until mounting.

5.3.4 Mounting and Cover-slipping

A large Petri dish was filled halfway to the top with 0.01M acetate buffer pH 3.3. The sections for a single brain were removed from the 12-well tray using a paintbrush and placed in the centre of the dish. The sections were arranged around the perimeter of the dish, starting with the most rostral sections at the top of the

dish. The sections were placed one by one in the clockwise fashion, rostrally to caudally.

Following the organization of the sections, a Fisher Superfrost Plus glass microscope slide (Fisher Scientific; Hampton, NH) was labeled with the mouse number, mouse strain, and the staining procedure applied to the tissue. The sections were transferred from the Petri dish to the slide in the rostral to caudal direction in which they were organized in the dish. Once placed on the slide, the sections that were slightly folded were examined under the dissecting microscope in order to permit the tissue to be flattened more easily. Kimwipes (Kimberly-Clark Corporation; Irving, TX) were used to remove the excess buffer solution on the slide in order to prevent movement of the sections. Once the mounting was complete, the slide was placed on a covered slide warmer to air-dry overnight.

The slides to be cover-slipped were placed in a slide rack in a fume hood and rinsed for 15 s in dH_2O. The slides were then rinsed in the following sequence of solutions, with a five second drainage period after each rinse: 70% ethanol (15 s), 95% ethanol (15 s), 95% ethanol (15 s), 100% ethanol (15 s), 100% ethanol (15 s), xylene (30 s), and xylene (30 s). Following the final rinse in xylene, one slide was removed at a time from the slide rack and placed on a piece of paper towel. Six to eight drops of Cytoseal 60 (Fisher Scientific) were placed on top of the tissue and a cover glass was placed on top. After the slides had been cover-slipped, they were left in the fume hood overnight.

5.3.5 Imaging

Prior to taking photomicrographs of the stained tissue sections, the slides were examined using a Leica DME microscope with a Leica camera mounted on top. The sections were visualized with Leica FireCam software on an Apple (Cupertino, CA) monitor. Notes were taken in order to compile a record that qualitatively described whether pathology was present in the representative sections of the desired regions for each brain. The primary motor cortex (M1) and the hippocampus CA1 were examined. Following the initial qualitative assessment, photographs were taken of the representative sections for each brain using a Zeiss Axioplan 2 motorized microscope with a Zeiss Axiocam HRc digital camera using Axiovision 4.6 software (Carl Zeiss Canada Ltd., Toronto, Ontario, Canada). Photomicrographs were obtained at 10X.

The primary motor cortex (M1) was examined for evidence of Aβ plaques and NFTs at three levels: rostral (Bregma 1.78 mm), intermediate (Bregma 0.62 mm), and caudal (Bregma -0.58 mm). The hippocampus CA1 was also examined for the presence or absence of Aβ plaques and NFTs at three levels: rostral hippocampus CA1 (Bregma -1.22 mm), intermediate hippocampus CA1 (Bregma -2.46 mm), and caudal hippocampus CA1 (Bregma -3.80 mm). The brains from three male and three female 3xTg-AD and control mice at both 2- and 15-months of age were analyzed at three levels in both the M1 and the CA1; 144 sections were analyzed in total.

5.3.6 Qualitative analysis

Sections were qualitatively scored using the following schema (adapted from Mastrangelo and Bowers (2008): (0) indicated no staining present; (0/+) indicated a

limited number of cells showing evidence of staining; and (+) indicated consistent presence of cells showing staining. Staining was operationally defined as dark pigmentation, well-defined from the background. Aβ plaques were determined to be intracellular if the staining resembled a cell-like structure and had distinct borders, while extracellular plaques were defined as globular-like staining with rough edges.

5.4 Results

At 2-months of age, both male and female 3xTg-AD mice had positive staining for Aβ plaques in all three levels (rostral, intermediate, and caudal) in the primary motor cortex (M1) (Figure 5.1). In the CA1 region of the hippocampus, although 2-month old female 3xTg-AD mice had consistent positive staining for Aβ plaques, males were only found to have staining at the caudal hippocampus CA1 level, but not at the rostral or intermediate levels. Both male and female 3xTg-AD mice at 15-months of age had extensive positive staining for Aβ plaques in all three levels of the M1 and the hippocampus CA1. See Tables 5.1 and 5.2 and Figures 5.1 and 5.2.

For human tau staining, at 2-months of age there appeared to be a difference in the presence of human tau transgenes between male and female 3xTg-AD mice. Male mice at 2-months of age have almost no positive staining for tau, however female mice at 2-months of age were found to have positive tau staining in both the M1 and the hippocampus CA1. By 15-months of age, both male and female mice show positive tau staining in the CA1, but females appear to have more staining than males in the primary motor cortex. See Tables 5.1 and 5.2 and Figures 5.3 and 5.4.

As expected, control mice showed no positive staining for Aβ plaques or human tau (Table 5.1). However, it should be noted that one of the 2-month old

female mice control mice was found to have positive staining for Aβ plaques only (not for tau) in both the M1 (caudal) and the CA1 (rostral) areas.

5.5 Discussion

The hypotheses that 3xTg-AD mice at 2- and 15-months of age would have more Aβ plaque deposition and human tau transgenes compared to age-matched controls, were supported. 3xTg-AD mice at both ages showed intracellular Aβ plaque deposition and positive staining for human tau in both the primary motor cortex and the hippocampus CA1.

The hypothesis that there would be no differences between male and female 3xTg-AD mice at either age was not supported. Female 3xTg-AD mice were found to have positive human tau staining in both the primary motor cortex and the hippocampus CA1 at 2-months of age, while male 3xTg-AD mice showed almost no positive staining in either region. This finding contradicts the results of both Oddo et al. (2003b) and Clinton et al. (2007). Both studies reported finding no differences in the presence of Aβ deposits or tau pathology between male and female 3xTg-AD mice.

Finally, based upon the results of Mastrangelo and Bowers (2008), it was hypothesized that intracellular Aβ plaques would be detected in the brains of 2-month old 3xTg-AD mice, but there would be no extracellular Aβ plaques or tau pathology present. The results from the present study support this hypothesis; intracellular plaque deposition was detected in 3xTg-AD mice at 2-months of age, but there were no extracellular Aβ plaques or tau pathology. The hypothesis that 15-month old 3xTg-AD mice would exhibit both intra- and extracellular Aβ plaques as

well as tau pathology was also supported. Although 15-month old male 3xTg-AD mice showed very limited tau staining in the motor cortex, both male and female mice were positive for tau staining in the hippocampus and females showed positive staining for tau in the motor cortex.

The meaning and significance of these findings are discussed in further detail in Chapter 6: Meaning and implications.

Chapter 6: Meaning and Implications

The purpose of the present study was to thoroughly characterize the behavioural phenotype of the 3xTg-AD-AD mouse model of Alzheimer's disease. Using a cross-sectional design, both male and female 3xTg-AD-AD and B6129SF2/J (control) mice at five different ages (2, 6, 9, 12, and 15 months) were compared on a selection of cognitive and non-cognitive behaviours.

In the 8-arm radial maze, mice were tested for both working memory and reference memory. Triple-transgenic mice were found to make significantly more working memory errors and reference memory errors than controls. Additionally, male mice were found to have impaired working and reference memory compared to female mice in the RAM. Surprisingly, there was no effect of age found, with mice showing similar memory abilities at each of the five ages tested.

The accelerating rotarod measured motor coordination and learning ability. Triple-transgenic mice showed superior coordination and learning, consistently outperforming control mice. After weight was included in the analyses as a covariate, there were no differences in coordination or learning between male and female mice. There was also no effect of age on rotarod performance.

Anxiety was investigated using the open field test and the elevated plus maze. Triple-transgenic mice were more anxious in both tests, freezing significantly more often than control mice, however no consistent sex differences or age effects were found.

Finally, depression was measured in the present study using the forced swim test. Interestingly, 3xTg-AD-AD mice engaged in less depression-related behaviour

(immobilization) than control mice. Female mice were found to engage in more depression-related behaviour than males, however there was no effect of age in the FST.

Not surprisingly, given the conflicting findings of previous research, the results of the present study both supported and contradicted previous research. Overall it appears as though the 3xTg-AD-AD mouse model exhibits increased working and reference memory deficits (Billings at al., 2005 & 2007; Clinton et al., 2007; Giménez-Llort et al., 2007; McKee et al., 2008; Gabbita et al., 2012; Stover, 2012), superior motor performance (Filali et al., 2012; Stover, 2012), decreased locomotion (Giménez-Llort et al., 2007; Sterniczuk et al., 2010; Filali et al., 2012), and increased anxiety (Giménez-Llort et al. 2007; Sterniczuk et al., 2010; Filali et al., 2012) compared to controls.

While some of these behavioural differences replicate the behaviours seen in humans with AD (e.g., increased memory deficits and anxiety), others directly contradict the symptoms of AD (e.g., improved motor performance and decreased depression-related behaviour). Similarly, the only consistent sex effects found in the present study also support and contradict human studies. Female 3xTg-AD mice were found to engage in more depression-related behaviours in the FST, a result that mimics the finding in humans that females with AD are at a higher risk of depression than males (Lyketsos & Olin, 2002). However, male 3xTg-AD mice made significantly more working and reference memory errors than female 3xTg-AD mice; a finding that contradicts the increased severity of cognitive deficits in females with AD compared to males with AD (Carter et al., 2012). Additionally, the sex differences

in neuropathology in the present study directly contradict the memory impairment seen in the RAM. Human tau was found in both the M1 and the hippocampus CA1 in female 3xTg-AD mice at 2-months of age, while male 3xTg-AD mice had almost no tau present in either brain region at 2-months of age.

Notably, in the present study, no effect of age was found for any of the behaviours. The present study included five different ages, selected to correspond with the advancement of the AD-related neuropathology, however no difference was found on any measure even between the youngest (2-months of age) and oldest (15-months of age) cohorts. The lack of any age effects may have been dictated by the presence of behavioural differences in 2-month old 3xTg-AD mice. Only one previous report of differences between 3xTg-AD and control mice at 2-months of age could be found (Stover, 2012). In an experiment conducted in the same lab as the present study, Stover (2012) reported increase motor performance and a deficit in learning in the MWM in 2-month old 3xTg-AD mice.

While the behaviour differences found at 2-months of age in the 3xTg-AD mice (both in the present study and in Stover, (2012)) do not correspond with the behavioural data previously reported (Billings et al., 2005; Billings et al., 2007; Giménez-Llort et al., 2007), the neuropathology of the 3xTg-AD mice in the present study supports the behavioural findings. Both Aβ plaques and human tau were present in both the primary motor cortex and the hippocampus CA1 in 2-month old 3xTg-AD mice. Although the presence of neuropathology at 2-months of age was not reported by Oddo et al. (2003b), Mastrangelo and Bowers (2008) found plaques and tau present in limited quantities in mice at 2-months of age. It should be noted that

many studies that have reported no behavioural differences between 3xTg-AD mice and controls at 2-months of age have not independently investigated the neuropathology of the mice used. Additionally, Mastrangelo and Bowers (2008) only investigated the neuropathology of the mice and not the behaviour. However, the neuropathology in the present study replicates the findings of Mastrangelo and Bowers (2008) and supports the behavioural differences present at 2-months of age.

One of the most peculiar findings of the 3xTg-AD mouse model is the superior motor coordination and learning seen on the rotarod. It is unclear what underlying mechanism might be driving the superior motor functioning, however it is possible that the same variable may have also influenced performance in the forced swim test. Both tests require involuntary, continuous participation. On the rotarod, mice that continue walking for the longest period of time before falling off are considered to have superior motor coordination. In the FST mice that continue to swim and engage in the fewest pauses are considered to be "less depressed". In both of these tests of continuous, involuntary participation, the 3xTg-AD mice outperformed controls. However, when placed into a voluntary participation situation, for example the OF and EPM, the 3xTg-AD mice were much less active and appeared more anxious. It is possible that the results from the present study may not directly indicate superior motor performance or lower levels of depression in the 3xTg-AD mice, but rather a difference in their behavioural response to an involuntary task. Perseveration, the continuance of a response without an appropriate intervening stimulus, is a common symptom seen in Alzheimer's disease patients (Pekkala et al.,

2008). It is possible that the results from the rotarod and FST are reflecting a symptom of perseveration in the 3xTg-AD mice.

Results from the present study introduce many possible directions for future research. The absence of progressive age deficits could be investigated by testing older cohorts of mice on the same battery of tests used in the present study. It is possible that the progression of deficits did not advance significantly enough to be detected by 15-months of age. Additionally, more complex and challenging cognitive tasks could be employed. It is possible that the cognitive tasks used in the present study were not taxing enough to parse out age-related differences. Older 3xTg-AD mice might show increased impairments as the tasks increase in difficulty. Finally, future studies may want to focus on the superior motor coordination and learning ability in the 3xTg-AD mice. Testing the 3xTg-AD mice on multiple motor-related tasks may help to elucidate the underlying mechanisms leading to the increased ability. Finally, the possibility that the 3xTg-AD mice may exhibit a form of perseveration when performing on the rotarod or other involuntary tasks should be taken into consideration and possibly studied in more detail.

Chapter 7: Conclusions

The 3xTg-AD mouse model was designed as a rodent analogue to mimic the symptoms of Alzheimer's disease seen in humans. As with any animal model of a human disease, it is unlikely that all behaviours will be exactly replicated, however the more closely a model resembles disease pathology, the more likely researchers are to be able to develop functioning treatments for humans. The 3xTg-AD mouse model of AD has increased working and memory deficits, increased anxiety, and exhibits similar neuropathology to that seen in humans with AD. It is also possible that it exhibits a cognitive deficit similar to the perseverance seen in individuals with AD. This collection of behavioural and neurological pathology suggests that the 3xTg-AD mouse model is one of the best options available for AD research. However, in the present study at least, the 3xTg-AD mice did not show any progressive change in deficits; mice at 2-months of age behaved similarly to those at 15-months of age, despite differences in neuropathology. Alzheimer's disease is characterized primarily by a progressive decline in cognitive deficits and a model that does not replicate this characteristic must be found wanting.

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Appendix A: Tables

Table 1.1 – Number of mice tested for each genotype, sex, and age.

		Age (months)						
		2	**6**	**9**	**12**	**15**	**Σ**	**Total**
3xTg-AD	M	8	7	4	5	3	**27**	**69**
	F	10	5	9	8	10	**41**	
B6129SF2/J	M	8	6	10	12	11	**46**	**89**
	F	8	5	8	10	11	**42**	
Total		**34**	**23**	**31**	**35**	**35**		**158**

Table 2.1 – Correlation of mean latency to fall each day (collapsed across all groups) with mean weight on each day (collapsed across all groups).
*p<0.05, **p<0.01

	n	Weight Day 1	Weight Day 2	Weight Day 3	Weight Day 4	Weight Day 5
Latency Day 1	157	-.381**				
Latency Day 2	157		-.273**			
Latency Day 3	157			-.211*		
Latency Day 4	157				-.198*	
Latency Day 5	157					-.149

Table 2.2 – Correlation of mean latency to fall (collapsed across days) and mean weight (pooled across days) for both genotype and sex.
*p<0.05, **p<0.01

	Sex	Genotype	n	Weight
Latency	M	Control	47	-.406**
		3xTg-AD	27	.257
	F	Control	41	-.174
		3xTg-AD	42	-.255

Table 2.3 – Correlation of mean latency to fall (collapsed across days) and mean weight (collapsed across days) for genotype, sex, and binned ages. Data from mice ages 6-7 months and 9-10 months were combined to create the "Adult" bin and data from mice ages 12-13 months and 15-16 months were combined to create the "Old" bin (data from mice ages 2-3 months of age made up the "Young" bin, but were not combined with any other age groups).
*p<0.05, **p<0.01

	Latency											
	Young (2-3m)				Adult (6-10m)				Old (12-16m)			
	Male		Female		Male		Female		Male		Female	
	Control	3xTg-AD	Control	3xTg-AD	Control	3xTg-AD	Control	3xTg-AD	Control	3xTg-AD	Control	3xTg-AD
Weight	–.116	–.507	.028	–.379	.238	.163	.468	–.255	–.396	.730*	–.419	–.550*
n	8	8	8	10	16	11	13	14	23	8	20	18

Table 3.1. Main effects of genotype, sex, and age on locomotor-related behaviours in the OF and EPM.

Open field	**Genotype**	**Sex**	**Age**
Distance	3xTg **<** Control	–	–
Centre rears	–	M **>** F	12 **<** All others
Wall rears	3xTg **<** Control	–	12 **>** All others
Elevated Plus Maze	**Genotype**	**Sex**	**Age**
Distance	3xTg **<** Control	–	–
Line crosses	3xTg **<** Control	–	–
Rears	3xTg **<** Control	M **<** F	–

Table 3.2. Main effects of genotype, sex, and age on anxiety-related behaviours in the OF.

Open field		**Genotype**	**Sex**	**Age**
Centre square	Entries	–	–	–
	Time	–	–	–
SAPs		–	–	6 **>** 12, 15
Freezing	Bouts	3xTg **>** Control	–	6, 12 **>** 15
	Time	3xTg **>** Control	–	–
Grooming	Bouts	–	–	–
	Time	–	–	–
Defecation		–	M **<** F	–
Urine spots		–	–	–
Elevated Plus Maze		**Genotype**	**Sex**	**Age**
Open arm	Entries	3xTg **<** Control	–	2 **>** 6, 15
	% Time	3xTg **>** Control	–	–
SAPs		–	–	2 **>** All others
Head dips		–	–	–
Freezing	Bouts	3xTg **>** Control	–	–
	Time	3xTg **>** Control	–	–
Grooming	Bouts	–	–	–
	Time	–	–	–
Defecation		–	–	–
Urine spots		–	–	–

Table 5.1 – Qualitative observations of the presence of Aβ plaques and the human tau transgene in both the primary motor cortex (M1) and the hippocampus of 2- and 15-month old male and female 3xTg-AD and control mice, at three levels: rostral (R), intermediate (I), and caudal (C).
"0" = no staining present; "0/+" = limited number of cells showing evidence of staining; "+" = consistent presence of cells showing staining; "n/a" = section damaged or incomplete

Table 5.1

			Amyloid-beta						Neurofibrillary tangles (tau)					
			M1			CA1			M1			CA1		
Age	**Geno**	**Sex**	**R**	**I**	**C**	**R**	**I**	**C**	**R**	**I**	**C**	**R**	**I**	**C**
2	**Control**	**M**	0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0
		F	0	0	+	0	0	n/a	0	0	0	0	0	0
			0	0	0	0	0	n/a	n/a	0	0	0	0	n/a
			0	0	0	0	0	0	0	0	0	0	0	0
	3xTg-AD	**M**	0/+	+	+	0	0	0/+	0/+	0	0	0	0/+	+
			0/+	0/+	0	0	0	+	0/+	0	0	0	0	+
			+	+	n/a	0	0	+	0	0	0	0	0	0
		F	+	+	+	0/+	n/a	+	0	0	0	0	0	+
			+	+	+	+	+	+	+	0/+	0	0	0/+	+
			+	+	+	+	+	+	+	0/+	0	0	+	+
15	**Control**	**M**	0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0
		F	0	0	0	0	0	0	0	0	0	0	0	0
			n/a	0	0	0	0	0	0	0	0	0	0	0
			0	n/a	0	0	0	n/a	0	0	0	0	0	0
	3xTg-AD	**M**	+	+	+	0/+	n/a	+	0/+	0/+	0	0/+	+	+
			+	+	0/+	0/+	0/+	+	0/+	0	0	0/+	+	+
			+	+	+	+	+	+	0/+	0	0	0	+	+
		F	+	0/+	0/+	n/a	n/a	+	0/+	n/a	0/+	n/a	0	0
			+	+	+	+	+	+	0/+	0/+	0/+	0	0	0/+
			+	+	+	n/a	+	+	+	0/+	0/+	+	+	+

Table 5.2 – Summarized qualitative observations of the presence of Aβ plaques and the human tau transgene in both the primary motor cortex (M1) and the hippocampus of 2- and 15-month old male and female 3xTg-AD mice, at three levels: rostral (R), intermediate (I), and caudal (C).
"0" = no staining present; "0/+" = limited number of cells showing evidence of staining; "+" = consistent presence of cells showing staining

		Amyloid-beta						Neurofibrillary tangles (tau)					
		M1			CA1			M1			CA1		
Age	**Sex**	**R**	**I**	**C**	**R**	**I**	**C**	**R**	**I**	**C**	**R**	**I**	**C**
2	**M**	0/+	+	0/+	0	0	+	0	0	0	0	0	+
	F	+	+	+	+	+	+	+	0/+	0	0	0/+	+
15	**M**	+	+	+	0/+	+	+	0/+	0	0	0/+	+	+
	F	+	+	+	+	+	+	0/+	0/+	0/+	0/+	0/+	0/+

