Effects of Maternal Environment on Behavioural Development in Young Adult 3xTg-AD and B6129S/F2 Mice

by

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Submitted in partial fulfilment of the requirements
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DALHOUSIE UNIVERSITY

DEPARTMENT OF PSYCHOLOGY AND NEUROSCIENCE

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Abstract

The 3xTg-AD mouse model of Alzheimer's disease (AD) has three transgenes, one human gene from familial AD (APP^{swe}), one mutated mouse gene ($PS1^{M1461}$), and one human gene associated with tau pathology (tau^{P301L}). Transgenic and wildtype (B6129S/F2) mice must be bred as separate lines. We cross fostered litters of transgenic and wildtype mice and measured their behaviour at two and six months of age. We found that transgenic mice had lowered anxiety-like behaviours, decreased locomotion, and improved motor performance at two and six months of age. Transgenic mice had a deficit in visuo-spatial learning, but not memory at two and six months. There were several differences between mice reared by wildtype and transgenic mothers, though there was no overall pattern of differences on any measures. At two and six months of age this strain is beginning to demonstrate some behavioural changes that would be expected in a mouse model of AD.

List of Abbreviations Used

AD	Alzheimer's Disease
ANOVA	Analysis of Variance
APP	Amyloid Precursor Protein
MWM	Morris Water Maze
PND	Post Natal Day
PPI	Prepulse Inhibition
PS	Presenilin

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Chapter 1. Introduction

1.1 Alzheimer's Disease

1.1.1. Behavioural Symptoms

Alzheimer's disease (AD) is a progressive neurological disorder characterized by deficits in cognitive function. Patients with AD develop age related deficits in cognition, social behaviour, and motor function. Alzheimer's disease is the most common form of dementia; over 300,000 Canadians suffer from AD and this is projected to increase dramatically over the next few decades (Alzheimer Society of Canada, 2010). While disease progression can differ significantly between patients, the typical progression involves increasing memory impairments followed by deficits in language, motor function or vision (McKhann et al., 1984). Several sub-types of AD have been identified with distinct patterns of progression of neuropathology and symptoms, which may be related to genetic differences (Murray et al., 2011). The memory impairments in AD typically begin with deficits in the acquisition of episodic memory and over time progress to effect working memory and long term memory retrieval (Albert, 2011).

Patients with AD exhibit a number of changes in personality and emotionality. Many patients with AD exhibit increased levels of anxiety compared to healthy controls, though few have symptoms severe enough to meet diagnostic criteria for an anxiety disorder (Chemerinski et al., 1998; Ferretti et al. 2001). It is unclear whether the increased levels of anxiety in AD patients are a direct result of the disease or are secondary to the development of other symptoms, though the presence of anxiety is typically associated with other neuropsychological symptoms (Porter et al., 2001; Teri et al., 1998). Apathy, a loss of motivation, is one of the most common symptoms of AD,

and may be confused with loss of ability (Landes et al., 2001).

The motor deficits in AD begin early in the disease as very mild deficits in fine and complex motor control and progress to deficits in gross motor functioning late in the disease (Kluger et al., 1997; Pettersson, Olsson, and Wahlund, 2005). Despite the deficits in motor functioning, motor learning seems to remain relatively intact in AD patients (Dick et al., 1995; Eslinger and Damasio, 1986).

Circadian rhythm disruption is a common symptom of Alzheimer's disease, and is one of the leading reasons for institutionalization of AD patients (Vitiello et al, 1992). Patients with AD develop progressive dis-regulation in their circadian rhythms, leading to sleep disruption, with fragmented sleeping patterns, and a disruption in the normal cycle of body temperature (Harper et al., 2005; van Someren et al., 1996; Wulff et al., 2010). These sleep disruptions may contribute to the cognitive and emotional deficits that develop AD, and are a significant cause of stress for caregivers (Vitiello et al, 1992).

Olfactory deficits are a common symptom of Alzheimer's disease; early in the disease patients with AD have deficits in olfactory detection threshold and they develop deficits in odour identification as the disease progresses (Doty, Reyes, and Gregor, 1987; Koss et al., 1988). The presence of olfactory deficits in early mild cognitive impairments is predictive of progression to AD (Devanand et al., 2000) and the degree of olfactory threshold impairment is associated with the severity of dementia in AD (Murphy et al., 1990). These olfactory deficits may be due to the buildup of amyloid beta plaques and neurofibrillary tangles in the olfactory bulb (Kovacs, Cairns, and Lantos, 1999).