Appendix B: Figures

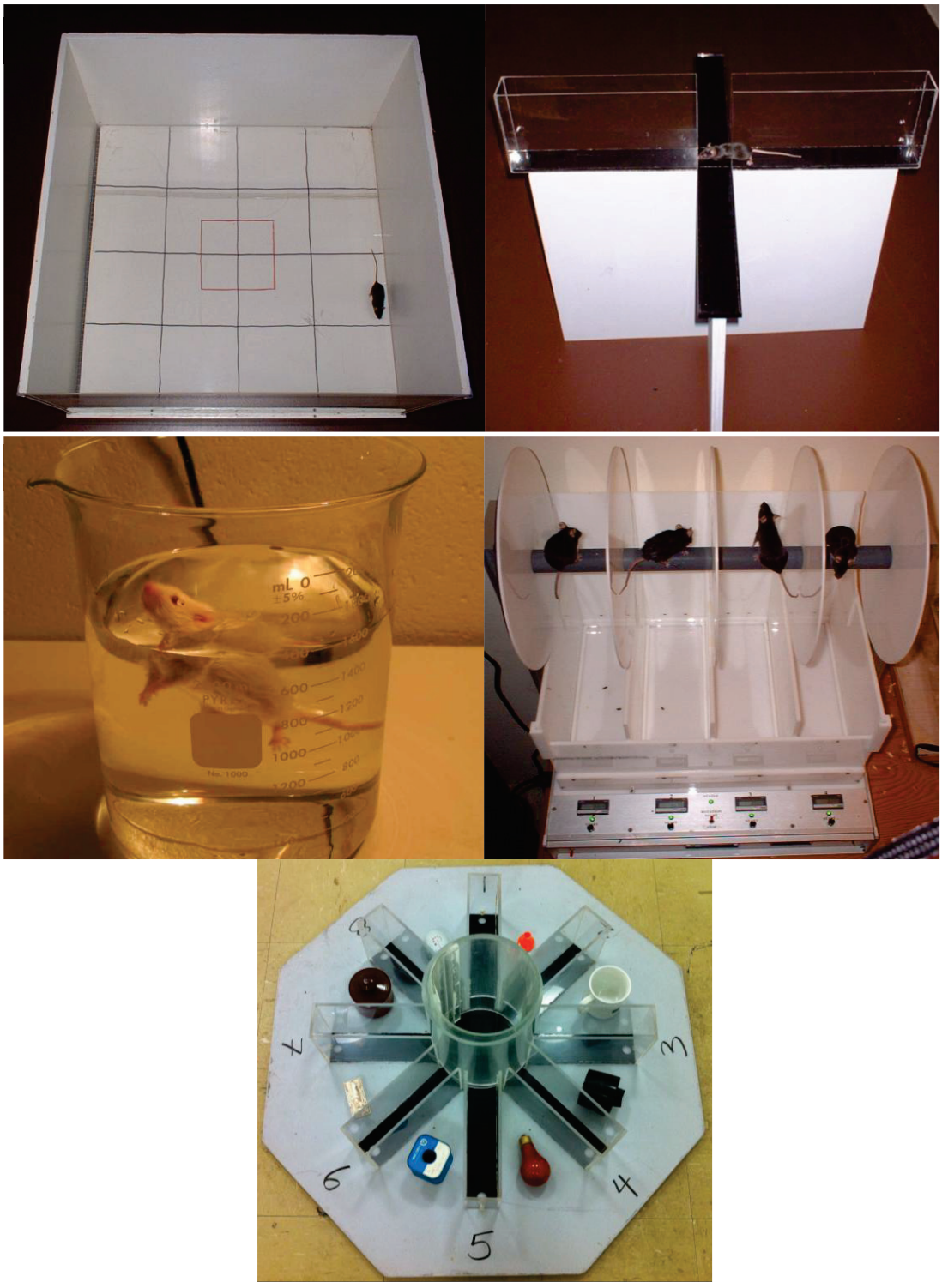


Figure 1.1 Five types of apparatus were used in the present study: (a) Open field; (b) Elevated plus maze; (c) Forced swim test; (d) Rotarod; (e) Radial arm maze (8-arm).

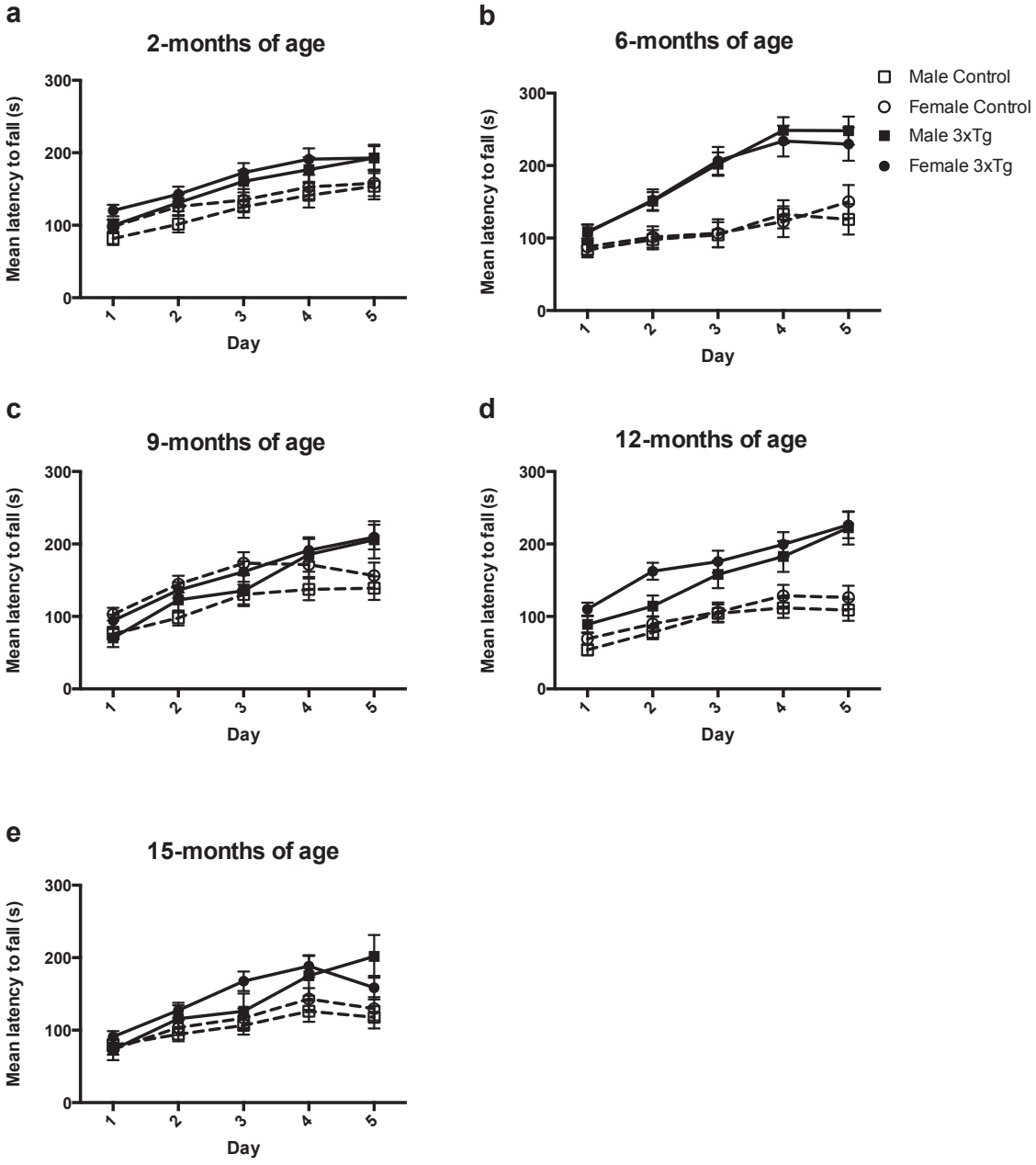
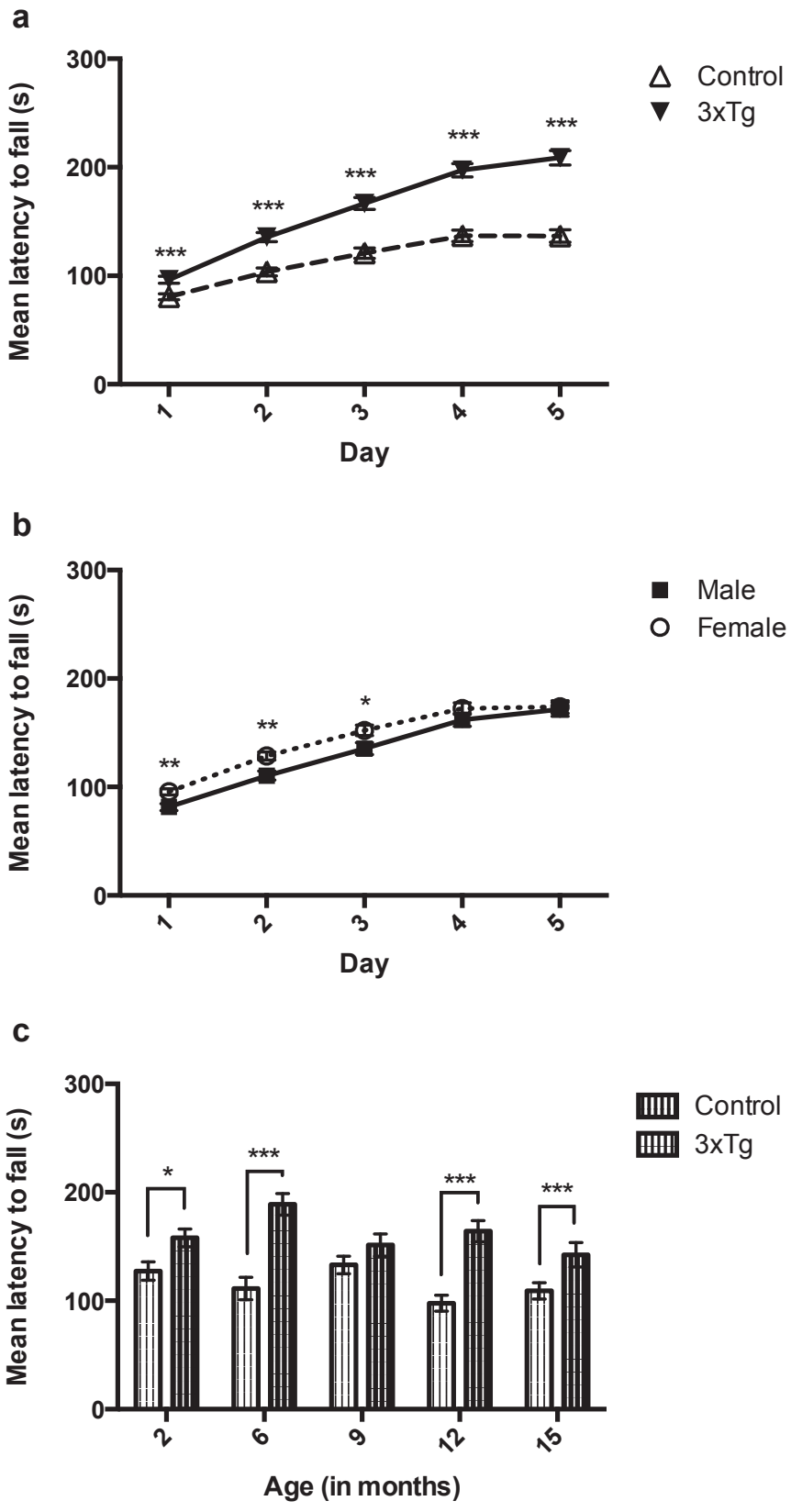


Figure 2.1. Mean (± SEM) latency to fall (s) for male and female control and 3xTg mice over five days of rotarod trials, at: [(a) 2-3 months of age; (b) 6-7 months of age; (c) 9-10 months of age; (d) 12-13 months of age; (e) 15-16 months of age]. * = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 2.2. Rotarod summary analyses (mean ± SEM latency to fall): (a) Genotype differences over the five days of testing. A main effect of genotype showed that 3xTg mice significantly outperformed controls. A day x genotype interaction was also found. (b) Sex differences over the five days of testing. An effect of sex showed that females outperformed males. (c) No main effect of age was found, however a genotype x age interaction showed that 3xTg mice outperformed control mice at every age except at 9-months of age.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 2.2


a
Mean latency to fall (s)
300
200
100
0
1
2
3
4
5
Day
***
***
***
***
***
Control
3xTg
b
Mean latency to fall (s)
300
200
100
0
1
2
3
4
5
Day
**
**
*
Male
Female
c
Mean latency to fall (s)
300
200
100
0
2
6
9
12
15
Age (in months)
*
***
***
***
Control
3xTg

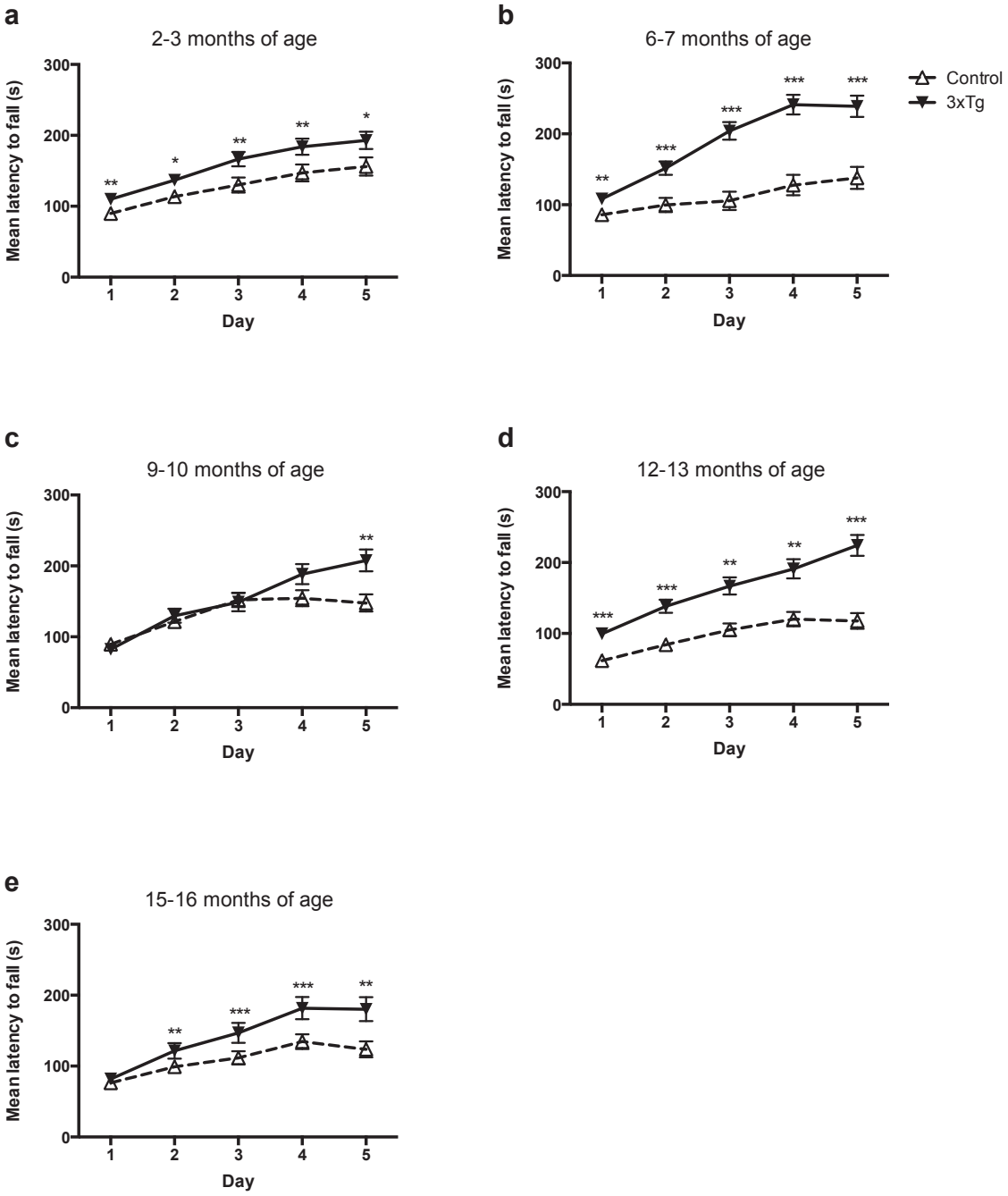


Figure 2.3. Mean (± SEM) latency to fall at each age (data are pooled across sex). Shows a significant day x age x genotype interaction. (a) 2-months of age; (b) 6-months of age; (c) 9-months of age; (d) 12-months of age; (e) 15-months of age. * = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

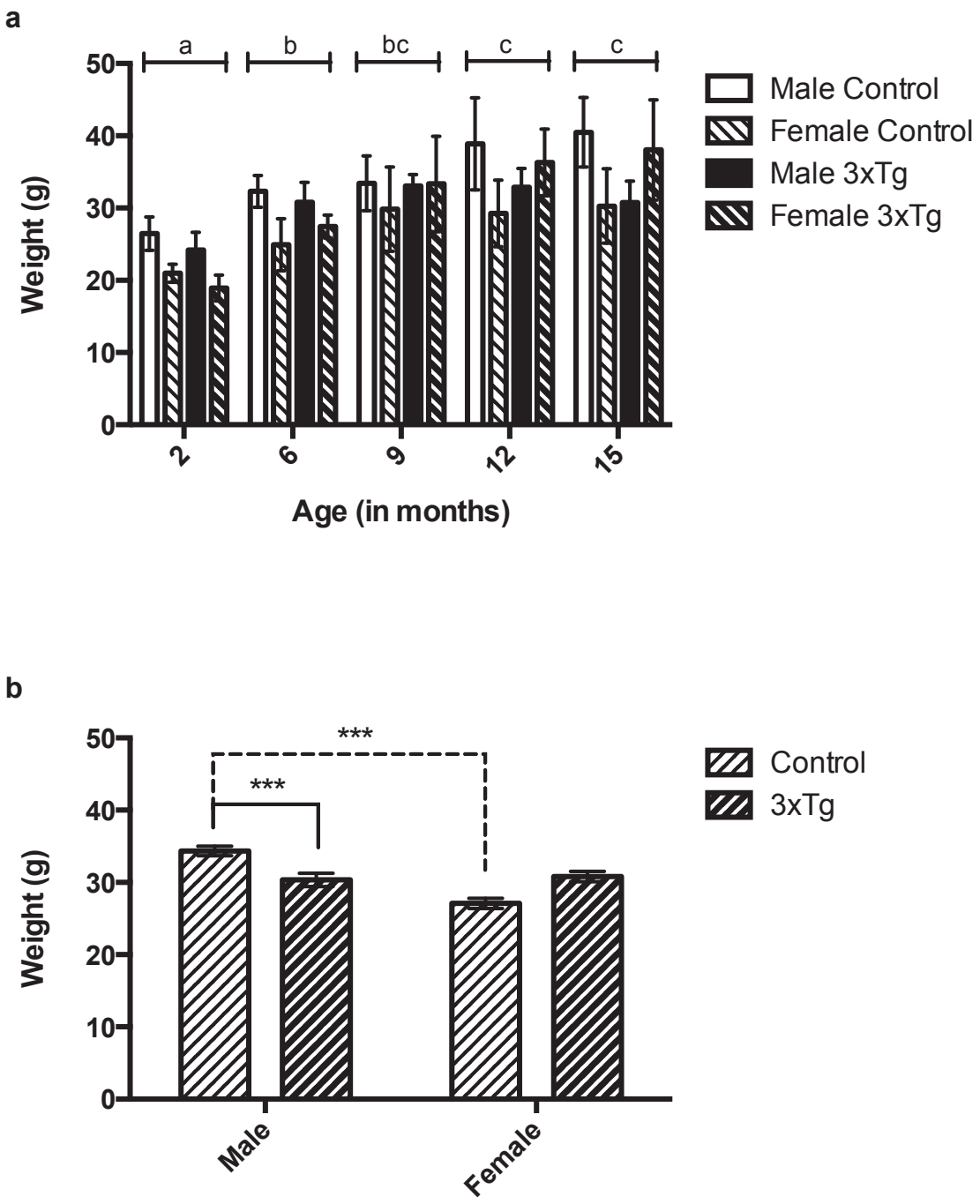


Figure 2.4. Mean (± SEM) body weight of male and female 3xTg and control mice, at each age. (a) A significant age effect showed that, in general, as age increased mean body weight increased. (b) Shows mean body weight (data pooled across age) of male and female 3xTg and control mice. Male control mice weighed more than both female control mice and male 3xTg mice.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

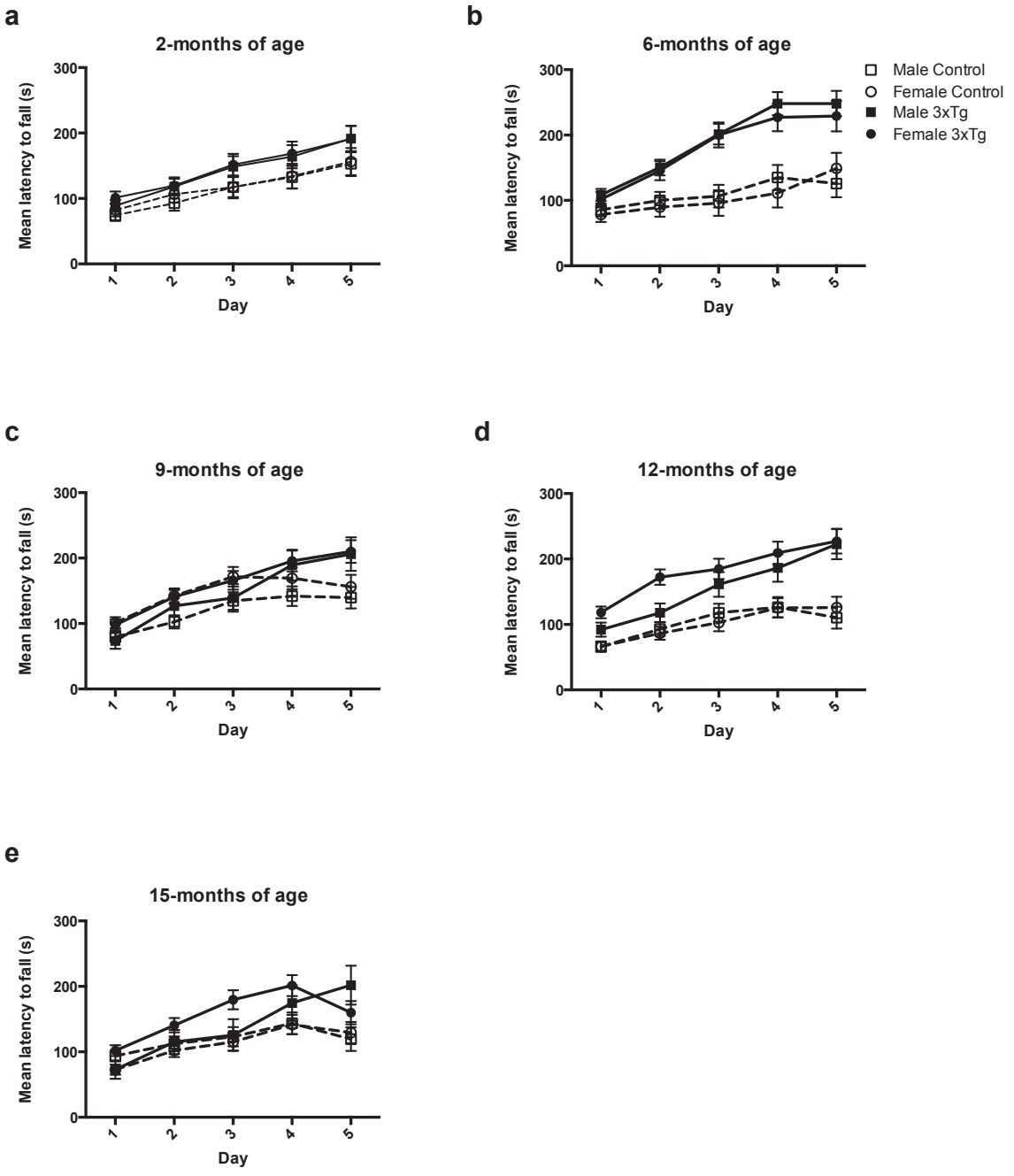


Figure 2.5. Mean (± SEM) latency to fall (s) for male and female control and 3xTg mice at all ages, including weight as a covariate: (a) 2-3 months of age; (b) 6-7 months of age; (c) 9-10 months of age; (d) 12-13 months of age; (e) 15-16 months of age. When weight was included as a covariate, sex no longer had a significant effect.