1.1.2. Neuropathology

The neuropathological hallmarks of AD are amyloid beta plaques composed of

amyloid beta protein, neurofibrillary tangles which are caused by phosphorylated tau protein, and cell death, leading to neurodegeneration (Shoghi-Jadid et al., 2002). Amyloid beta plaque deposition occurs extracellularly and tends to follow a specific pattern, beginning in the basal portions of the neocortex and spreading to more apical and sub-cortical areas as the disease progresses, with increasing plaque densities. High levels of amyloid beta plaques are not necessarily associated with high levels of neurofibrillary tangles, but cases with a high level of neurofibrillary tangles tend to have high levels of amyloid beta protein (Braak and Braak, 2001). Neurofibrillary tangles develop intracellularly and also tend to develop following a specific pattern, beginning in the entorhinal region, followed by the limbic areas, and lastly in the cortex (Braak and Braak, 2001). The number of neurofibrillary tangles is positively correlated with the severity of AD, as is their presence in the neocortex, which represents a more advanced stage of neurofibrillary pathology (Arriagada et al., 1992; Bierer, et al., 1995; Haroutunian et al., 1999), though there is evidence that amyloid beta plaques alone are associated with the early stages of AD (Tiraboschi et al., 2004).

1.1.3. Genetics and Familial Alzheimer's Disease

Alzheimer's disease has a strong genetic component and many genes have been identified as possible risk factors in the development of AD. Mutations in the Apolipoprotein E gene (ApoE) are associated with an increased susceptibility to develop AD, though the specific mechanism by which these mutations increase the susceptibility to develop AD has yet to be determined (Selkoe, 2011). Familial AD is a heritable variant of AD and has an earlier onset than the sporadic disease. The amyloid precursor protein (APP) and presenilin (PS) genes have been associated with familial AD (Price & Sisodia,

1998). The amyloid precursor protein is cleaved first by a beta secretase protein and then by a gamma secretase protein, of which presenilin is a component (Takasugi et al., 2004). The majority of APP is cleaved into amyloid beta 40, but some amyloid beta 42 is formed, which is the form associated with the development of amyloid beta plaques. Mutations in the both the presenilin and APP genes can increase the relative production of amyloid beta 42, which leads to its aggregation and the development of amyloid beta plaques (Theuns et al., 2008; Citron et al., 1997; Schenuer et al., 1996). These genes have been used in the creation of transgenic mouse models of AD.

1.2 Early Life Environment and Development

1.2.1 Alzheimer's Disease and Early Life Environment

In addition to the genetic risk factors, a number of environmental risk factors for the development of AD have been identified. The disease process in AD may begin decades before the development of noticeable symptoms and may arise as an interaction between genes and environmental factors (Miller & O'Callaghan, 2008). Many of these risk factors are in the early life environment, from development in the uterus to the early post-natal environment. Higher levels of education are associated with a lowered risk for the development of AD, as is having a higher socioeconomic status (Bilbul and Schipper, 2011). Low birth weight, poor early life nutrition, and retarded early life body growth have all been associated with an increased risk for the development of AD (Borenstein et al., 2006). Early life factors have been associated with the development of other diseases later in life; for example low birth weight is associated with an increased risk for the development of heart disease and diabetes later in life (Simmons, 2009). These early life factors may influence the development of AD by epigenetic mechanisms. Early life

exposure to lead has been shown to increase amyloidogenesis, possibly though epigenetic mechanisms (Wu et al., 2008). Epigenetic modulation of development begins prenatally and continues throughout the lifespan, so epigenetic modifications may play a role in later neurodevelopment (Marques et al., 2011).

1.2.2 Early Life Environment and Development in Mice

In mice, as in humans, the early life environment can significantly impact later life development, including cognitive functioning. Several environmental factors have been shown to interact with the development of cognitive deficits and neuropathology in mouse models of AD (Chouliaras et al. 2010). Maternal care can differ significantly between strains of mice and this variation in care may interact with the genes introduced in transgenic mice to effect development and thus later life cognitive performance (Francis et al., 2003; Brown et al., 1999). Epigenetic mechanisms have been demonstrated to underlie the lasting changes caused by early life environment (Szyf et al., 2007; Champagne & Curley, 2009). As transgenic mouse models of AD are sometimes bred with the transgenic and wildtype lines separately, there may be differences in maternal care between these lines, which may interact with the effect of the transgenes, and therefore is it is important to study the effects of maternal care and the early life environment in these mice.

1.3 The 3xTg-AD Mouse Model of Alzheimer's Disease

1.3.1 Development and Genetics

The 3xTg-AD (B6;129-Psen1tm1Mpm Tg(APPSwe,tauP301L)1Lfa/J, JAX# 004807, now at the Mutant Mouse Regional Resource Center MMRRC #: 034830-JAX) mouse model of AD has three mutations, APPswe, $PS1^{M146V}$, and tau^{P301L}. APPswe is a

human gene encoding for amyloid precursor protein which contains the Swedish double mutation linked to familial AD. PS1^{M146V} is a mouse gene with a human mutation associated with human familial AD inserted and the tau^{P301L} gene is a human gene with the P301L missense mutation associated with tau pathologies. The transgenes are controlled by the thy1 promoter which is mainly expressed in the central nervous system (Oddo et al., 2003). The mice were created by Oddo et al. (2003) by inserting the APP and tau mutations into the embryo of a PS1^{M146V} transgenic mouse. Transgenic mice with the APPswe and PS1^{M146V} mutations develop amyloid pathology, and inclusion of the human tau gene recreates the tau pathology present in human AD. These mutations interact to create a number of behavioural and neuropathological deficits (Oddo et al., 2003).