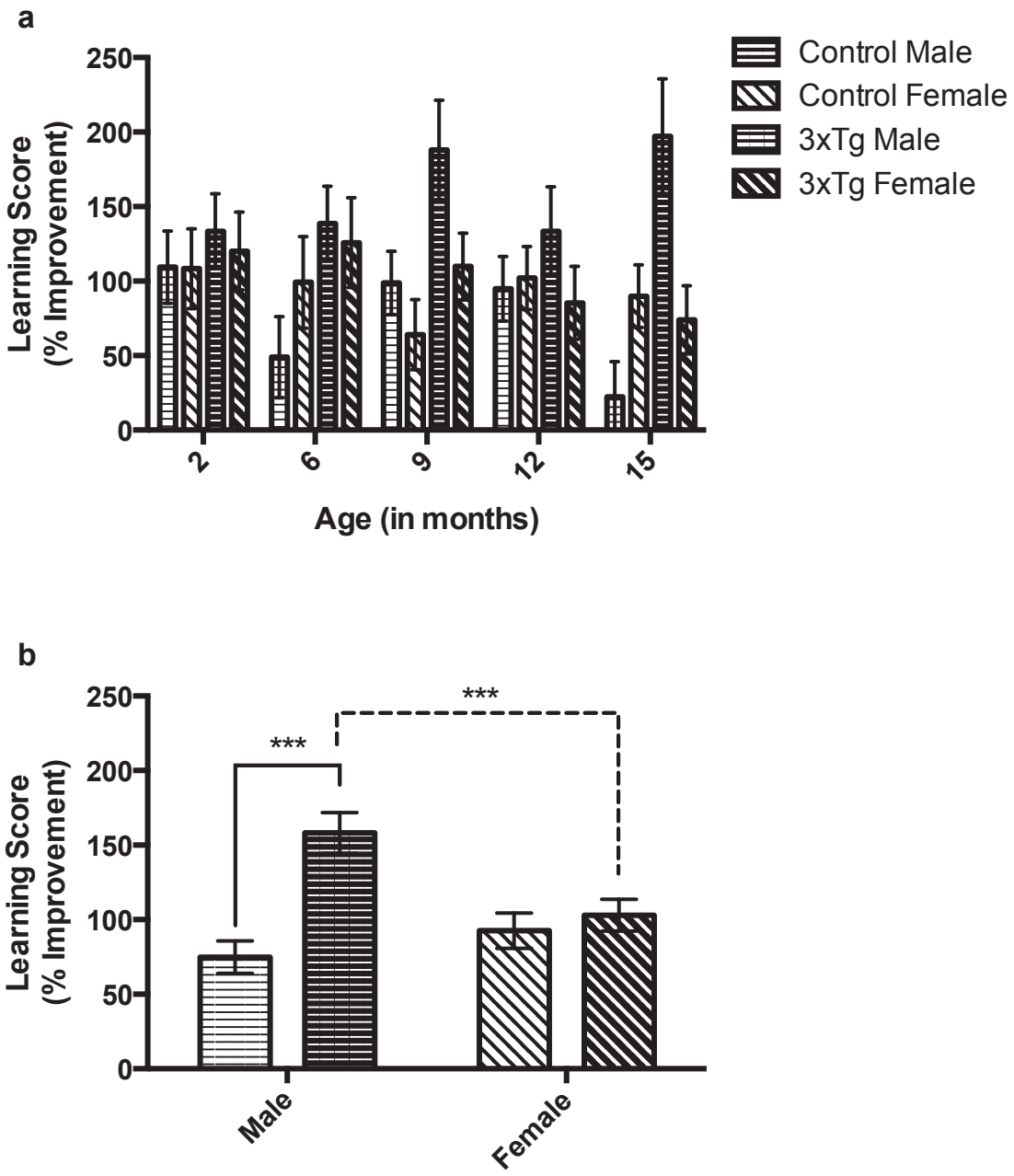


Figure 2.6. Learning score: Percent improvement. (a) Percent improvement (Learning score) for all groups. No effect of age was found, but a main effect of genotype was found for percent improvement, with 3xTg mice showing a higher percent improvement than controls. (b) A significant genotype x sex interaction was also found. Male 3xTg mice had a significantly higher percent improvement than control males, but no difference was found between 3xTg and control females. A significant difference was also found between male and female 3xTg mice but not between male and female controls. * = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 3.1. Mean (± SEM) distance travelled (cm) in the OF by male and female 3xTg and control mice, at each age. (a) A significant main effect of genotype was found. There were no main effects of age or sex on distance travelled. (b) Shows the significant genotype x age interaction. (c) Shows the significant sex x age interaction. * = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 3.1

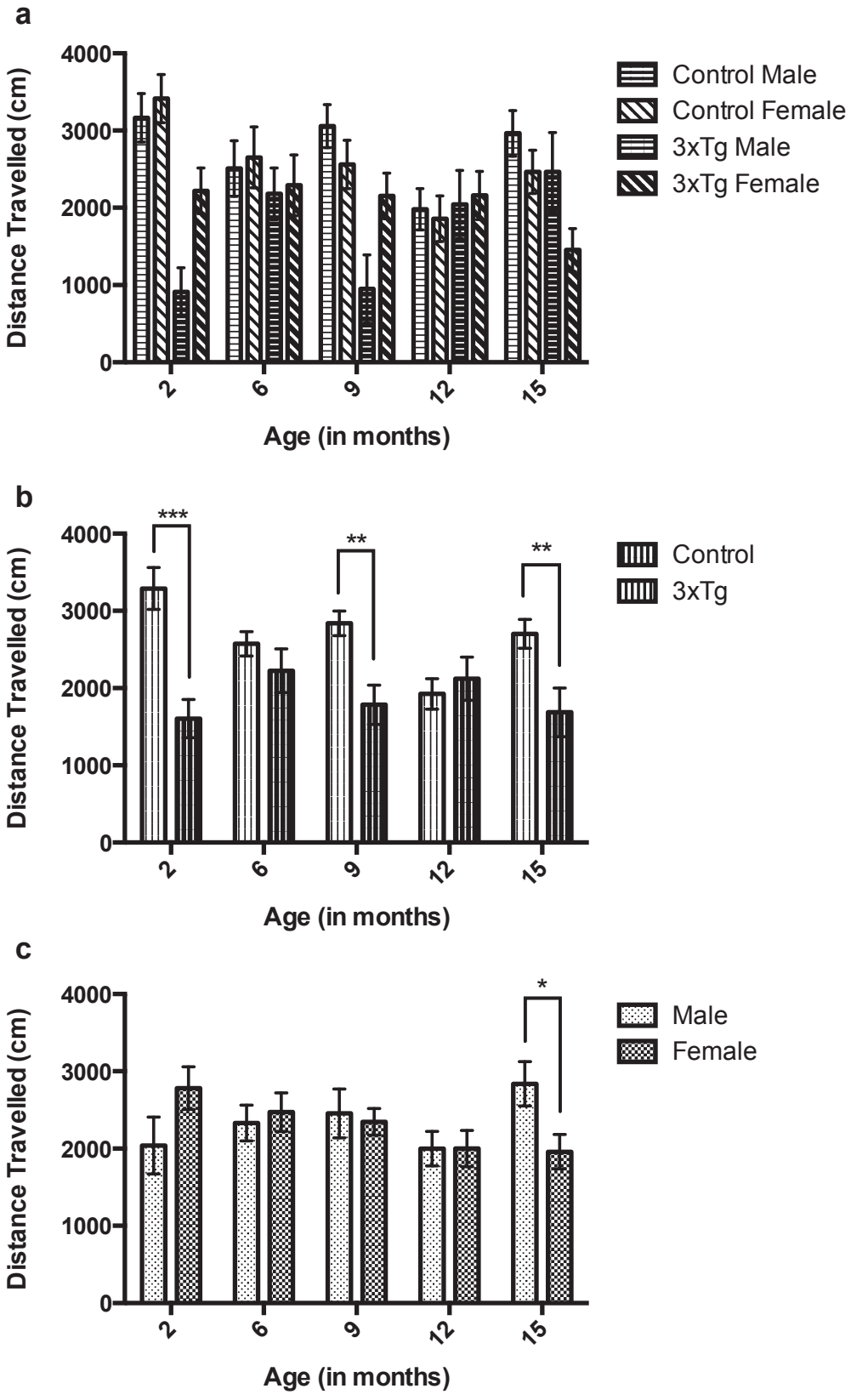
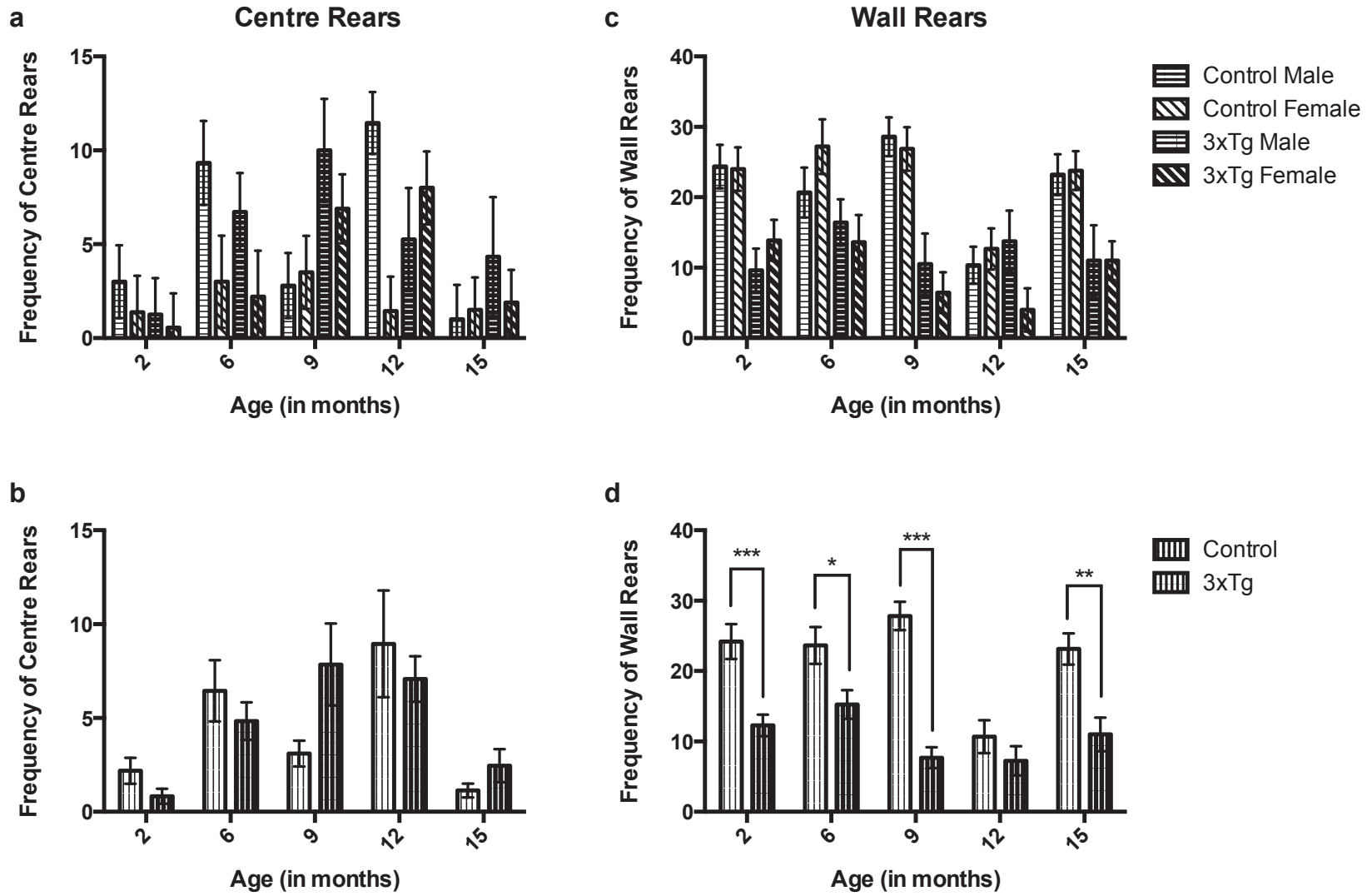

a
Distance Travelled (cm)
4000
3000
2000
1000
0
2
6
9
12
15
Age (in months)
Control Male
Control Female
3xTg Male
3xTg Female
b
Distance Travelled (cm)
***
**
**
Age (in months)
Control
3xTg
c
Distance Travelled (cm)
*
Age (in months)
Male
Female

Figure 3.2. Mean (± SEM) centre and wall rearing in the OF by male and female 3xTg and control mice, at all ages. (a) There was no effect of genotype on the frequency of centre rears. Males were found to engage in more wall rears than females, and there was a significant age effect. There was also a genotype x sex x age interaction for centre rears. (b) Shows the frequency of centre rears collapsed across sex. (c) There was a significant effect of genotype and age on wall rears, but no effect of sex. (d) Shows genotype x age interaction for frequency of wall rears.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 3.2


a
Centre Rears
Frequency of Centre Rears
15
10
5
0
2
6
9
12
15
Age (in months)
c
Wall Rears
Frequency of Wall Rears
40
30
20
10
0
2
6
9
12
15
Age (in months)
Control Male
Control Female
3xTg Male
3xTg Female
b
Frequency of Centre Rears
15
10
5
0
2
6
9
12
15
Age (in months)
d
Frequency of Wall Rears
40
30
20
10
0
***
*
***
**
2
6
9
12
15
Age (in months)
Control
3xTg

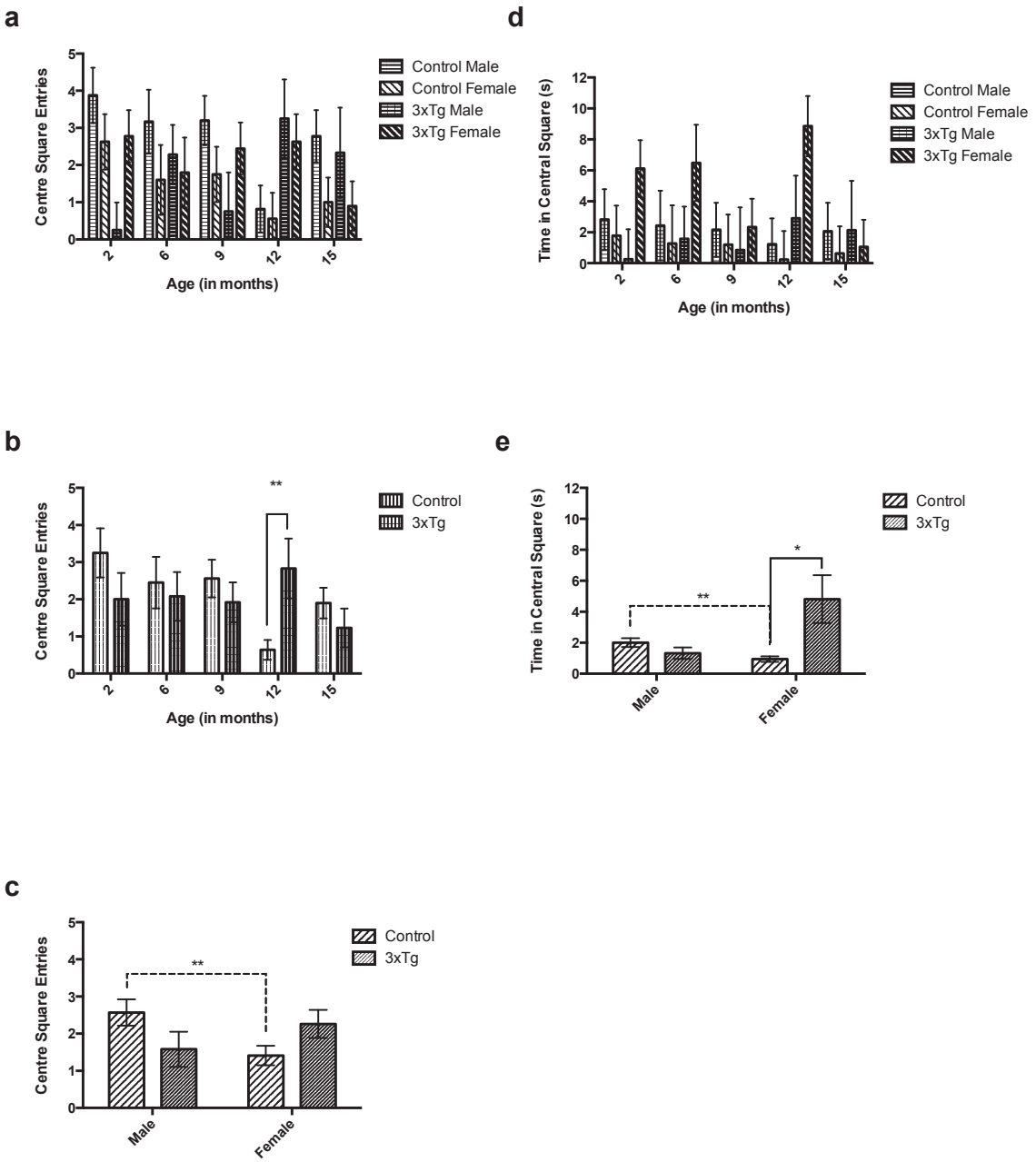


Figure 3.3. Mean (± SEM) entries and time spent in the centre square of the OF by male and female 3xTg and control mice, at all ages. (a) There were no main effects of genotype, sex, or age on entries into the centre square. (b) Shows the genotype x age interaction for entries into the centre square. (c) Shows the genotype x sex interaction for time spent in the centre square. (d) There were no main effects of genotype, sex, or age on time spent in the centre square. (e) Shows the genotype x sex interaction for time spent in the centre square.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