1.3.2 Neuropathology

The 3xTg-AD mouse has a number of neuropathological deficits as a result of its transgenes. At two months of age the only reported overt neuropathology is an abnormality in the myelination of the Schaffer collaterals in the hippocampus, which increases in severity until at least six months of age. This is similar to the myelin pathology observed in human AD (Desai et al., 2009). The gene product of APPswe is also detectable at two months in the pyramidal neurons of the hippocampus, the entorhinal cortex and the primary motor cortex; however this is not the cleaved amyloid beta protein that is associated with neuropathology. Intracellular amyloid beta 40 and 42 are detectable in the neocortex beginning at three months of age and are detectable in the hippocampus beginning between three and six months of age. At six months of age the first extracellular plaques are detectable in the neocortex and spread to the hippocampus

before twelve months (Oddo et al., 2003; Mastrangelo and Bowers, 2008). Beginning at four months of age in males and nine months in females, there is a decrease in neurogenesis in the dentate gyrus (Rodríguez et al., 2008).

Tau pathology appears to begin later than amyloid pathology, with phosphorylated tau, which is the form of tau protein associated with neurofibrillary tangles, first detected in small amounts at six months of age in the hippocampus and amygdala, with more robust detection occurring at nine months of age. Phosphorylated tau is first detectable in the motor cortex at nine months of age and in the entorhinal cortex at only twenty-six months of age (Mastrangelo and Bowers, 2008). These neuropathologies lead to behavioural deficits.

1.3.3 Behavioural Deficits

The current behavioural characterization of these mice, while incomplete, indicates that the 3xTg-AD mice appear to be similar to the original wildtype background control strain (C7BL/6;129X1/SvJ;129S1/Sv) mice early in life. The behavioural characterization completed thus far provides conflicting reports about the presence, direction, and age of onset of differences between transgenic and wildtype mice. Between 2 and 2.5 months of age there have been no reported differences between the transgenic and wildtype mice in the Morris water maze (MWM), a visually dependent test of learning and memory (Billings et al., 2005; Billings et al., 2007; Gimenez-Llort et al., 2007). By four months of age the 3xTg-AD mice may begin to show deficits in memory of the platform location in the MWM. In experiments completed by Billings et al. (2005) and Clinton et al. (2007) during the probe trial transgenic mice crossed the platform location fewer times and spent less time in the

platform quadrant than B6129S/F2 mice at six months of age, which indicates that 3xTg-AD mice have deficits in memory retention, but not learning, as their latency to find the platform in training trials decreased between trials and days, though in a study by Gimenez-Llort et al. (2007) transgenic mice were impaired in both learning and memory at six months of age, as demonstrated by a higher latency to the platform and no preference for the platform quadrant during the probe trials. From nine months of age on there are reports of significant deficits in both acquisition and recall in several studies (Billings et al., 2005; Billings et al., 2007; Gimenez-Llort et al., 2007), however others have reported no deficits in the Morris water maze at six and twelve months of age (Gimenez-Llort et al., 2010; Pietropaolo et al., 2008). Pietropaolo et al. (2008) found that the 3xTg-AD mice had better performance compared to wildtype mice in the cued version of the MWM, using a visible platform, and Gimenez-Llort et al. (2010) found that the transgenic mice swam faster than wildtype mice in the Morris water maze.

In contextual fear conditioning the 3xTg-AD mice have no deficits at 2 months of age, but by four and six months of age they are impaired on recall 24 hours after the test (Billings et al., 2007). In active and passive avoidance learning Sterniczuk et al., (2010) found no difference between transgenic and C57BL/6J mice at seven to eleven months of age. Unfortunately the control strain used in this study was incorrect (C57Bl/6J), and the age of control and transgenic mice was significantly different (6.5-7.5 and 7.5-11 months of age respectively) so the results of this study should be interpreted with caution.

In the light/dark box, a measure of anxiety, the 3xTg-AD transgenic mice had fewer entries into the light section and more freezing, indicating a higher level of anxiety than B6129S/F2 mice at six months of age (Billings et al., 2007). There are conflicting

reports of anxiety levels in the open field and elevated plus maze. In the open field Sterniczuk et al. (2010) reported that transgenic mice spent more time in the centre of the maze than C57BL/6J mice, which indicates a lower level of anxiety. Gimenez-Llort et al. (2007, 2010) report a higher level of anxiety in the 3xTg-AD mice in the open field at six and twelve months of age compared to wildtype mice, and Pietropaolo et al. (2008) found no differences in anxiety levels between 3xTg-AD and wildtype mice in the open field at six or twelve months of age. In the elevated plus maze, Sterniczuk et al. (2010) found that 3xTg-AD transgenic mice spent less time than C57BL/6J mice in the closed arms, indicating a lower level of anxiety. On the other hand, Gimilez-Llort et al. (2007) and Pietropaolo et al. (2008) found no differences between genotypes in the elevated plus maze.

Sterniczuk et al. (2010) also found no deficits in 3xTg-AD mice on the Rotarod or the wire hang test, both measures of motor functioning, in mice tested between seven and eleven months of age. Gimenez-Llort et al. (2007) found that at 2.5 months of age transgenic mice had better performance than wildtype mice on the wire hang test, but no differences at six months of age. In the acoustic startle test, Pietropaolo et al. (2008) found that transgenic mice had a larger acoustic startle response than wildtype mice at stimulus intensities over 90dB.

1.4 Mouse Models of Alzheimer's Disease

1.4.1 Rationale For Using Mouse Models Of Alzheimer's Disease

While animal models of a complex human disease can never perfectly replicate all aspects of the disease, they can model some aspects of the disease, and so be useful tools in the study of those human diseases. Mouse models have provided valuable insight

in to the mechanisms of disease such as AD (Luo et al., 2001), but there are several limitations to using a mouse model of human disease. Mouse models of disease can be created by many different methods, including treatment with drugs, surgery, infection with pathogens, and genetic modification, and if the mechanism used to create the symptoms in the mouse model is substantially different from that of the human disease then it is possible that any treatments which are effective in the mouse model may not translate to humans. The mechanisms of disease could also be substantially different in mouse models than in humans, as humans and mice have different physiology. These differences mean that treatments which are effective in mice may not necessarily translate well to human disease, or may have unexpected side effects. For example, immunization therapy for the clearance of amyloid beta plaques in a mouse model of AD cleared most AB pathology and reversed some behavioural deficits, but in human clinical trials a significant number of patients developed meningoencephalitis, though the treatment showed promising results in patients who did not develop meningoencephalitis (Hock et al., 2003; Orgogozo et al., 2003). These limitations necessitate the careful choice of an animal model, and a detailed study of a wide range of behaviours and other characteristics, to ensure that the model is both exhibiting the desired symptoms and has no additional, possibly confounding, differences from the control strain.

1.4.2 Variation in Behavioural Assessment of Mice

Behavioural assessments of mouse models of Alzheimer's disease are used to confirm the presence of symptoms similar to those in human AD, and to measure the effects of drugs or other treatments of the symptoms of AD. Behavioural assessments must be carefully chosen to ensure that the correct measure is being tested and that any

differences between groups are a result of differences between those groups, and not variation in testing procedures. Variation in testing procedures or other unknown factors between laboratories can result in significant differences in findings. The laboratory environment can have a significant effect on behaviour, even when all testing, handling, and animal husbandry procedures are kept consistent (Crabbe et al., 1998; Wahlsten et al., 2003; Whalsten et al., 2006). These studies have demonstrated that some behavioural assessments, mainly those of locomotor behaviour and anxiety, in the elevated plus maze are variable between laboratories, others, such as locomotor activity in the open field, performance in the Morris water maze, and ethanol preference were stable between laboratories. These studies also found that significant differences with larger effect sizes were generally more stable between locations than differences with small effect sizes (Crabbe et al., 1998; Wahlsten et al., 2003; Whalsten et al., 2006). These findings must be taken into consideration when interpreting the results of behavioural assessments of mice.

Chapter 2. Rationale and Hypotheses

2.1 Rationale

As described above, the current literature provides an incomplete assessment of the behavioural phenotype of the 3xTg-AD mouse model of AD. Previous attempts to provide a behavioural characterization have used only one sex, have used incorrect control strains, or have studied only a relatively restricted age range (Billings et al., 2005; Gimenez-Llort et al., 2007; Pietropaolo et al., 2008; Sterniczuk et al,. 2010). Testing mice that span a large age range as a single group may involve mice with significantly different levels of neuropathology, which could confound findings. Testing at only an advanced age when neuropathology is already developed will not allow the dissociation between deficits that develop as a result of neuropathology and differences between stains that are present throughout development. Incorrect control strains make results difficult to interpret. Differences between strains may be due to the effect of transgenes, or background strain differences, as there can be significant differences in behaviour between strains (Cook et al., 2002).

In order to better characterize the behaviour of the 3xTg-AD mouse model of AD we performed a longitudinal study of these mice at two and six months of age. This age range covers the period before any overt neuropathology is present (two months of age) and continues until there is a build-up of amyloid pathology (six months of age) (Mastrangelo and Bowers, 2008). The longitudinal nature of this study will allow us to characterize the changes in behaviour that occur over time and allow us to examine long term memory. We will study the learning and memory, anxiety, motor function, and social behaviour of these mice, to provide a more complete examination of these mice to

model more of behavioural changes in patients with AD. Having a behavioural characterization of this mouse strain is important as it will allow us to determine the reliability and validity of this mouse model of AD so that intervention studies are more accurate and have a better chance of translation to human disease. There is an issue with the 3xTg-AD mice and point of origin: there appear to be discrepancies in the development of intraneuronal amyloid development as well as a different rate of progression of pathology between a set of the mice housed at Georgetown University compared to those kept at the University of California, indicating that there may have been genetic alterations in the strain after the mice were distributed (Hirata-Fikae et al, 2008). It is therefore important to examine these mice to determine if they have a different behavioural phenotype than the first cohort produced.