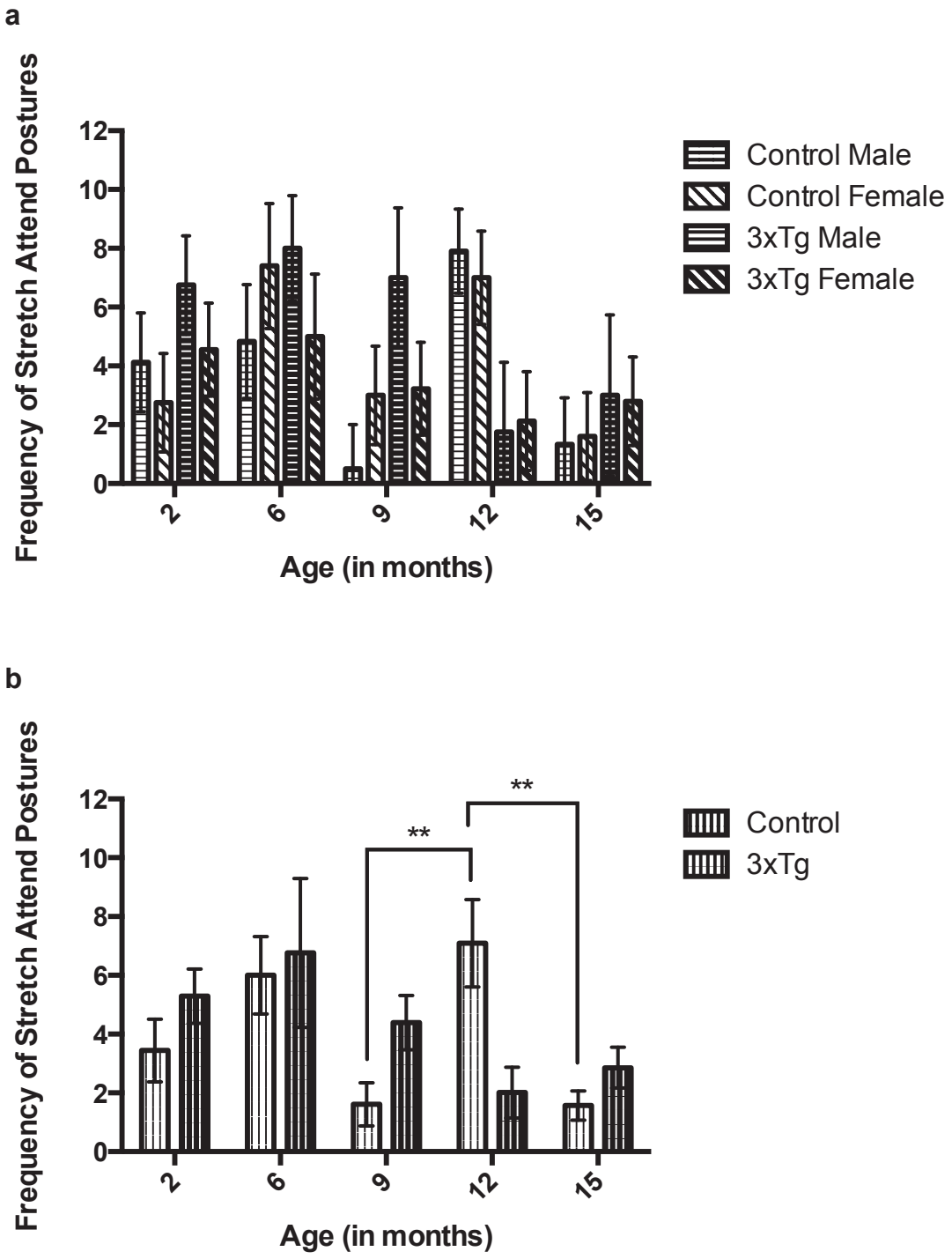
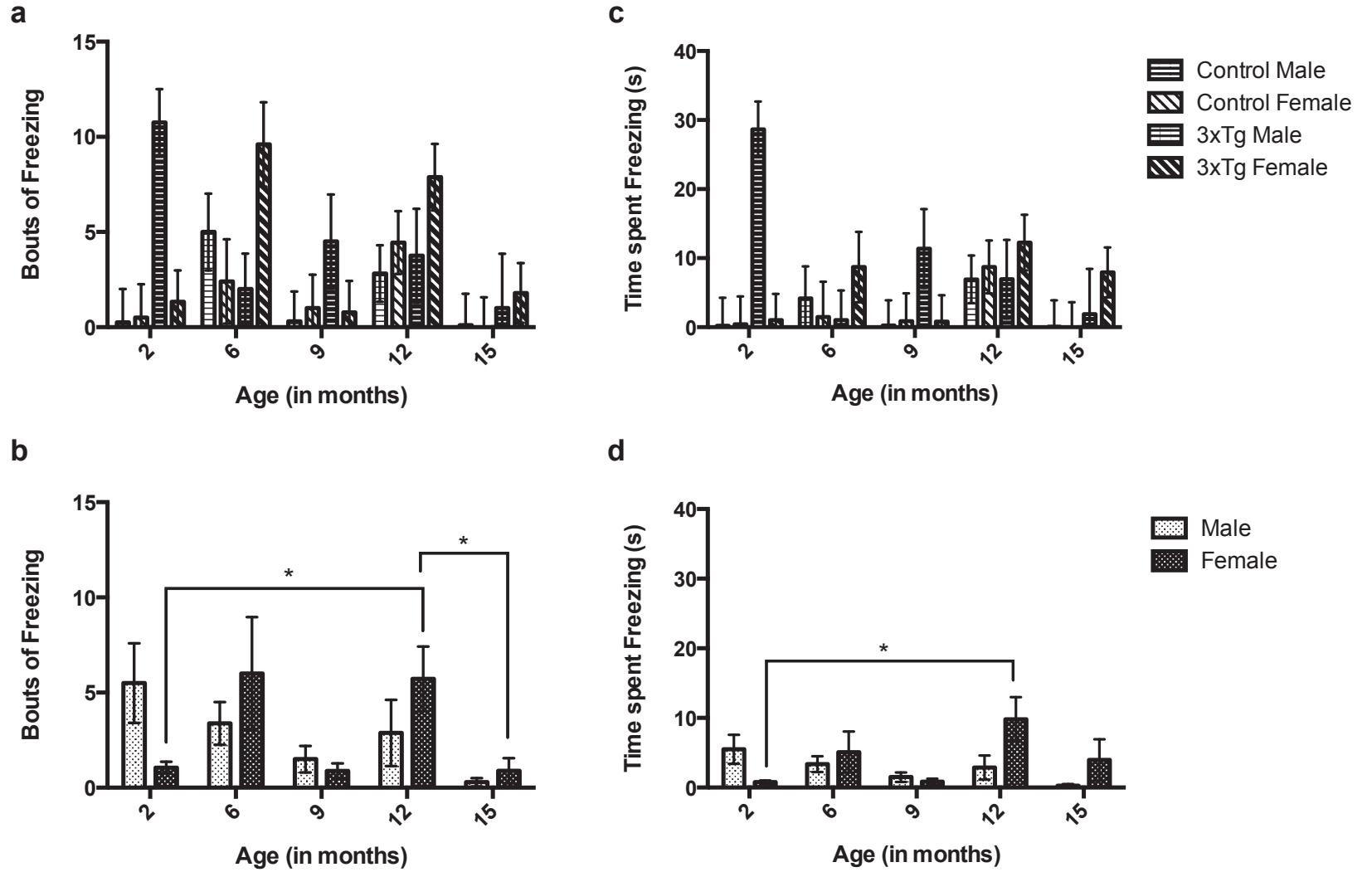


Figure 3.4. Mean (± SEM) frequency of stretch attend postures (SAPs) by male and female 3xTg and control mice at all ages. (a) There were no main effects of genotype or sex on SAPs, however there was a significant effect of age. (b) Shows the genotype x age interaction for frequency of SAPs.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 3.5. Mean (± SEM) frequency of freezing and time spent freezing (s) in the OF by male and female 3xTg and control mice, at all ages. (a) Genotype and age were both found to have significant effects on frequency of freezing, however sex did not have a significant effect. (b) Shows the age effect and sex x age interaction for bouts of freezing. (c) Genotype was also found to have a significant effect on time spent freezing, however there were no significant effects of sex or age. (d) Shows the sex x age interaction for time spent freezing.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 3.5


a
Bouts of Freezing
15
10
5
0
2
6
9
12
15
Age (in months)
c
Time spent Freezing (s)
40
30
20
10
0
Age (in months)
Control Male
Control Female
3xTg Male
3xTg Female
b
Bouts of Freezing
*
*
Age (in months)
d
Time spent Freezing (s)
*
Age (in months)
Male
Female

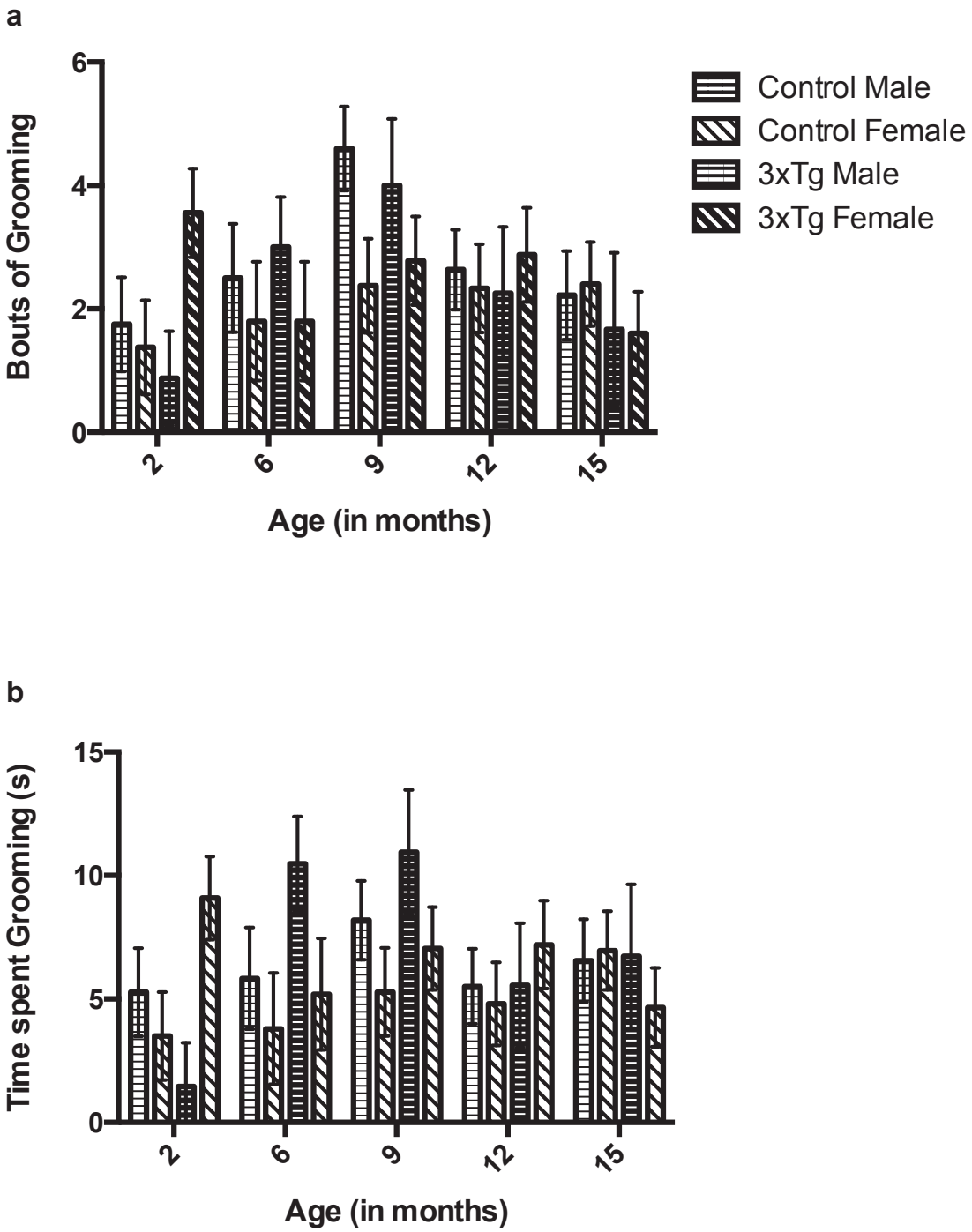


Figure 3.6. Mean (± SEM) frequency and duration of grooming in the OF by male and female 3xTg and control mice, at all ages. (a) Frequency of bouts of grooming. There were no significant effects of genotype, sex, or age. (b) Time spent grooming (s). There were no significant effects of genotype, sex, or age.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

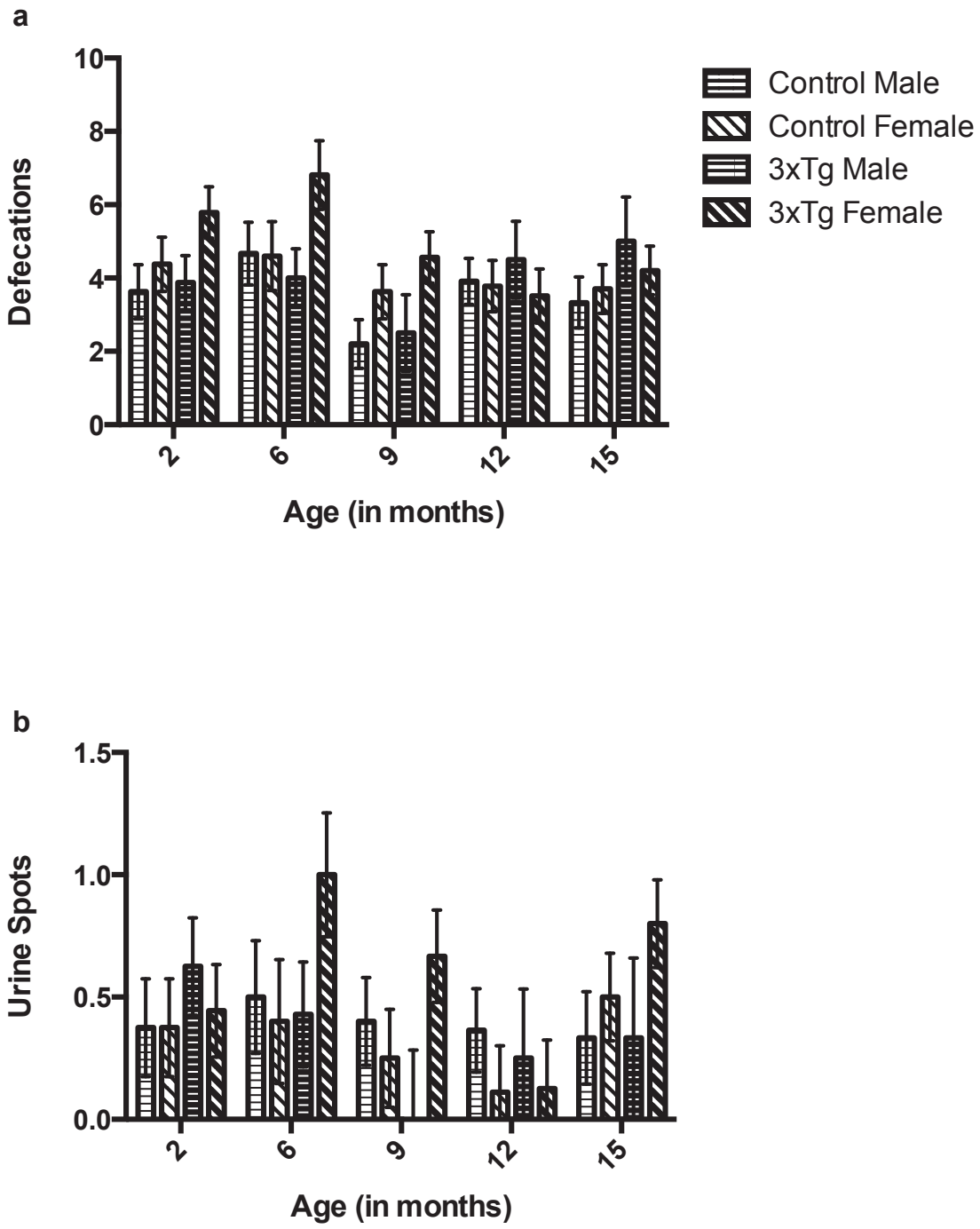


Figure 3.7. Mean (± SEM) number of defecations (a) and number of urine spots (b) in the OF by male and female 3xTg and control mice, at all ages. There were no significant effects of genotype, sex, or age for either defecations or number of urine spots. * = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

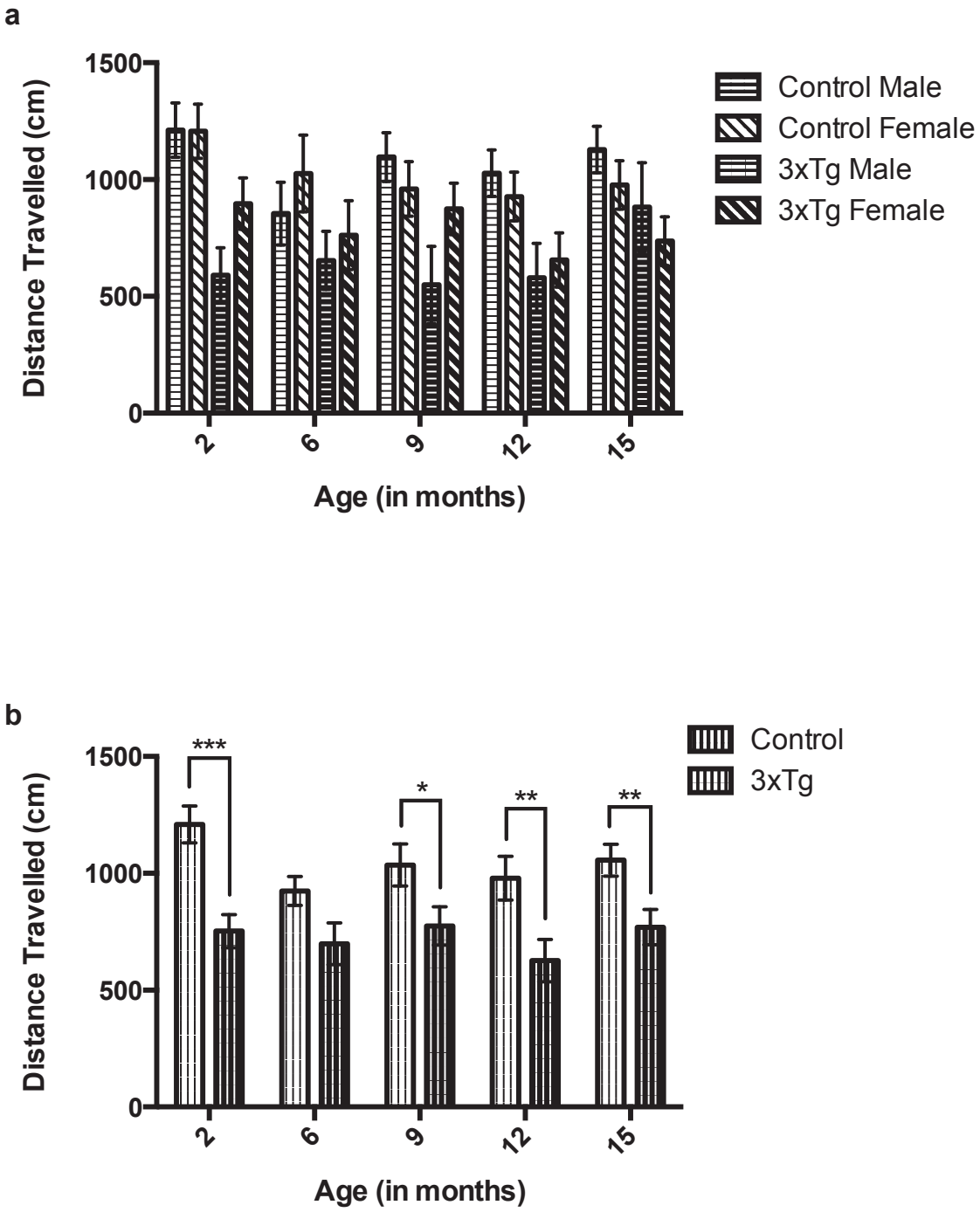


Figure 3.8. Mean (± SEM) distance travelled (cm) in the EPM by male and female 3xTg and control mice, at all ages. (a) There was a significant main effect of genotype, but not of sex or age. (b) Shows genotype effect by age (data is pooled across sex). * = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