Billings et al (2007) tested the 3xTg-AD mice longitudinally as well as cross-sectionally in order to determine what effect repeated testing had on neuropathology and behavioural deficits. They found that repeated testing ameliorated memory deficits and delayed the onset of neuropathology compared to the naive mice; however differences between the 3xTg-AD and wildtype mice were still detectable. We expect that our longitudinal test design may increase the cognitive abilities of the mice on all our tasks at six months of age, but not to sufficiently to make the difference between the transgenic and wildtype mice undetectable.

Finally there is the issue of the effect of early life environment and its interaction with the effect of the transgenes. The 3xTg-AD mice must be bred separately from their wildtype controls as the PS1 ^{M146V} gene can segregate independently from the APP^{swe} and tau^{P301L} transgenes, which results in 3xTg-AD and control (B6129S/F2) mice being

reared by mothers of their own genotype (Oddo et al., 2003). The mice of each genotype may receive differences in quantity or quality of maternal care or other aspects of the early life environment, which could lead to differences between phenotypes later in life that are a result of early life environment, or an interaction of early life environment and genotype (Szyf et al., 2007; Champagne & Curley, 2009). In order to separate the effects of pup genotype and maternal genotype we cross-fostered litters of pups between wildtype and 3xTg-AD mothers and measured the effect of pup genotype and foster mother genotype, as well as interactions between the two.

2.2 Hypotheses

Generally we expect that 3xTg-AD and wildtype mice will differ in development across the ages tested. As there have been no studies on maternal effects in these mice we are unable to make specific predictions about the effect of maternal genotype, but we generally expect that maternal genotype will interact with pup genotype in development. We also expect that the sex of the pup may modulate both genotype and maternal effects.

2.2.1 Body Weight

We expect that males will weigh more than females across ages, and that transgenic mice will weigh more than wildtype mice, as previous experiments have reported that 3xTg-AD mice weigh more than wildtype mice at four and six months of age (Gimenez-Llort et al., 2010; Julien et al., 2010)

2.2.2 Home Cage Observations

We hypothesize that in home cage observations transgenic mice will exhibit more agonistic and fewer affiliative behaviours than wildtype mice as AD tends to cause social dysfunction and increases aggression (Chemerinski et al., 1998; Deutsch et al., 1991;

Ferretti et al. 2001). We also hypothesize that males will exhibit more agonistic and fewer affiliative behaviours than females as this is typical in mice (Terranova et al., 1994), and that there will be no differences in non-social behaviour, as neither transgene nor sex are expected to influence non-social behaviour.

2.2.3 Elevated Plus Maze

Some researchers have found decreased anxiety at 7-11 months of age while others found no differences at six or twelve months of age, and so we expect that transgenic mice may display a lowered level of anxiety on the elevated plus maze, if there is a difference between genotypes (Pietropaolo et al., 2008; Sterniczuk et al., 2010 Gimenez-Llort et al., 2007). Previous studies have reported that the transgenic mice display increased locomotor behaviour compared to wildtype mice in both the elevated plus maze and open field, and so we expect increased locomotor behaviour (Pietropaolo et al., 2008; Gimenez-Llort et al., 2007; Gimenez-Llort et al., 2010).

2.2.4 Open Field

Previous studies have reported decreased, increased, and unchanged anxiety levels in the open field test, and increases in locomotor behaviour have been reported, and so we expect increased locomotor behaviour and possibly changes in anxiety levels in transgenic mice (Pietropaolo et al., 2008; Gimenez-Llort et al., 2007; Gimenez-Llort et al., 2010).

2.2.5 Auditory Startle and Prepulse Inhibition

We expect that the 3xTg-AD mice will show a deficit compared to wildtype mice in prepulse inhibition with increasing age, as other mouse models of Alzheimer's disease display decreased prepulse inhibition relative to control mice (McCool et al., 2003).

Pietropaolo et al. (2008) found that transgenic mice had a greater acoustic startle response than wildtype mice at six and twelve months of age and so we expect similar results. We expect the only difference in the no stimulus response will be that males will have a larger response than females, as they generally weigh more and thus will transduce more force to the sensor during normal movements.

2.2.6 Rotarod

Sterniczuk et al. (2010) found no difference between 3xTg-AD mice and C57BL/6 mice in motor coordination and motor learning on the Rotarod at seven to eleven months of age and Gimenez-Llort et al. (2010) found that at 2.5 months of age 3xTg-AD mice had better performance than wildtype mice on the wire hang test but there was no difference at six months of age. We expect transgenic mice may have increased motor abilities relative to controls at two months, but no differences at later ages.

2.2.7 Morris Water Maze

No deficits have been reported in visuo-spatial learning and memory in 3xTg-AD mice at two months of age, but some studies have demonstrated an impairment in probe trial performance at four months of age, and impairments in acquisition at six months of age (Billings et al., 2005; Billings et al., 2007; Clinton et al., 2007; Gimenez-Llort et al., 2007), though others have reported no differences at six or twelve months of age (Pietropaolo et al., 2008; Gimenez-Llort et al., 2010), and so we expect that there will be no differences at two months of age and that the transgenic mice may develop a deficit in learning and memory with age.

In the visible trials of the MWM we expect no differences between groups, as the

majority of previous studies have reported no differences at any age tested (Billings et al., 2005; Billings et al., 2007; Clinton et al., 2007; Gimenez-Llort et al., 2007), though Peitropaolo et al. (2008) report improved performance in the transgenic mice relative to controls at six and twelve months of age on the first day of testing in a cued MWM task.

2.2.8 Social Dominance Tube Test

We expect that the transgenic mice will be more aggressive than the non-transgenic mice, and will become more aggressive with age, as human patients with AD can exhibit changes in emotional state, emotional lability, and aggressive behaviour (Chemerinski et al., 1998; Deutsch et al., 1991; Ferretti et al. 2001).

2.2.9 Social Preference/Novelty

In the social novelty/social preference task we expect that transgenic mice may have a decreased preference for social novelty as they develop impairments in memory with age and therefore will be unable to recognize other mice and develop a preference.

2.2.10 Conditioned Odour Preference Task

We expect that the mice will show a deficit in long term olfactory memory, as they have deficits in other tests of learning and memory, and that these deficits will increase with the age of the animal tested and the latency between learning and memory tests (Billings et al., 2005; Billings et al., 2007; Gimenez-Llort et al., 2007)

Chapter 3. Methods

3.1 Breeding, Cross-Fostering & Pre-Weaning Treatment of Mice

Four breeding pairs of 3xTg-AD mice (JAX # 004807) and four breeding pairs of B6129S/F2 mice (JAX# 101045) were purchased from Jackson Laboratories (Bar Harbor, Maine) and bred in our laboratory. All mothers had successfully reared at least one litter before this experiment began. The protocol for this experiment was approved by the Dalhousie University Committee on Animal Care (Protocol # 11-042).
The 3xTg-AD and B6129S/F2 mice are bred as separate lines, so transgenic mice are always reared by 3xTg-AD mothers and control mice by B6129S/F2 mothers. To study the effects of postnatal environment and maternal effects, all litters were cross fostered at post natal day 0 (the day of birth) or 1 so that half the mice of each genotype had a 3xTg-AD foster mother and half had a B6129S/F2 foster mother, ensuring that pups grew up in mixed genotype environments. The 3xTg-AD mothers gave birth to a mean (±S.E.M.) of 7.00 ± 0.59 pups per litter and the wildtype mothers to 8.55 ± 0.86 pups per litter. When pups were cross fostered, each mother was given the same number of pups they gave birth to, approximately half wildtype and half transgenic pups, none of their own.

The pups in each litter were tested on alternating days, from postnatal day (PND) 2 to 24 using a neurodevelopmental test battery by Caitlin Blaney as part of her honours thesis (Blaney et al., 2012). During this time, pups were tested for the development of neurodevelopmental milestones, sensorimotor reflexes, activity levels and cognition. The neurodevelopmental milestones measured were the day of eye opening, the day of pinnae detachment, and body weight. Sensorimotor reflexes measured included: forelimb and hindlimb grasp reflexes (the days on which mice responded to their limb being stroked

by a 1mm wire by grasping that wire); the vibrissae response (the day when mice responded to being lowered toward a table by truing their heads); the acoustic startle response (the day on which mice responded to a 70dB sound by startling); the tactile orientation reflex (the day when mice respond to having their heads stroked with a cotton swab by turning in the direction of stimulation); the righting reflex (the pups' latency to right themselves when placed on their back; PND2-10); and the day of loss of the crossed extensor reflex (the day in which pinching one hindlimb caused the other hindlimb to contract instead of extend). To study neuromotor development, negative geotaxis (latency to turn 180º after being placed face down on a wire mesh at 45º; PND2-10) and grip strength (after gripping a 1 mm wire with forelimbs, the latency to fall; PND6-16) were assessed. Activity levels were measured using an automated open field, which recorded the number of horizontal and vertical beam breaks from PND12-24. The homing test was used as a measure of olfactory-dependent memory, in which the latency to reach home cage bedding in a clean arena was recorded (PND14). Cognition was also measured using an object investigation test on PND22 and 24 after testing in the open field, in which the time spent interacting with a novel object and habituation to that object was recorded. A summary of the results of the neurodevelopment study is given in Table 1, and data from this study will be discussed in Section 5.2 (Discussion). The level of maternal care was measured, though there were no differences observed between genotypes (Blaney et al., 2012).