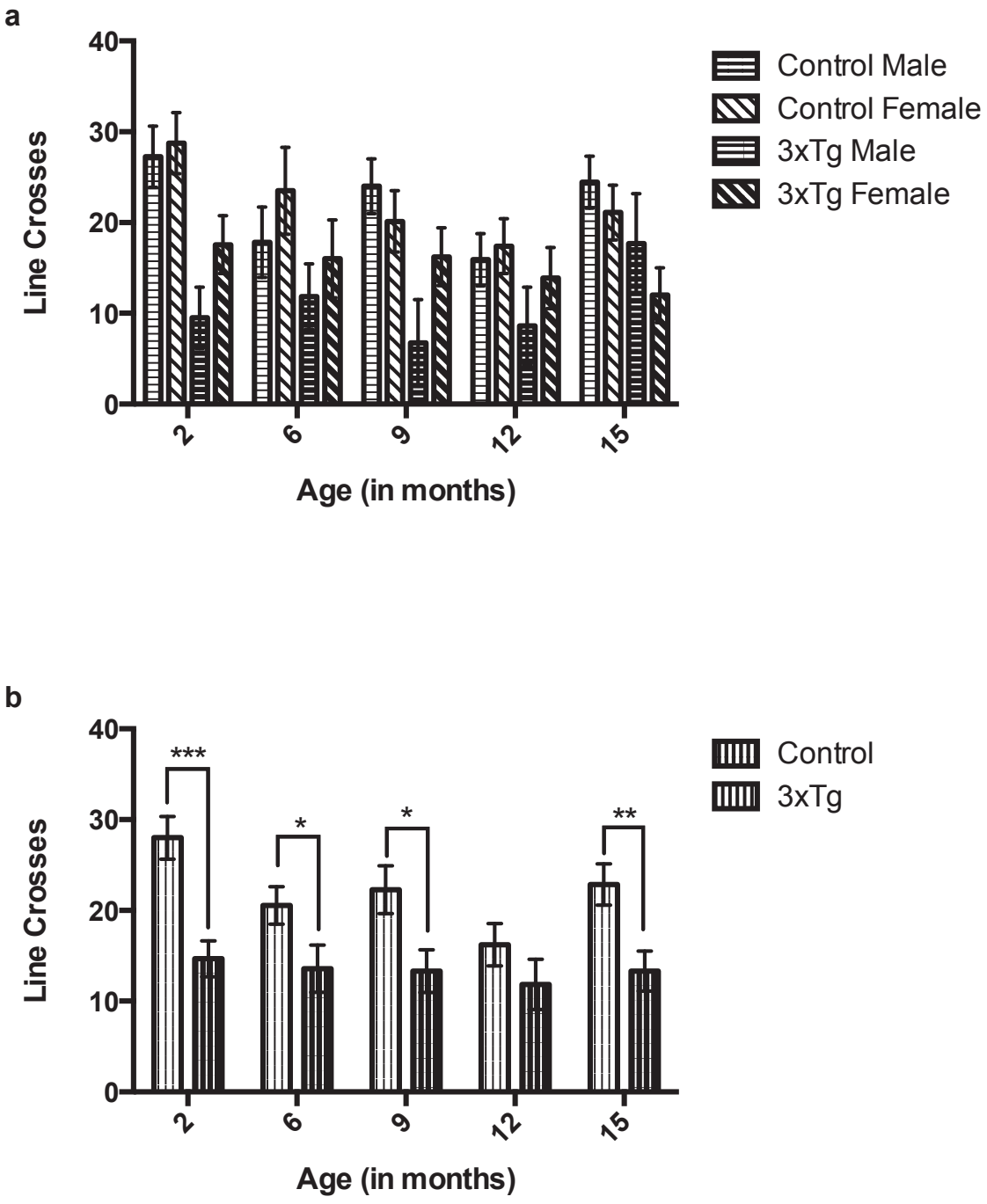


Figure 3.9. Mean (± SEM) line crosses in the EPM by male and female 3xTg and control mice, at all ages. (a) There was a significant genotype effect, but no effect of sex or age. (b) Shows the genotype effect by age (data pooled across sex).
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

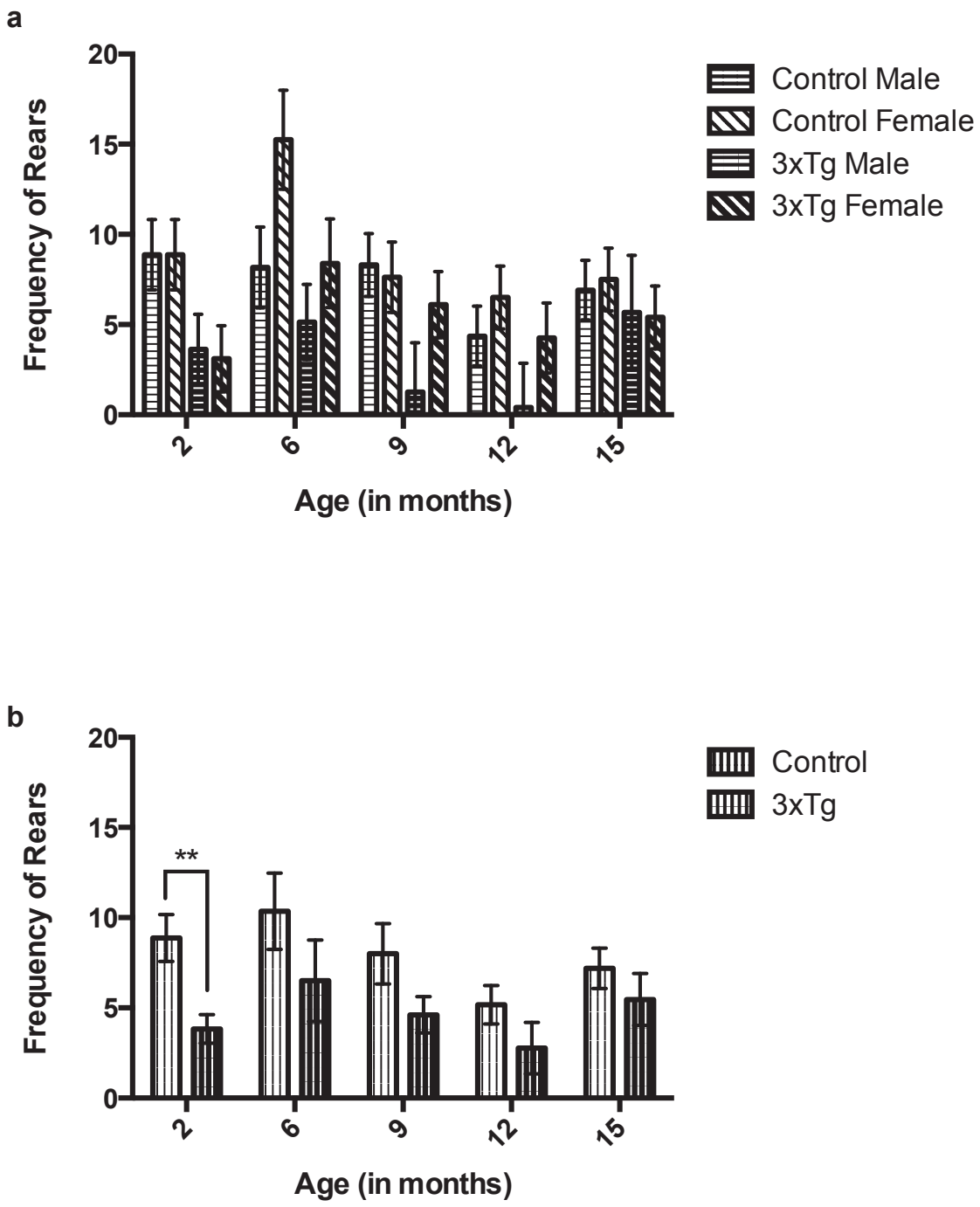


Figure 3.10. Mean (± SEM) frequency of rearing in the EPM by male and female 3xTg and control mice, at all ages. (a) Genotype, sex, and age all had significant main effects on the data. (b) Shows the genotype and age effects (data is pooled across sex). * = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

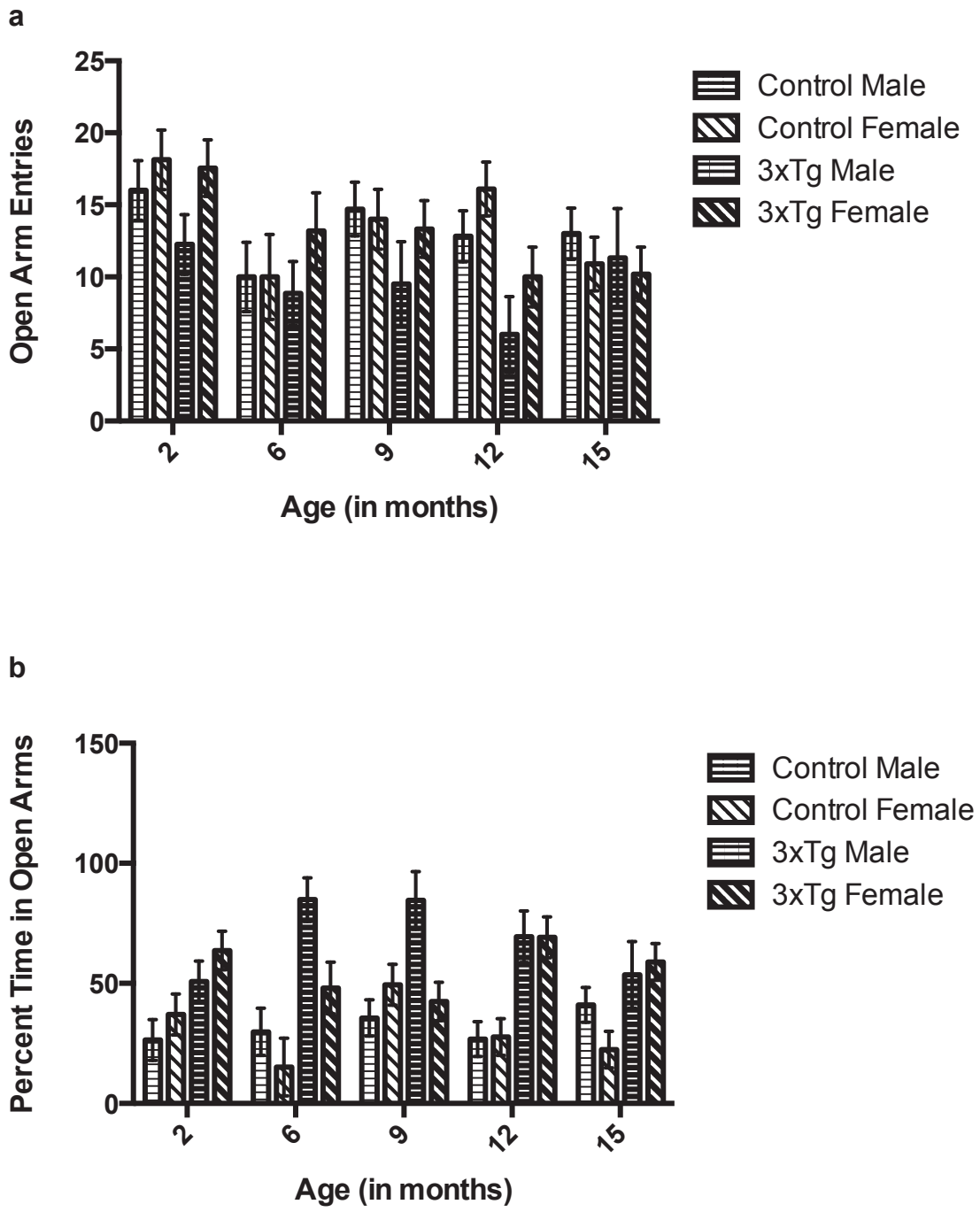
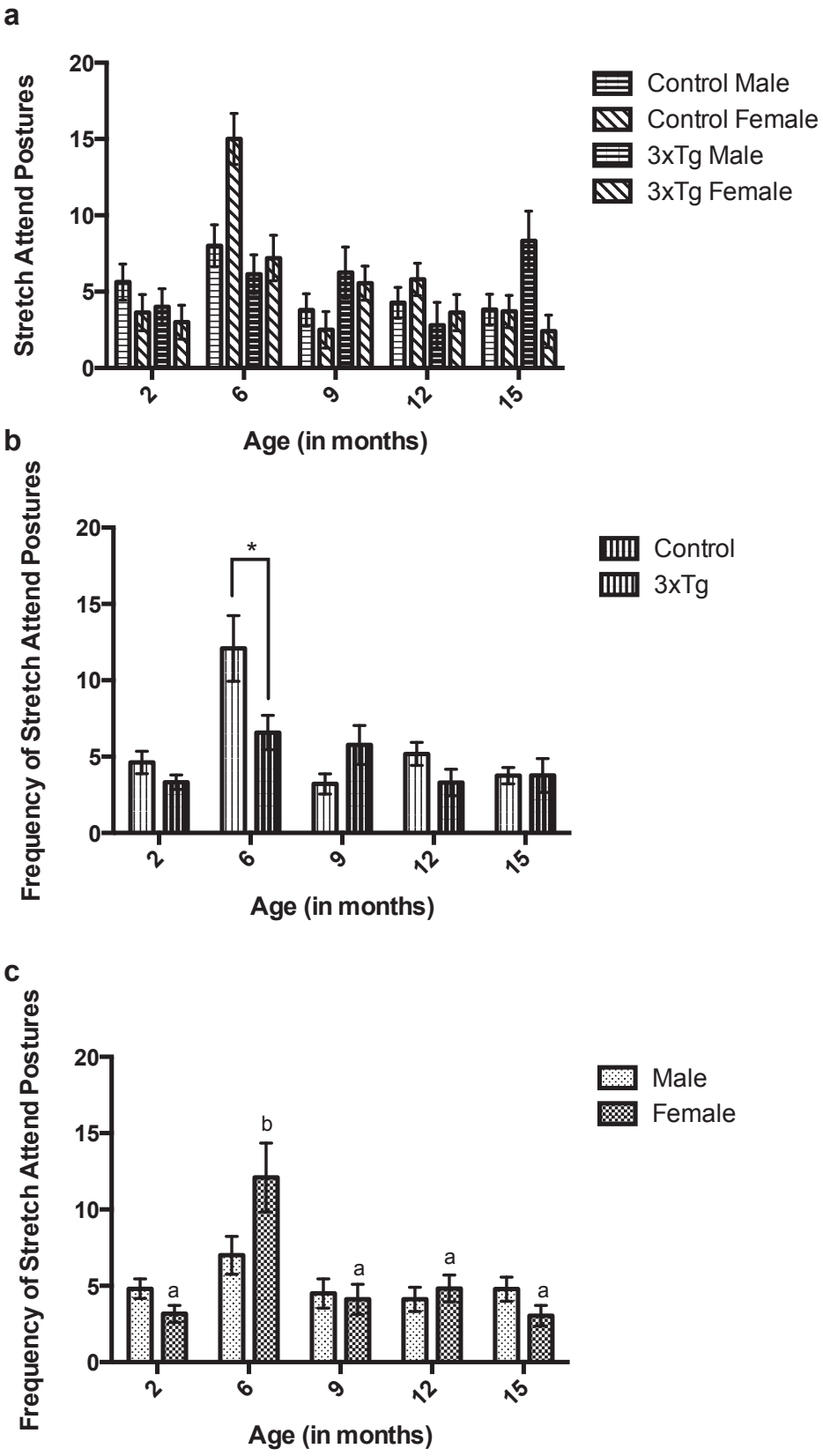


Figure 3.11. Mean (± SEM) open arm entries and percentage of time spent in the open arms in the EPM, by male and female 3xTg and control mice, at all ages. (a) There was a significant effect of genotype and age on open arm entries, but no effect of sex. (b) There was a significant effect of genotype on the percentage of time spent in the open arms, but no effects of either sex or age.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 3.12. Mean (± SEM) frequency of stretch attend postures (SAPs) in the EPM, by male and female 3xTg and control mice, at all ages. (a) There were no significant effects of either genotype or sex on SAPs, however there was a significant effect of age. (b) Shows the effect of age and the genotype x age interaction. (c) Shows the age effect and the sex x age interaction.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 3.12


a
Stretch Attend Postures
20
15
10
5
0
2
6
9
12
15
Age (in months)
Control Male
Control Female
3xTg Male
3xTg Female
b
Frequency of Stretch Attend Postures
*
Age (in months)
Control
3xTg
c
Frequency of Stretch Attend Postures
a
b
a
a
a
Age (in months)
Male
Female

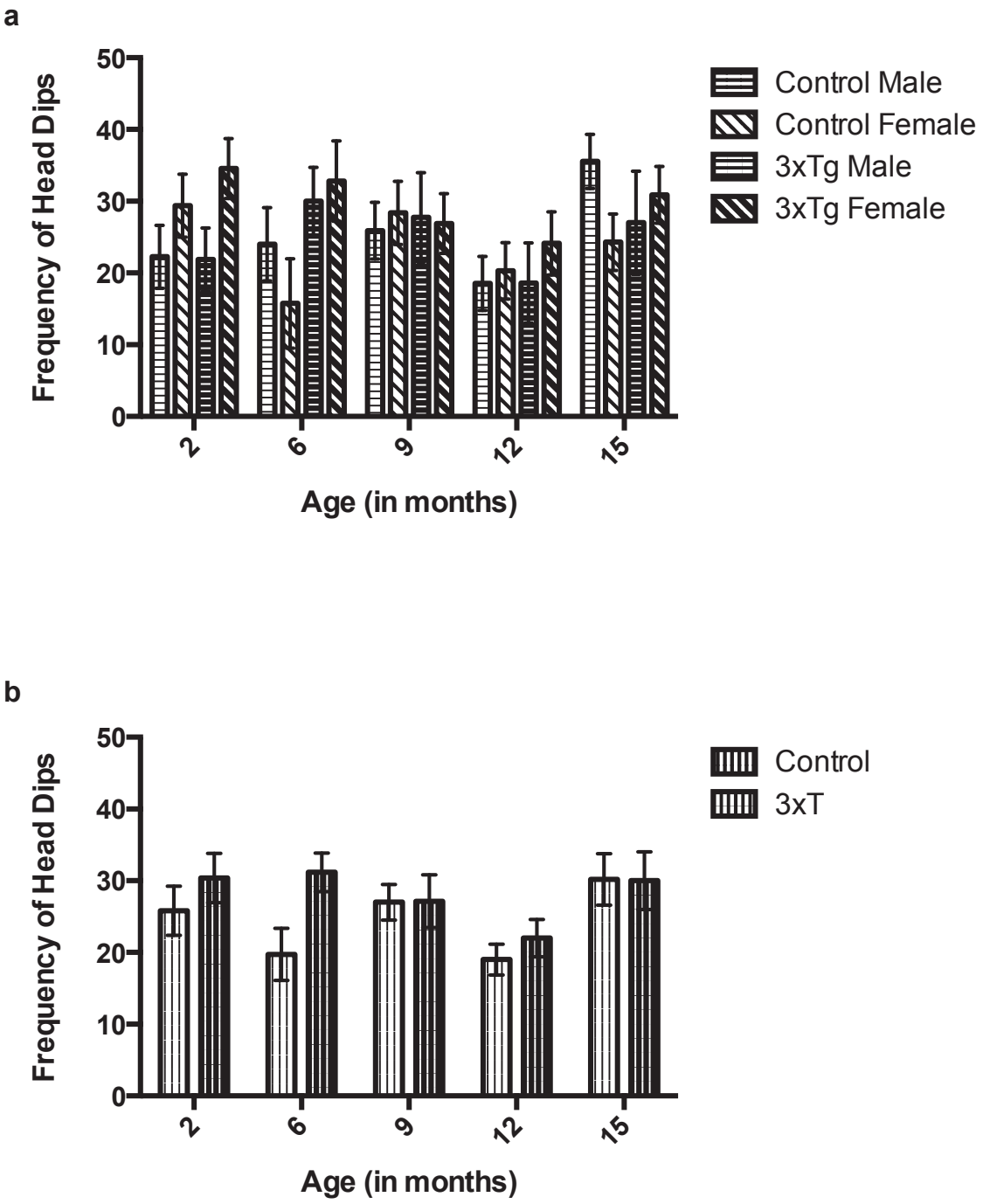


Figure 3.13. Mean (± SEM) frequency of head dips in the EPM by male and female 3xTg and control mice, at all ages. (a) There were no significant effects of genotype, sex, or age. (b) Shows the data pooled across sex.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

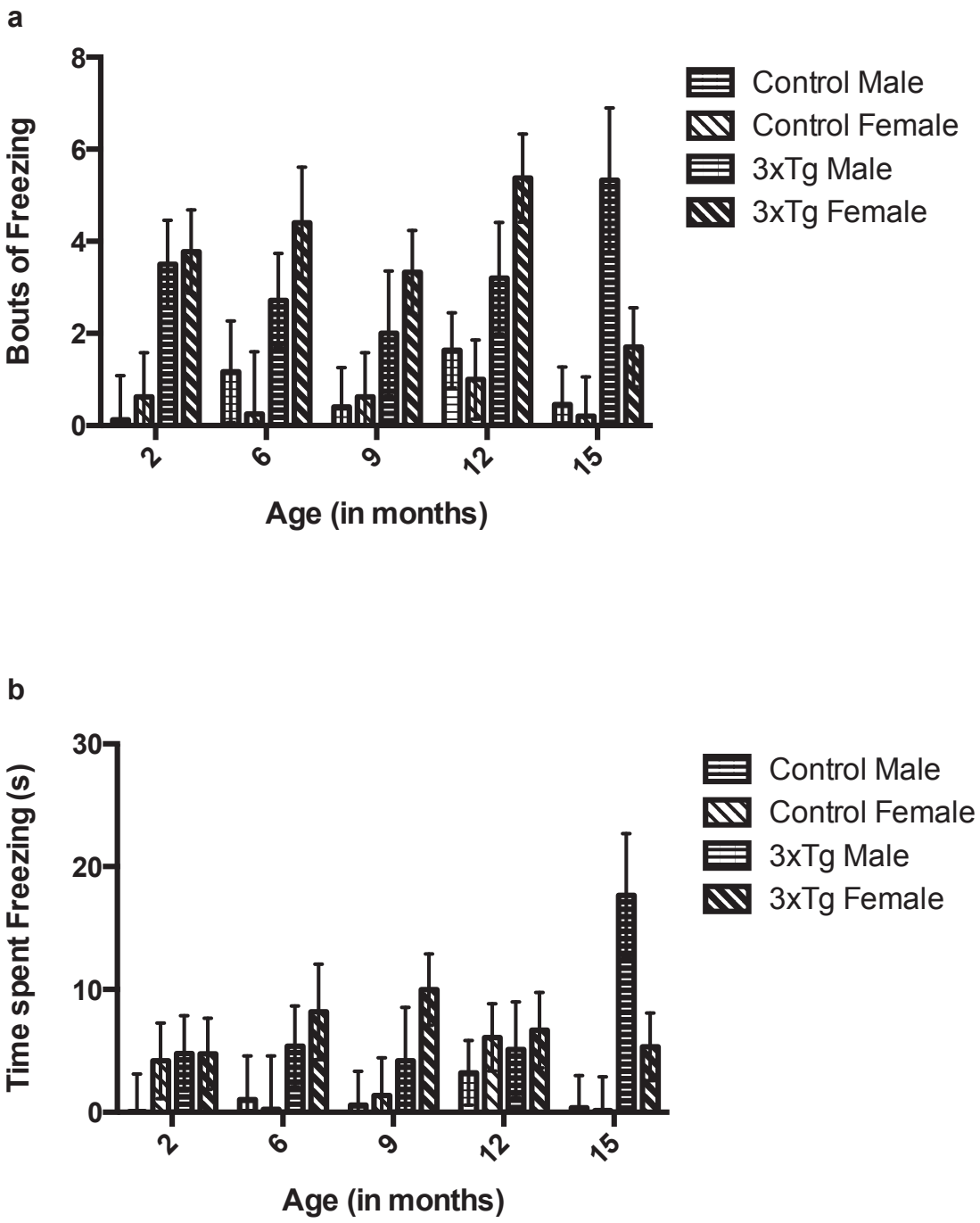


Figure 3.14. Mean (± SEM) frequency and time spent freezing (s) in the EPM by male and female 3xTg and control mice, at all ages. (a) There was a significant effect of genotype on bouts of freezing, however there were no significant effects of sex or age on bouts of freezing. (b) There was a significant effect of genotype on the time spent freezing, however there were no effects of sex or age on time spent freezing. * = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

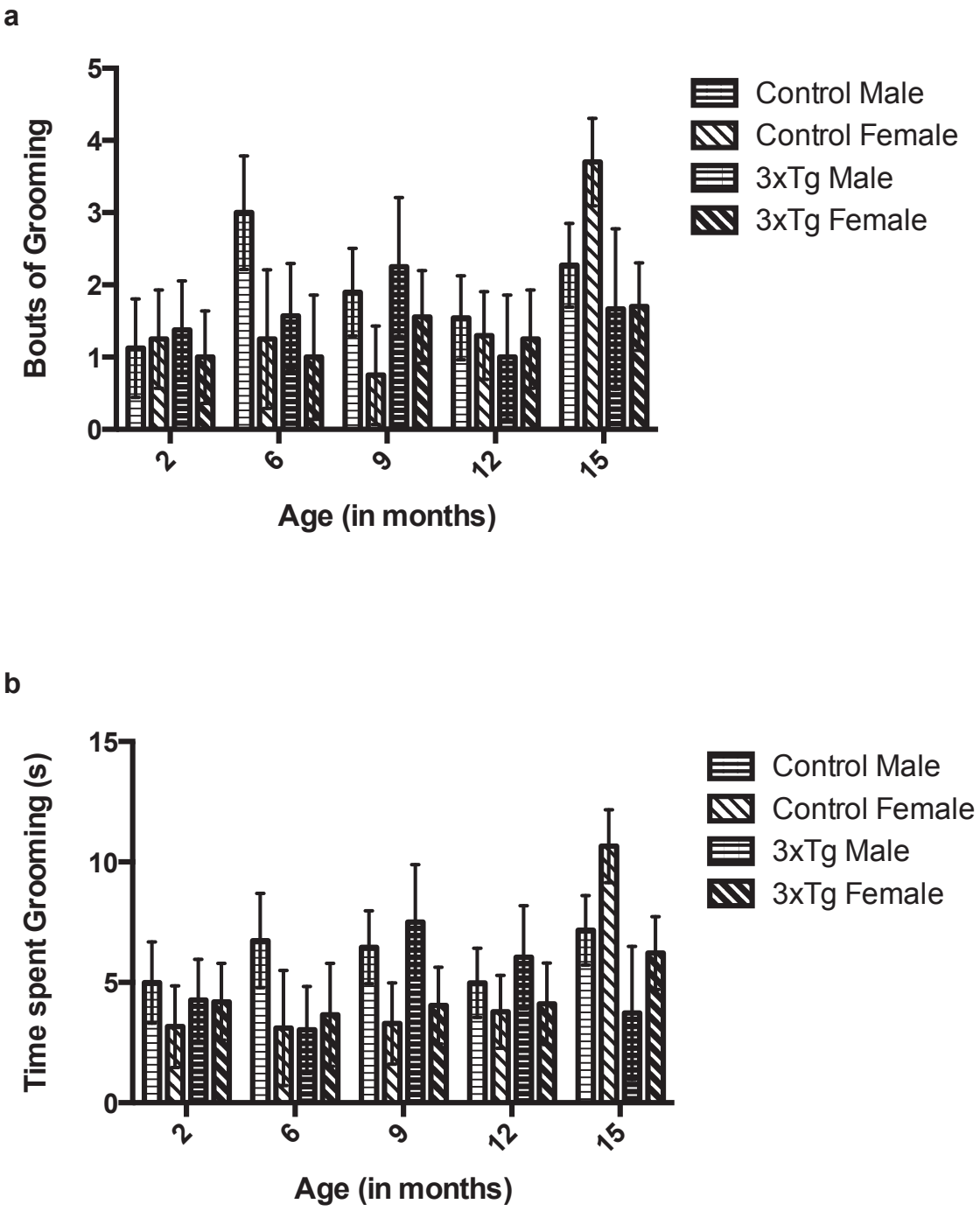


Figure 3.15. Mean (± SEM) frequency (a) and time spent (s) grooming in the EPM by male and female 3xTg and control mice, at all ages. There were no effects of genotype, sex, or age on either frequency of grooming or time spent grooming.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

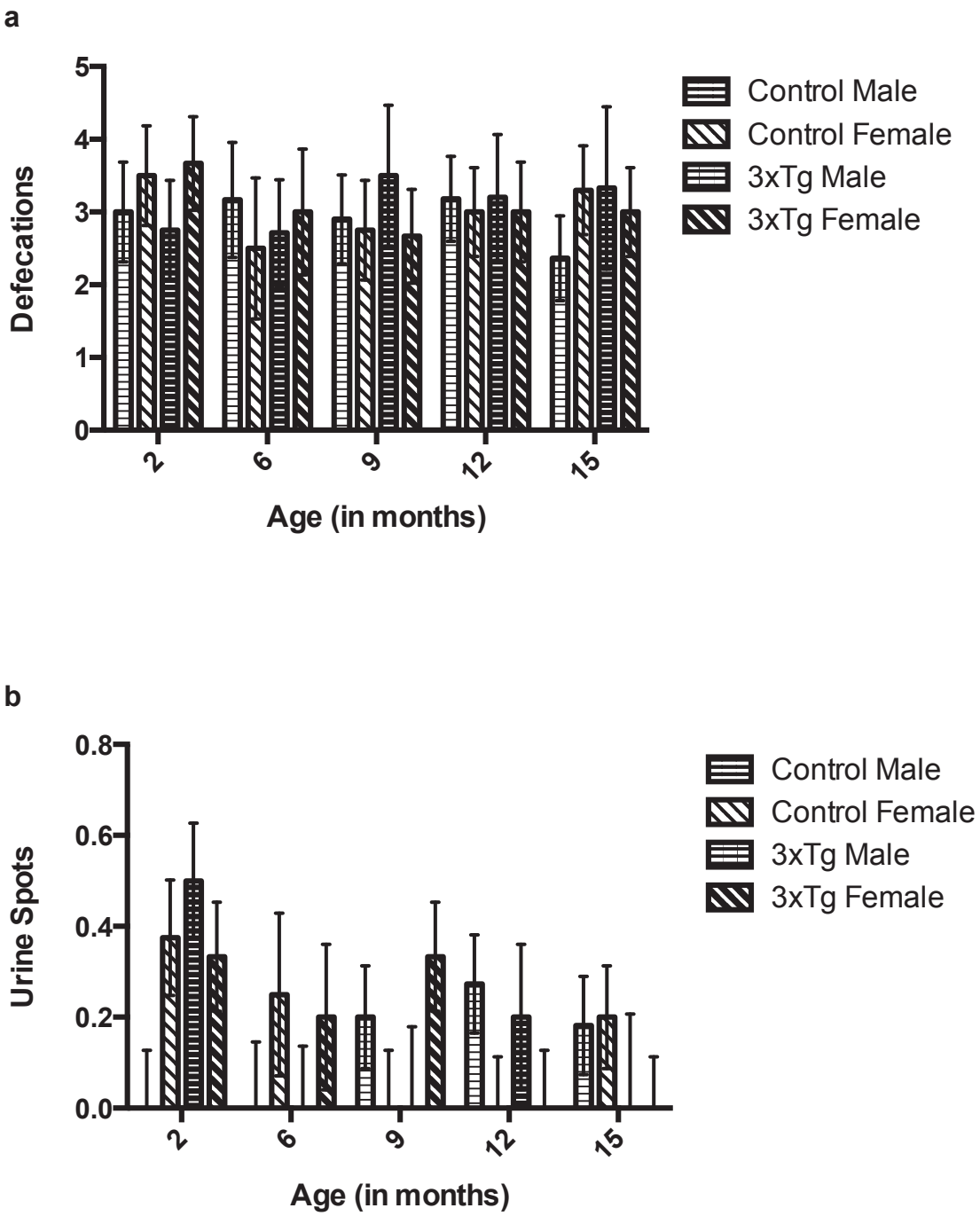


Figure 3.16. Mean (± SEM) defecations (a) and number of urine spots (b) in the EPM by male and female 3xTg and control mice, at all ages. There were no significant effects of genotype, sex, or age on either defecations or urine spots.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 3.17. Mean (± SEM) frequency and duration (s) of immobilization in the FST by male and female 3xTg and control mice, at all ages. (a) There were significant effects of genotype, sex, and age on the frequency of immobilization. (b) Shows the effects of genotype and age on frequency of immobilization, as well as the genotype x age interaction (data is pooled across sex). (c) Shows the genotype and sex effects on frequency of immobilization, as well as the genotype x sex interaction (data is pooled across ages). (d) There were also significant effects of genotype and sex on duration of immobility, however no effect of age. (e) Shows the genotype effect at each age (data is pooled across sex) for duration of immobilization. (f) Shows the effects of genotype and sex on the duration of immobilization (data is pooled across ages).
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 3.17

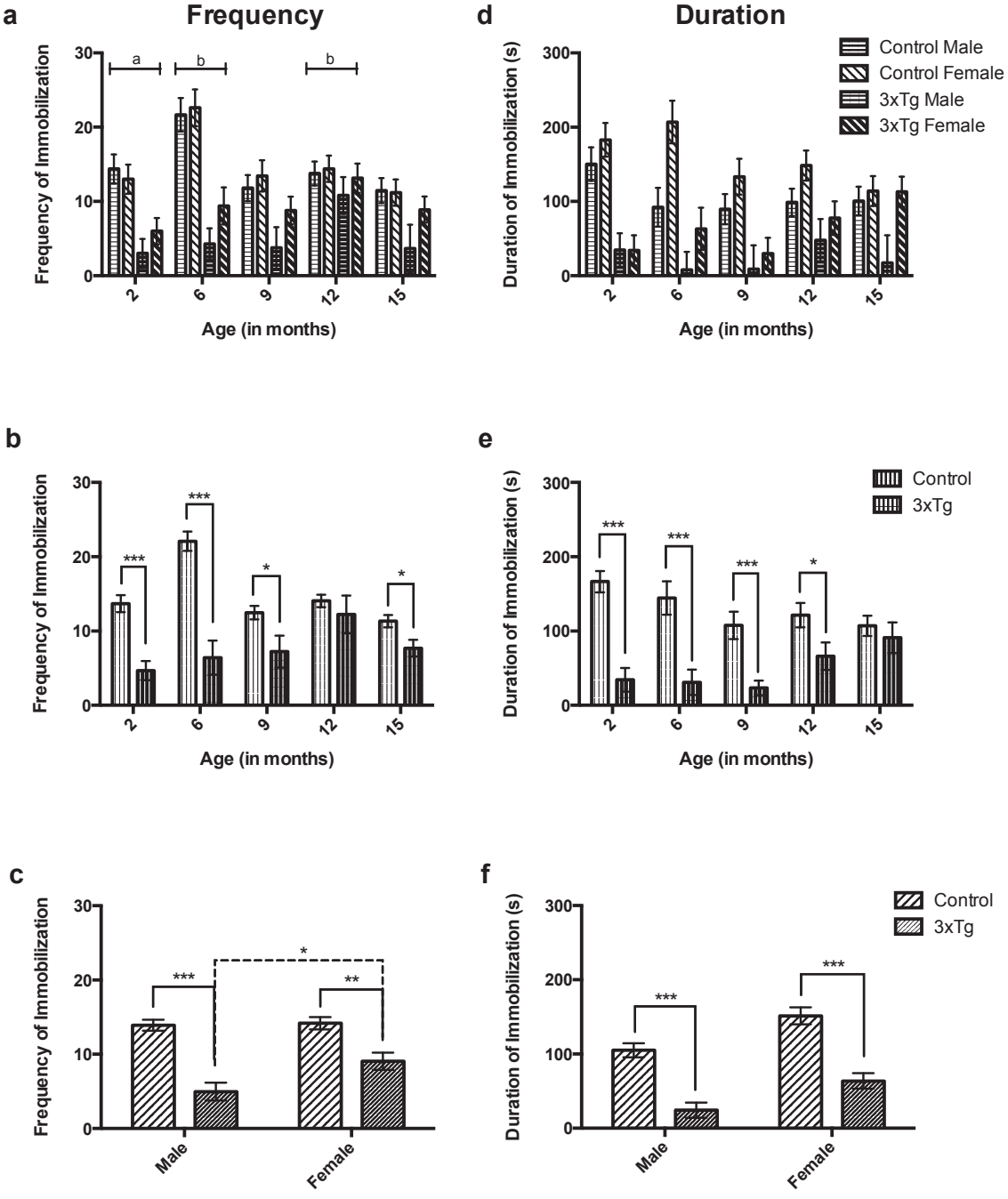
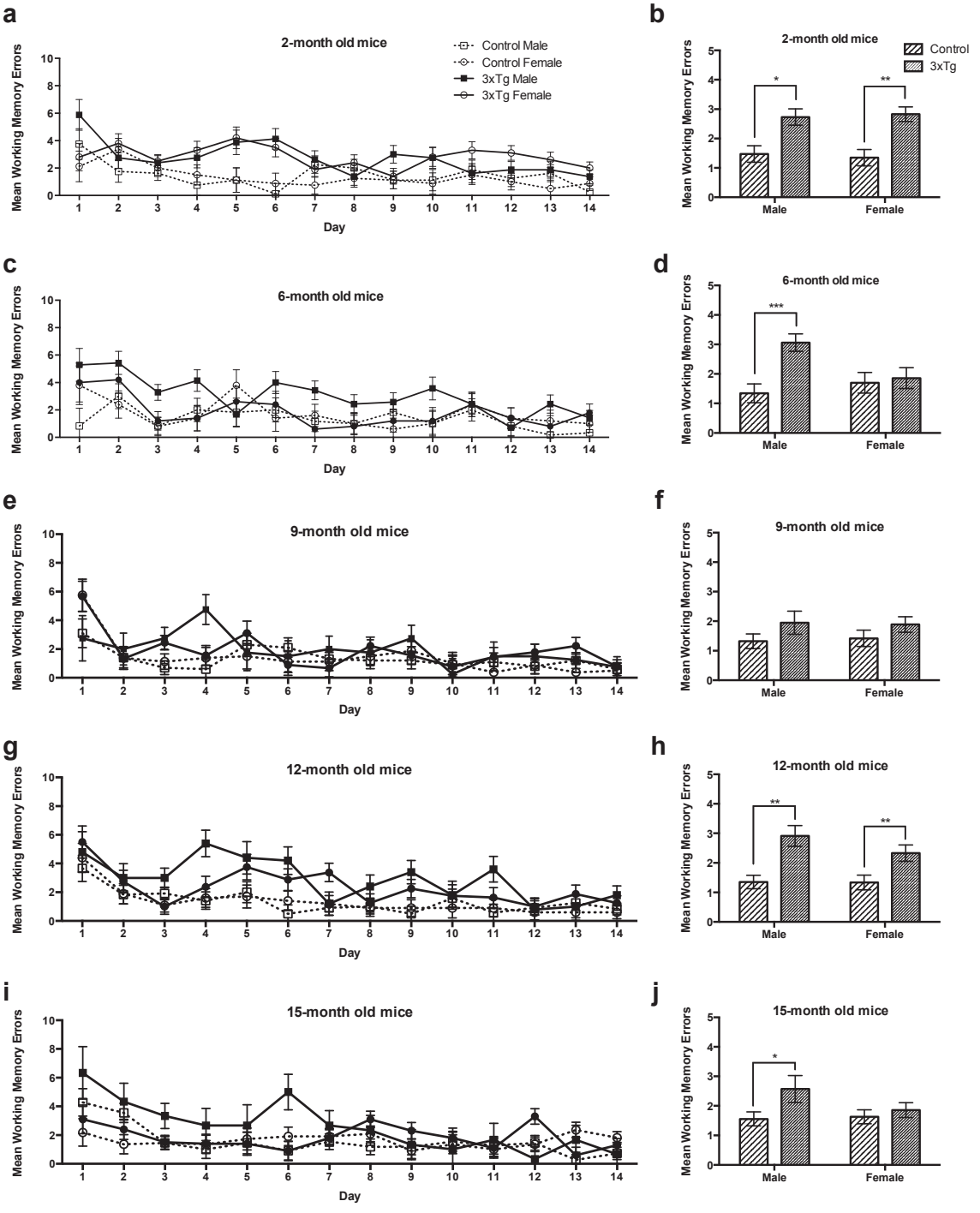

a
Frequency
Frequency of Immobilization
a
b
b
Age (in months)
d
Duration
Duration of Immobilization (s)
Control Male
Control Female
3xTg Male
3xTg Female
Age (in months)
b
Frequency of Immobilization
Age (in months)
e
Duration of Immobilization (s)
Control
3xTg
Age (in months)
c
Frequency of Immobilization
Male
Female
f
Duration of Immobilization (s)
Control
3xTg
Male
Female

Figure 4.1. Mean (± SEM) working memory errors across 14 days of training (a,c,e,g,i), and pooled across all 14 days (b,d,f,h,j) for control male, control female, 3xTg male, and 3xTg female mice at the five different age cohorts: (a,b) 2-months of age; (c,d) 6-months of age; (e,f) 9-months of age; (g,h) 12-months of age; (i,j) 15-months of age. A significant genotype effect was found, with 3xTg mice making more working memory errors than control mice. There was also a significant effect of day, with working memory errors deceasing over the 14 days of training.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 4.1


a
2-month old mice
Control Male
Control Female
3xTg Male
3xTg Female
Mean Working Memory Errors
Day
b
2-month old mice
Control
3xTg
Mean Working Memory Errors
Male
Female
c
6-month old mice
d
6-month old mice
e
9-month old mice
f
9-month old mice
g
12-month old mice
h
12-month old mice
i
15-month old mice
j
15-month old mice

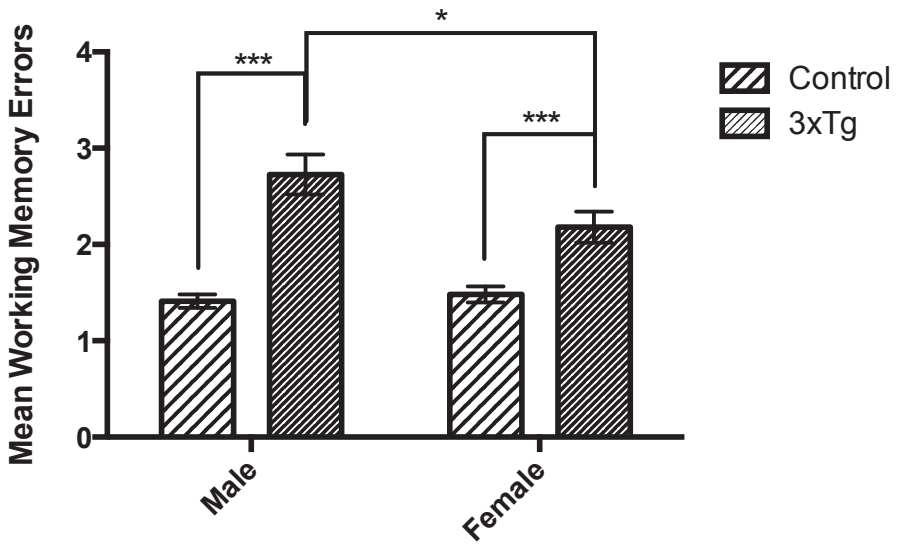


Figure 4.2. Mean (± SEM) working memory errors collapsed across all 14 days of training and at all ages. A significant genotype x sex interaction indicated that 3xTg males made more working memory errors than control males, and 3xTg females made more working memory errors than control females. The 3xTg males also made significantly more working memory errors than 3xTg females, however control male and female mice did not differ in the number of errors.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 4.3. Mean (± SEM) working memory errors with days grouped into bins: (a, d) bin 1 = Days 1-4; (b, e) bin 2 = Days 6-9; and (c, f) bin 3 = Days 11-14. (a-c) The 3xTg mice made significantly more working memory errors than control mice in all three bins. (a, b) Male mice made more errors than female mice in bins 1 and 2. (d-f) A significant genotype x sex interaction was found for bins 1 and 2, with 3xTg males making more errors than 3xTg females, but no difference between control male and female mice.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 4.3