Mice were weaned at 25 days of age and housed in same sex mixed genotype groups of two to four mice, in clear plastic cages measuring 18.75 x 28 x 12.5 cm, with wood chip bedding, a PVC tube (4 cm diameter x 7 cm length) for enrichment, and metal

wire covers. They were fed Purina 5001 rodent chow (Purina, St. Louis, Missouri) and tap water ad libitum, unless otherwise indicated. The housing room was kept at 22±2 °C on a reversed 12:12 light:dark cycle, with lights off at a10:00am.

3.2 Procedure

A total 78 mice, 40 3xTg-AD and 38 B6129S/F2, with approximately equal numbers of each sex, were used in this experiment (See Table 2). The mice were tested in three cohorts of approximately 27 animals each, using a longitudinal design. Groups of twenty-seven mice were chosen as this is the number of mice that could be feasibly tested in one day. Each cohort was tested at two and six months of age. This allowed us to test before, during, and after amyloid beta deposits and tau pathology develop in the brain (Billings et al., 2005; Oddo et al., 2003; Mastrangelo and Bowers, 2008). This experiment employed a 2 x 2 x 2 design (genotype x sex x foster mother genotype) at each of the two ages. Previous studies have used groups of between five and fifteen mice per genotype and have been able to detect deficits. We have 38-40 mice per genotype and 7-14 mice per group (genotype by sex by foster mother genotype), which should provide enough power to detect any differences between the groups. (Billings et al., 2005; Billings et al., 2007; Clinton et al., 2007; Sterniczuk et al., 2010). All testing was completed during the dark phase of the light:dark cycle. The experimenters were blind to the genotype and foster mother genotype of the mice, however it was not possible to blind the experimenters to the age of the mice as this was a longitudinal study and the same experimenters tested a cohort at more than one age. Cohort two was tested at two months of age by Daniel Ikpi, a visiting PhD student from the University of Calibar, and Cohort three was tested at six months of age by Michelle Hicks for her third year

independent research project, which I co-supervised. These other experimenters were trained by observing the testing of a cohort of mice before they began testing their cohorts, and were supervised during testing to ensure testing procedures between experimenters was consistent.

3.3 Test Battery

The mice were given each test in the order described below. This order was chosen so that tests were completed from least to most stressful in order to minimize the effect of stress on test results. The test battery took 26 days to complete at two months of age, and 27 days at six months as mice were re-tested on previous odours in the olfactory digging task.

3.3.1 Body Weight

The mice were weighed on each day of the Rotarod test (section 3.1.6), and the weight from the first day before the Rotarod testing began was used to compare between groups at each age.

3.3.2 Home Cage Observations

In order to study social behaviour we made home cage observations using the procedure of D'Andrea et al. (2007). Mice were uniquely identified with a non-toxic permanent marker an hour before the cage observations occurred. The home cage observations were completed in the colony room during the dark phase of the light:dark cycle using a 60 watt red light for illumination, which was only used during observations. Behaviours were scored by time sampling every two minutes, starting with the first mouse in each cage and continuing to the last mouse of each cage. A maximum of five cages were scored at once and the time between observations was 10 minutes per

cage. Breaks were added if fewer cages were observed to keep the time between observations consistent. If there were more than five cages, the remaining cages were observed in a second group immediately following the first. Three sets of observations were completed during the day, evenly spaced over the dark portion of the light:dark cycle. Behaviours were scored using one-zero sampling for three categories of behaviour: affiliative, agonistic, and non-social, as described by Grant and Mackintosh (1963). Affiliative behaviours included sniffing (touching another animal with their snout) or social grooming (stroking with paws or licking the fur of another animal) a cage mate. Agonistic behaviours included attacking (biting another animal), aggressive grooming (violently grooming another animal) offensive upright posture (on hind limbs facing another animal), defensive upright posture (on hind limbs pushing against an attacking mouse), submissive upright posture (on hind limbs turned away from an attacking mouse), crouched posture (lies on floor of cage with head touching cage), freezing (no movement except respiration), fleeing (moving quickly away from another animal), and tail rattling (pointing the tail upwards and moving it from one side to another). Non-social behaviours included wall rearing (one or both fore paws against the wall of the cage, and hind paws on the floor of the cage), self-grooming, immobility, brief contact, eating, and drinking. The frequency of each category of behaviour was analyzed at the two time points using a 2 x 2 x 2 x 3 (genotype x sex x foster mother genotype x time of day) mixed-design analysis of variance (ANOVA) at each age.