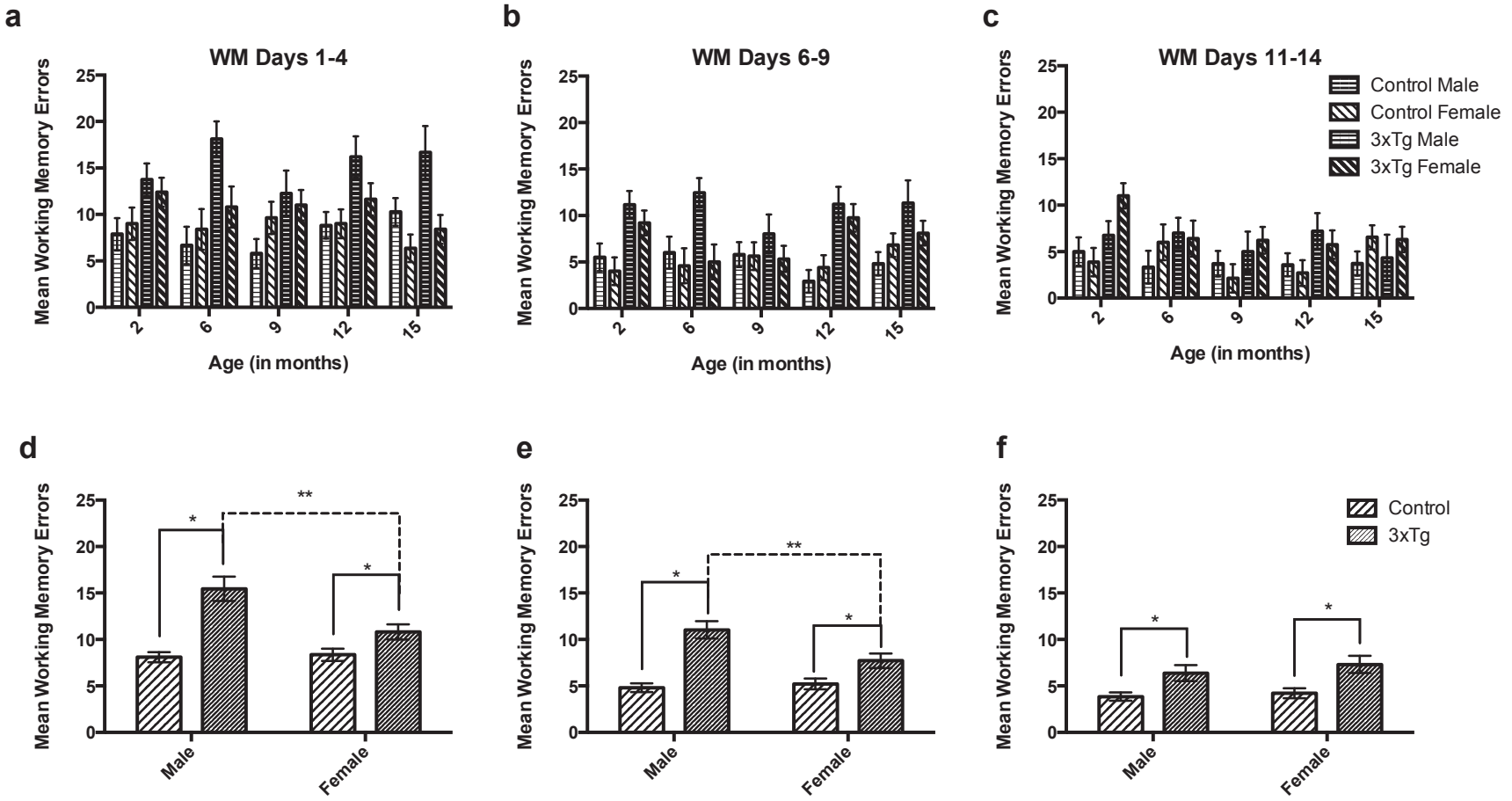

a
WM Days 1-4
Mean Working Memory Errors
Age (in months)
b
WM Days 6-9
Mean Working Memory Errors
Age (in months)
c
WM Days 11-14
Mean Working Memory Errors
Age (in months)
Control Male
Control Female
3xTg Male
3xTg Female
d
Mean Working Memory Errors
Male
Female
e
Mean Working Memory Errors
Male
Female
f
Mean Working Memory Errors
Control
3xTg
Male
Female

Figure 4.4. Mean (± SEM) reference memory errors across 14 days of training (a,c,e,g,i), and pooled across all 14 days (b,d,f,h,j) for control male, control female, 3xTg male, and 3xTg female mice at the five different age cohorts: (a,b) 2-months of age; (c,d) 6-months of age; (e,f) 9-months of age; (g,h) 12-months of age; (i,j) 15-months of age. A significant genotype effect was found, with 3xTg mice making more reference memory errors than control mice. There was also a sex effect, with male mice making more reference memory errors than female mice. Finally, there was a significant effect of day, with reference memory errors deceasing over the 14 days of training.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 4.4

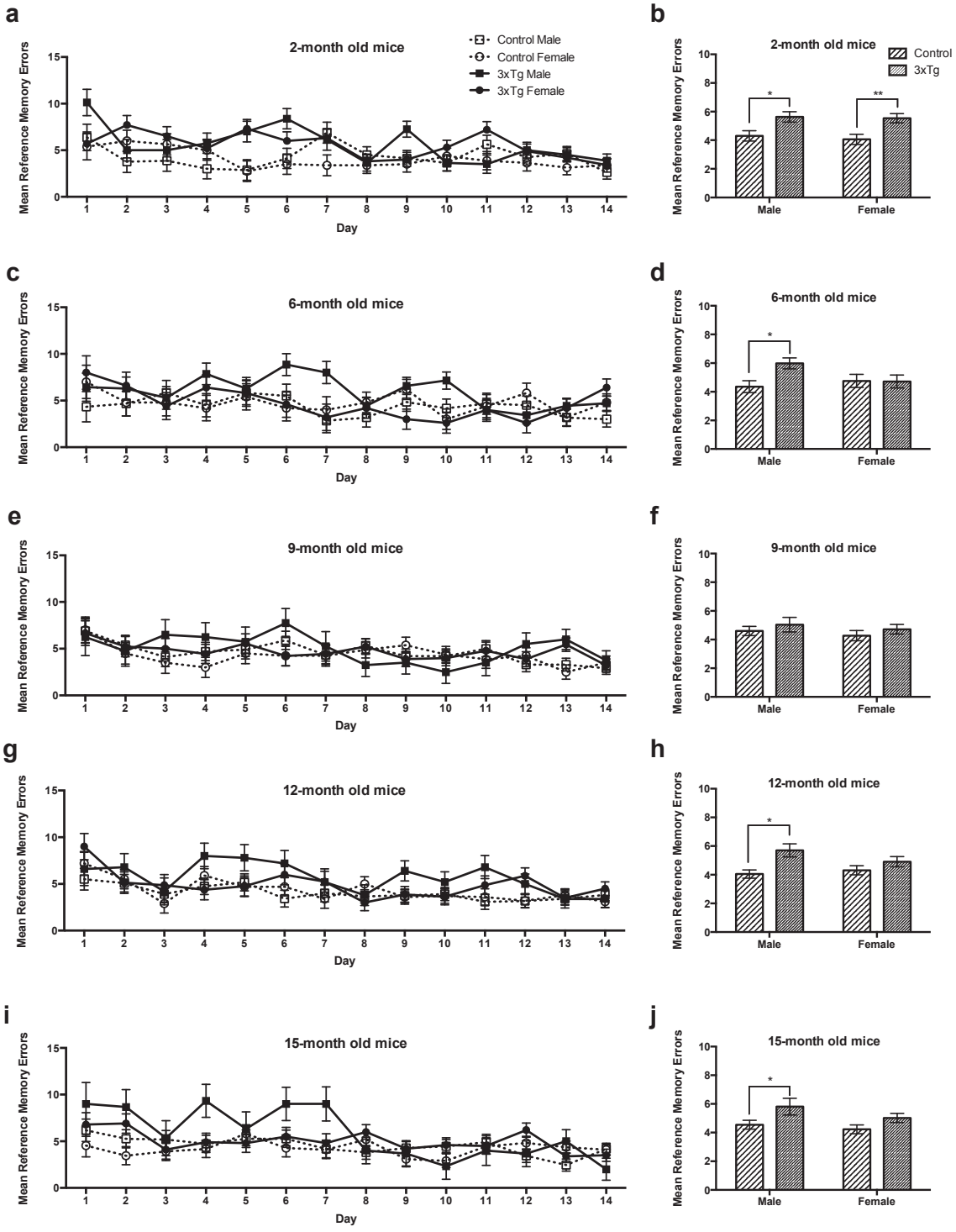

a
2-month old mice
Control Male
Control Female
3xTg Male
3xTg Female
Mean Reference Memory Errors
Day
b
2-month old mice
Control
3xTg
Mean Reference Memory Errors
Male
Female
c
6-month old mice
d
6-month old mice
e
9-month old mice
f
9-month old mice
g
12-month old mice
h
12-month old mice
i
15-month old mice
j
15-month old mice

Figure 4.5. Mean (± SEM) reference memory errors with days grouped into bins: (a, d) bin 1 = Days 1-4; (b, e) bin 2 = Days 6-9; and (c, f) bin 3 = Days 11-14. (a-c) The 3xTg mice made significantly more reference memory errors than control mice in all three bins. (b) Male mice were also made more errors than female mice during bin 2. (e) A significant genotype x sex interaction was found for in bin 2, with 3xTg males making more errors than 3xTg females, but no difference between control male and female mice.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 4.5

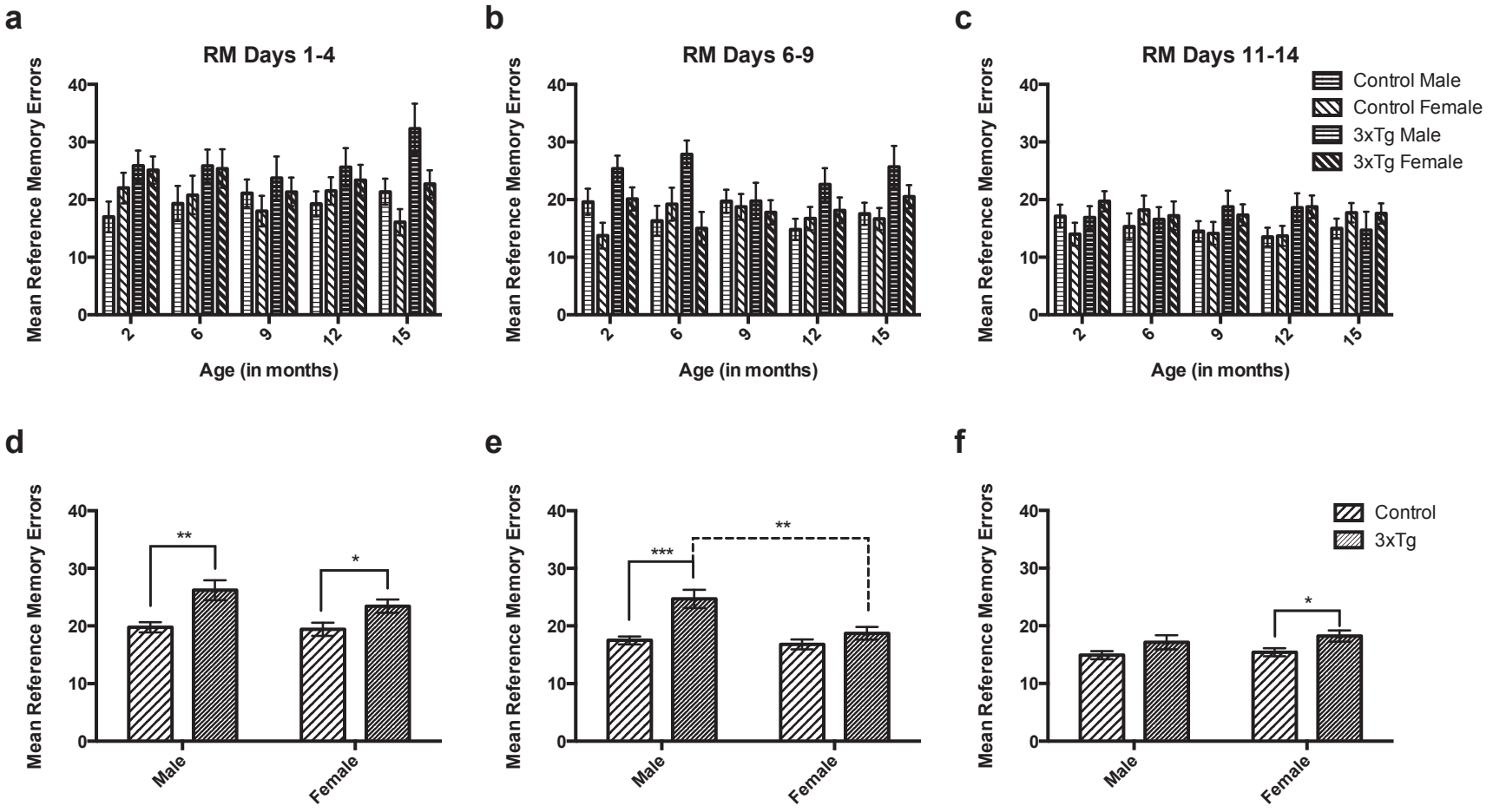

a
RM Days 1-4
b
RM Days 6-9
c
RM Days 11-14
Mean Reference Memory Errors
Age (in months)
Control Male
Control Female
3xTg Male
3xTg Female
d
e
f
Male
Female
Control
3xTg

Figure 4.6. Mean (± SEM) number of correct arm entries out of the first four arms entered across 14 days of training (a,c,e,g,i), and pooled across all 14 days (b,d,f,h,j) for control male, control female, 3xTg male, and 3xTg female mice at the five different age cohorts: (a,b) 2-months of age; (c,d) 6-months of age; (e,f) 9-months of age; (g,h) 12-months of age; (i,j) 15-months of age. A significant genotype effect was found, with 3xTg mice entering fewer correct arms in the first four entries than control mice. There was also a significant effect of day, with e number of correct arm entries increasing over the 14 days of training.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 4.6

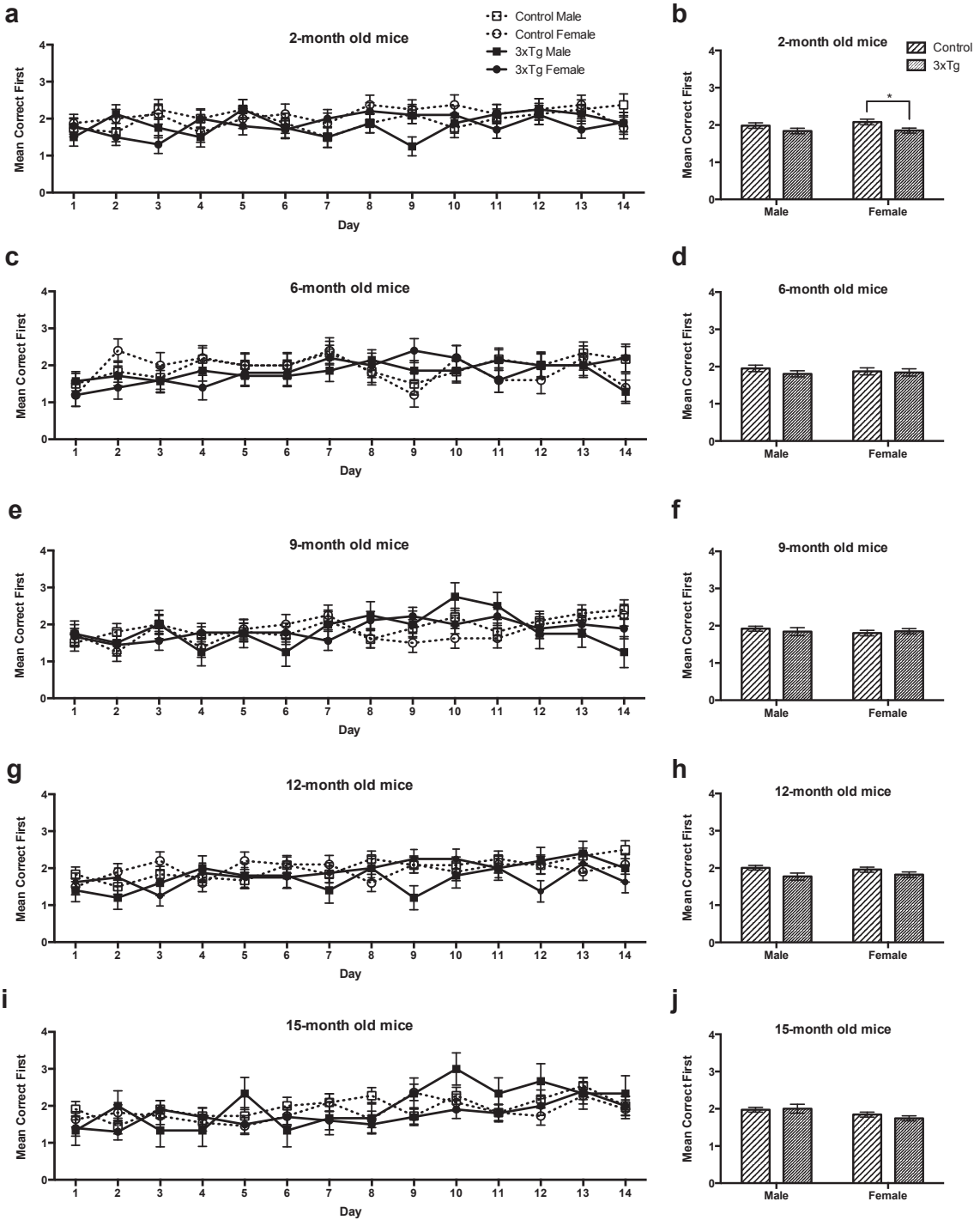

a
2-month old mice
Control Male
Control Female
3xTg Male
3xTg Female
Mean Correct First
Day
b
2-month old mice
Control
3xTg
*
Mean Correct First
Male
Female
c
6-month old mice
Mean Correct First
Day
d
6-month old mice
Mean Correct First
Male
Female
e
9-month old mice
Mean Correct First
Day
f
9-month old mice
Mean Correct First
Male
Female
g
12-month old mice
Mean Correct First
Day
h
12-month old mice
Mean Correct First
Male
Female
i
15-month old mice
Mean Correct First
Day
j
15-month old mice
Mean Correct First
Male
Female

Figure 4.7. Mean (± SEM) total arm entries across 14 days of training (a,c,e,g,i), and pooled across all 14 days (b,d,f,h,j) for control male, control female, 3xTg male, and 3xTg female mice at the five different age cohorts: (a,b) 2-months of age; (c,d) 6-months of age; (e,f) 9-months of age; (g,h) 12-months of age; (i,j) 15-months of age. A significant genotype effect was found, with 3xTg mice making more entries than control mice. There was also a significant effect of sex, with males making more entries than females. A significant day effect showed a decrease in the number of entries over the 14 days of training.
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

Figure 4.7

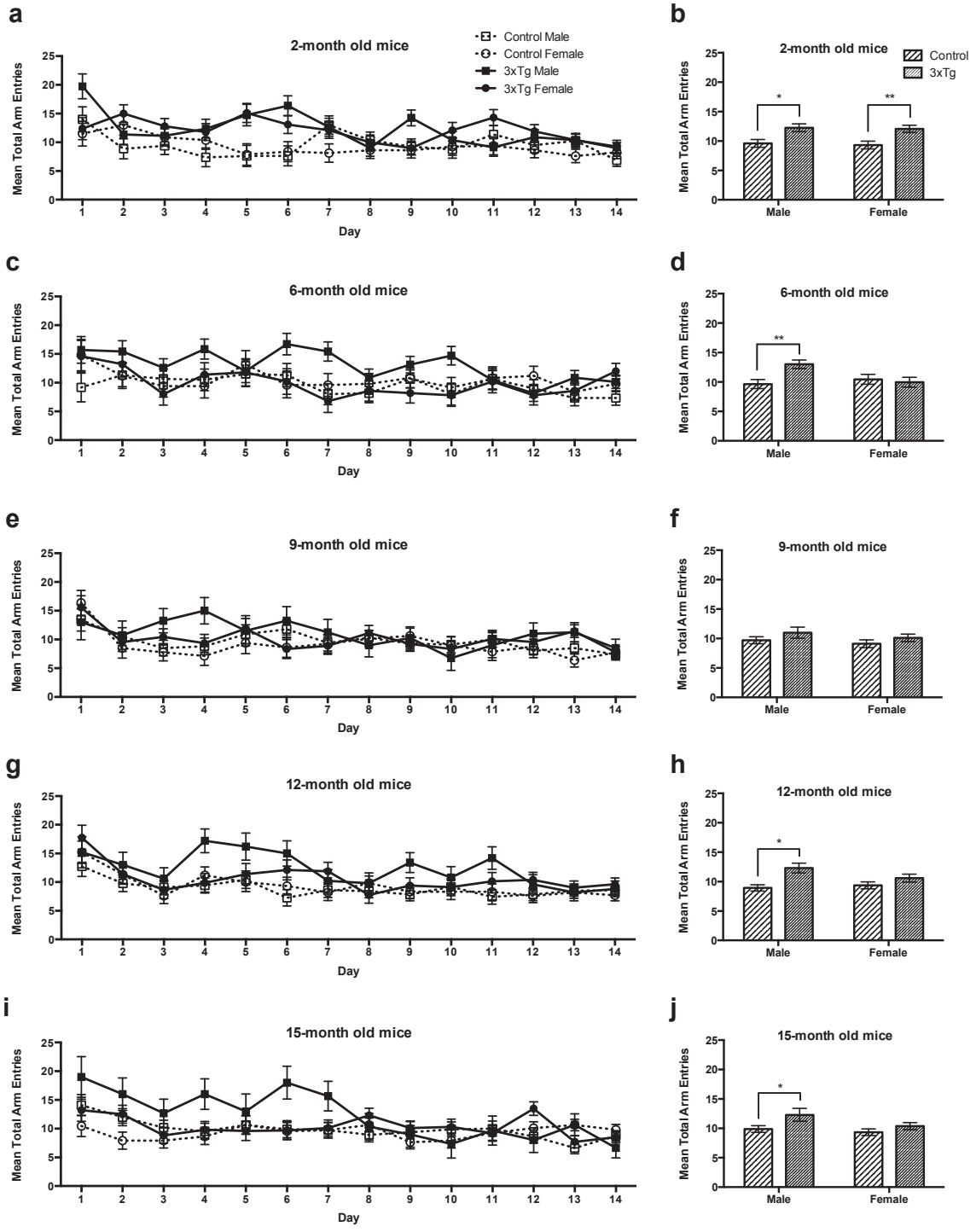

a
2-month old mice
Control Male
Control Female
3xTg Male
3xTg Female
Mean Total Arm Entries
Day
b
2-month old mice
Control
3xTg
Mean Total Arm Entries
Male
Female
c
6-month old mice
d
6-month old mice
e
9-month old mice
f
9-month old mice
g
12-month old mice
h
12-month old mice
i
15-month old mice
j
15-month old mice

Figure 5.1. Aβ plaque deposition in 2- and 15-month old, male and female 3xTg-AD and control (B6129SF2/J) mice in the primary motor cortex (M1). Coronal mouse brain sections (40 μm) were prepared from B6129SF2/J mice sacrificed at 2- (A, E, I, M, Q, U) and 15- (B, F, J, N, R, V) months of age and 3xTg-AD mice sacrificed at 2- (C, G, K, O, S, W) and 15- (D, H, L, P, T, X) months of age, and were processed using immunohistochemistry to detect the β-Amyloid precursor protein (β-APP) using the polyclonal rabbit anti β-Amyloid antibody. The images represent M1 sections at Bregma 1.78 mm (A-H), Bregma 0.62 mm (I-P), and Bregma -0.58 mm (Q-X). Scale bar in A represents X μm.

Figure 5.1

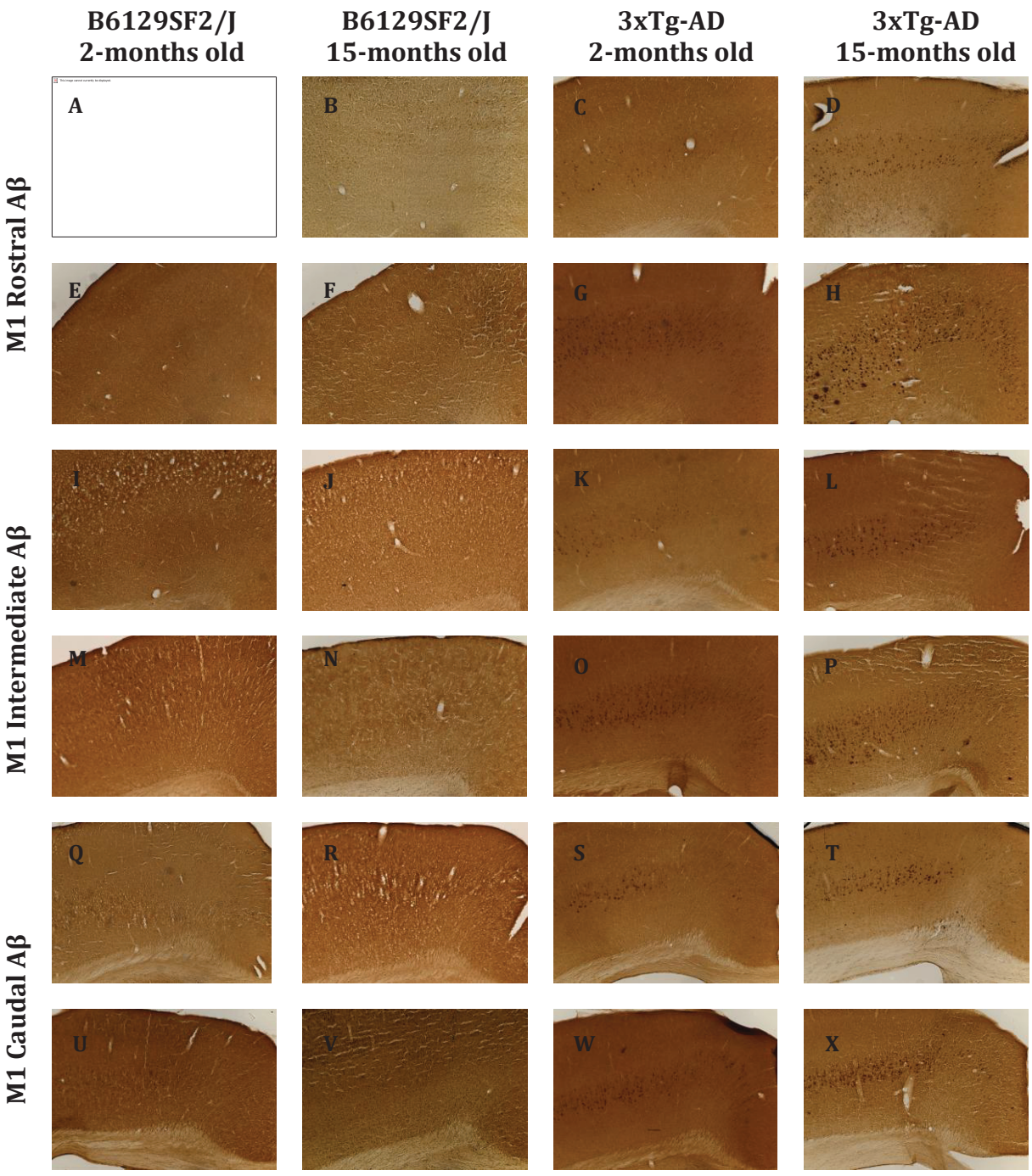

B6129SF2/J
2-months old
B6129SF2/J
15-months old
3xTg-AD
2-months old
3xTg-AD
15-months old
M1 Rostral Aβ
M1 Intermediate Aβ
M1 Caudal Aβ
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X

Figure 5.2. Aβ plaque deposition in 2- and 15-month old, male and female 3xTg-AD and B6129SF2/J (control) mice in the hippocampus CA1. Coronal mouse brain sections (40 μm) were prepared from B6129SF2/J mice sacrificed at 2- (A, E, I, M, Q, U) and 15 (B, F, J, N, R, V) months of age and 3xTg-AD mice sacrificed at 2- (C, G, K, O, S, W) and 15- (D, H, L, P, T, X) months of age and were processed using immunohistochemistry to detect the β-Amyloid precursor protein (β-APP) using the polyclonal rabbit anti β-Amyloid antibody. The images represent CA1 sections at Bregma -1.22 mm (A-H), Bregma -2.46 (I-P), and Bregma -3.80 (Q-X). Scale bar in A represents 200 μm.

Figure 5.2

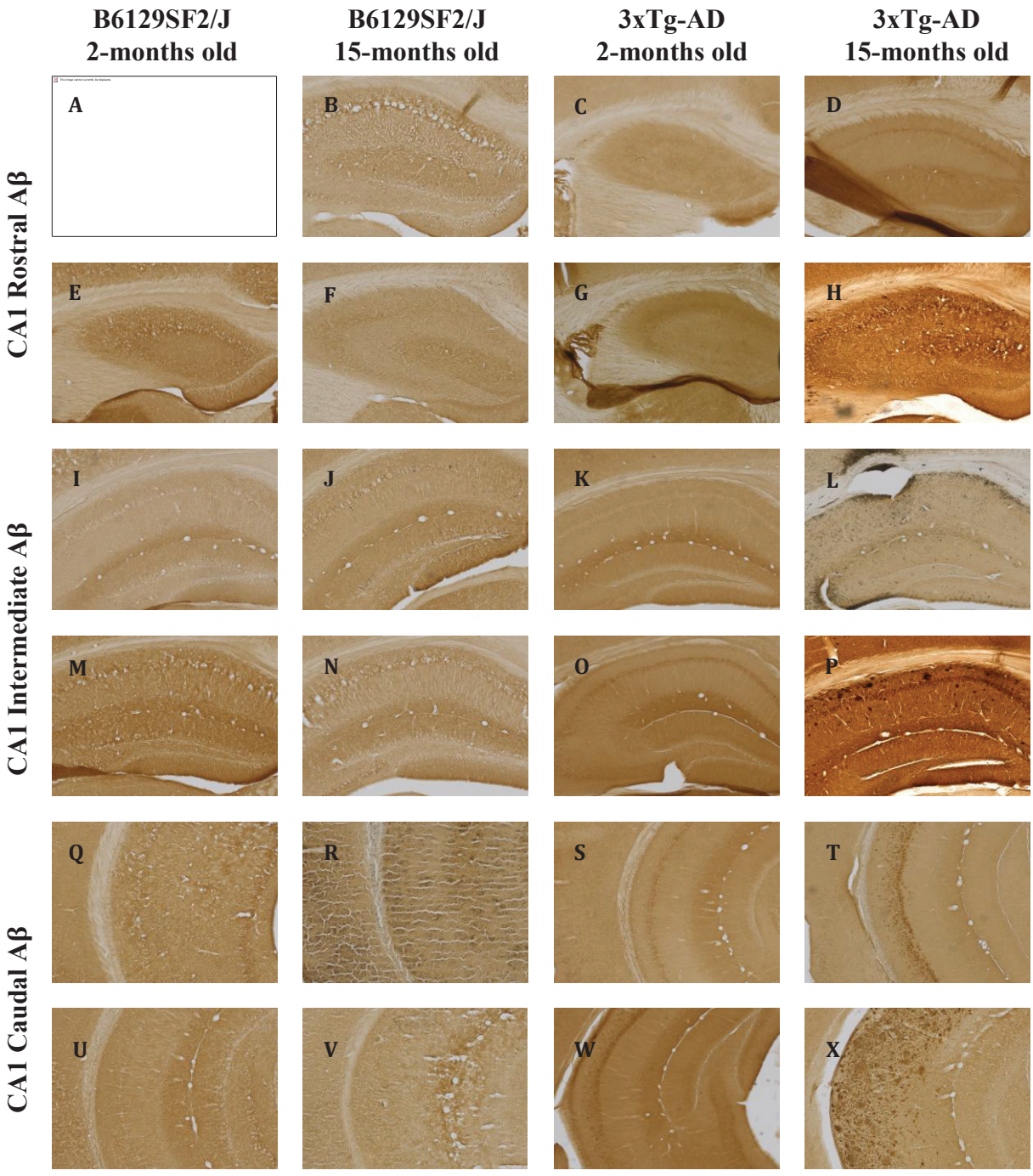

B6129SF2/J
2-months old
B6129SF2/J
15-months old
3xTg-AD
2-months old
3xTg-AD
15-months old
CA1 Rostral Aβ
CA1 Intermediate Aβ
CA1 Caudal Aβ
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X

Figure 5.3. Human tau_{P301L} in 2- and 15-month old, male and female 3xTg-AD and control (B6129SF2/J) mice in the primary motor cortex (M1). Coronal mouse brain sections (40 μm) were prepared from 3xTg-AD mice sacrificed at 2- (A, E, I, M, Q, U) and 15- (B, F, J, N, R, V) months of age and control mice sacrificed at 2- (C, G, K, O, S, W) and 15- (D, H, L, P, T, X) months of age and were processed using immunohistochemistry to detect Human tau^{P301L} transgene expression. The images represent M1 sections at Bregma 1.78 mm (A-H), Bregma 0.62 mm (I-P), and Bregma -0.58 mm (Q-X). Scale bar in A represents 200 μm.

Figure 5.3

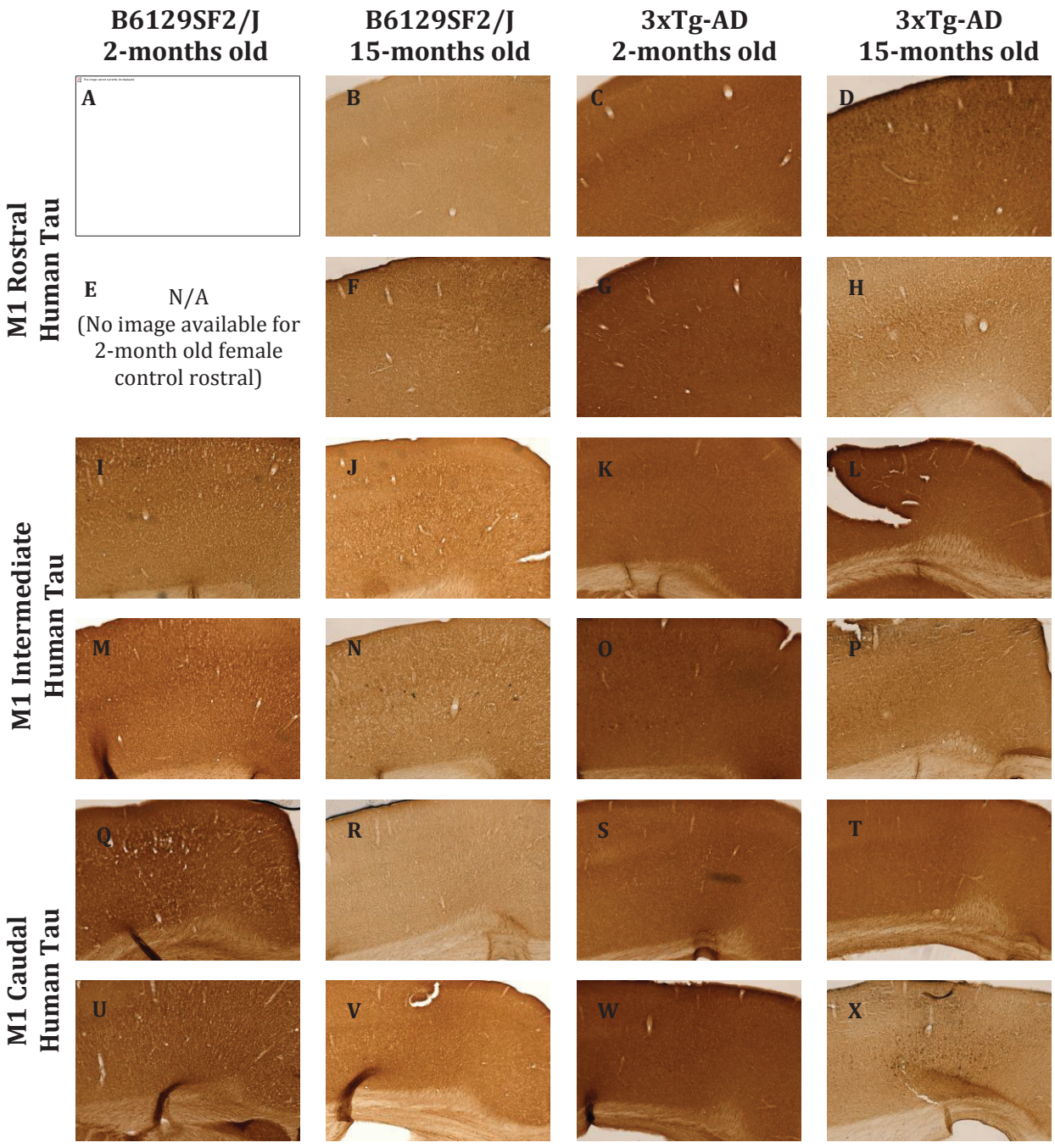
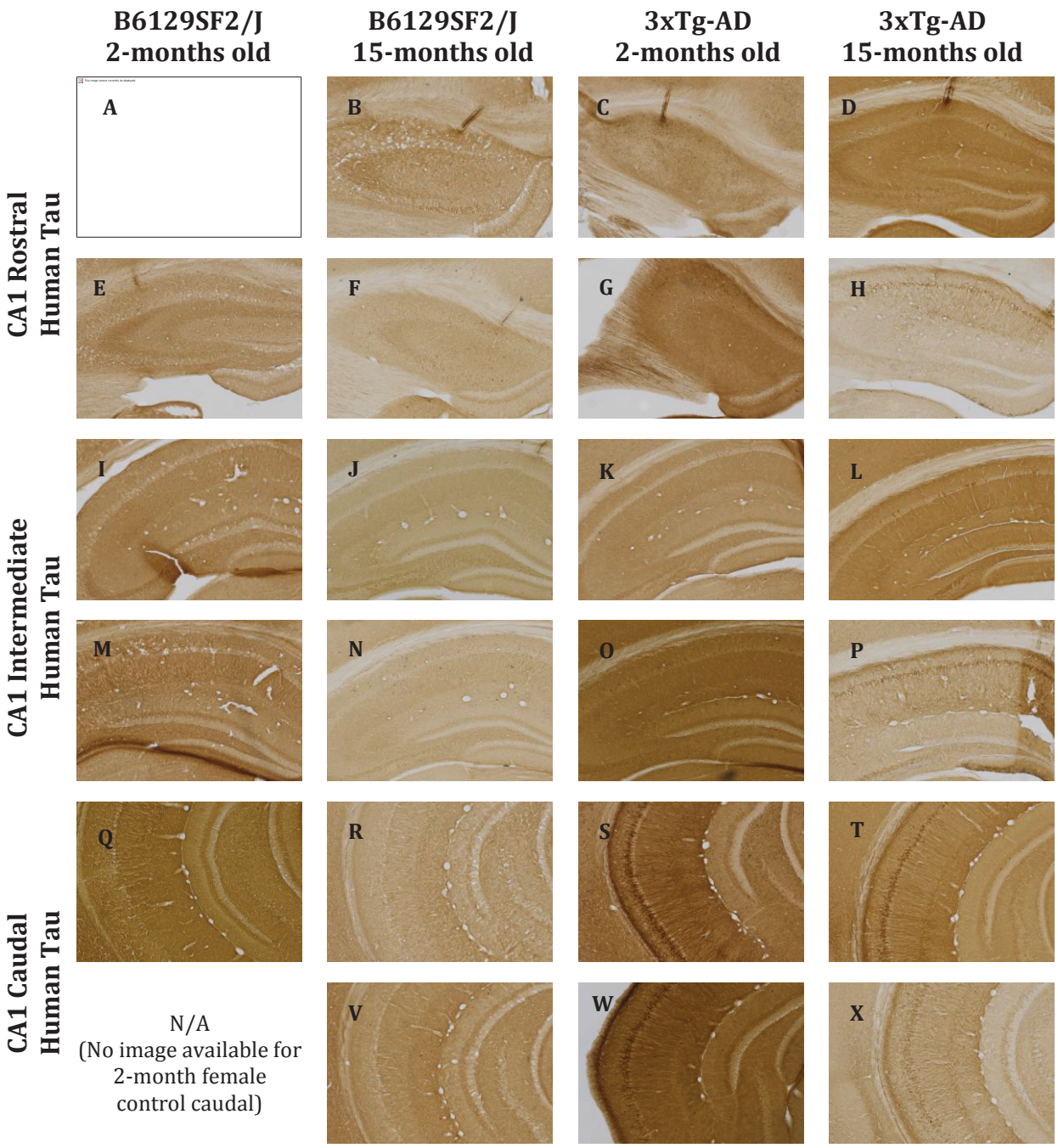

B6129SF2/J
2-months old
B6129SF2/J
15-months old
3xTg-AD
2-months old
3xTg-AD
15-months old
M1 Rostral
Human Tau
M1 Intermediate
Human Tau
M1 Caudal
Human Tau
A
B
C
D
E
N/A
(No image available for
2-month old female
control rostral)
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X

Figure 5.4. Human tau_{P301L} in 2- and 15-month old, male and female 3xTg-AD and B6129SF2/J (control) mice in the hippocampus CA1. Coronal mouse brain sections (40 μm) were prepared from B6129SF2/J mice sacrificed at 2- (A, E, I, M, Q, U) and 15- (B, F, J, N, R, V) months of age and 3xTg-AD mice sacrificed at 2- (C, G, K, O, S, W) and 15- (D, H, L, P, T, X) months of age and were processed using immunohistochemistry to detect Human tau_{P301L} transgene expression. The images represent M1 sections at Bregma -1.22 mm (A-H), Bregma - mm (I-P), and Bregma -3.80 mm (Q-X). Scale bar in A represents 200 μm

Figure 5.4


B6129SF2/J
2-months old
B6129SF2/J
15-months old
3xTg-AD
2-months old
3xTg-AD
15-months old
CA1 Rostral
Human Tau
CA1 Intermediate
Human Tau
CA1 Caudal
Human Tau
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
V
W
X
N/A
(No image available for
2-month female
control caudal)