3.3.3 Elevated Plus Maze

The elevated plus maze was used to measure anxiety and exploratory behaviour using the procedure described by Brown et al (1999). The maze was shaped like a plus

with two open arms (30 x 5 cm) and two closed arms (30 x 5 cm). The open arms had a 4mm lip to prevent the mouse from slipping off. The arms were connected by a center square (5 x 5 cm). The closed arms had transparent Plexiglas walls (15 cm high) and the floor of the maze was grey Plexiglas. The maze was in a room (2 x 5 m) illuminated by two 60 W white incandescent light bulbs. Mice were placed in the center square of the maze facing the open arms to begin a trial. Trials lasted five minutes and between trials the maze was cleaned with a 70% ethanol solution. The time in the open and closed arms, and distance travelled in the maze were recorded by a computerized tracking system (Limelight, Actimetrics Inc., Wilmette, IL) using a camera located 2.1 m above the maze. The experimenter used the Limelight program to record the frequency and duration of grooming and freezing (remaining completely immobile expect for movements caused by respiration). The number of bouts of rearing (supporting itself only by its hind legs), stretch attend postures (extending the head and then returning to its previous position), head dips (moving its head over the edge of the maze and pointing it downwards), and line crosses (crossing the lines painted on the maze dividing each arm in half) were also recorded. The number of defecations was recoded at the end of each trial. The percentage of time spent in the open and closed arms and amount of time spent engaging each behaviour were analyzed using a factorial ANOVA at each age, and a mixed-design ANOVA was used to compare across ages.

3.3.4 Open Field

The open field test was used to measure anxiety and exploratory behaviour using the procedure described in Brown et al (1999). The open field was a box with no top constructed from wood painted white measuring 72 x 72 cm with 36 cm high walls. The

floor had lines drawn to divide it into sixteen 18 x 18 cm squares, with a center 18 x 18cm square drawn in the middle and was covered by a piece of transparent Plexiglas. Mice were placed in a corner of the open field and allowed to explore the apparatus for five minutes. The amount of time spent near the walls (thigmotaxis), in the centre of the maze, and total distance travelled were measured by a computerized tracking system (Limelight, Actimetrics Inc., Wilmette, IL) from a video camera located 2.1 m above the maze. Using the Limelight program the experimenter scored the number of center square entries, time spent in the centre square, as well as the following behaviours as defined in section 3.3.3; rears against the wall and in the center of the maze (forepaws not touching any walls), the frequency and duration of bouts of grooming, stretch attend postures, and bouts of freezing. The number of defecations was recoded at the end of each trial. The maze was cleaned with a 70% ethanol solution between mice. A factorial ANOVA was used to analyze the behaviours at each age.

3.3.5 Auditory Startle and Prepulse Inhibition

Auditory startle and prepulse inhibition (PPI) were performed using the SR-Lab system (San Diego Instruments, San Diego, California, USA) using the procedure described in Martin and Brown (2010). The PPI chamber was a sound-attenuated box (38.1 x 40.6 x 58.4 cm) with a platform (12.8 x 20.3 cm) supporting a cylindrical restraining tube (12.8 x 5 cm, internal diameter of 3.5 cm), with a piezoelectric accelerometer mounted under the platform to record the startle response of the mice. A high frequency speaker was mounted 28 cm above the cylindrical restraint tube. Each mouse was given one session lasting approximately fifteen minutes. The sessions contained 42 trials with a 10-20 second inter trial interval, and throughout the session

there was a constant 65dB white background noise. The session began with a five minute acclimation period where only the background noise was produced. Following the acclimation period the mouse was given six 40ms acoustic startle trials with a 120dB stimulus and startle data were collected for 65ms after the stimulus. Following the startle trials 30 prepulse inhibition and 6 no-stimulus trials were presented in a semi-random order. During the no-stimulus trials no sound was presented other than the background noise and the startle response of the mouse was recorded for 65ms. Prepulse inhibition trials lasted a total of 185ms; first a prepulse stimulus tone of 74, 78, 82, 86, or 90 dB was presented for 20ms in a semi-random order, followed by 100 ms of background noise, then a 40 ms 120 dB startle tone. During prepulse inhibition trials, data were collected for 65 ms after the 120dB startle tone. The percentage of inhibition (difference in startle response between no-prepulse and prepulse trials) was analyzed using a mixed-design ANOVA at each age. Acoustic startle (response to only the startle tone) and response to the no-stimulus trials were analyzed using a mixed-design ANOVA at each age.

3.3.6 Rotarod

The accelerating Rotarod (Accuscan Instruments Inc. Columbus, Ohio) is a test of motor co-ordination and learning, which was performed as described by Brown & Wong (2007). The Rotarod was located in a testing room illuminated by a 60 W red light bulb and consisted of a rotating rod (3 cm diameter) made of textured plastic, divided into four 11 cm wide sections by Plexiglas dividers (15 cm diameter). An automatic timer started when the trial began and stopped automatically when two infra-red photo beams were broken (located 0.5 cm above the floor) by the mouse falling off the rod into the

holding chamber 39 cm below each section. The Rotarod was set to accelerate from 0 to 48 rpm over a 360 second trial. If a mouse did not fall off the Rotarod after 360 seconds the Rotarod was stopped and the mouse was placed into the holding chamber for the 60 second inter-trial interval. The mice were weighed each day before testing. The test was performed over five days with six trials per day; there was a one minute inter-trial interval during which mice were allowed to rest in the holding chambers before being replaced on the rod. The average latency to fall each day was analyzed using a mixed-design ANOVA. A learning score was calculated for each mouse by calculating the percent increase in latency to fall between day 1 and day 5 $\left(\frac{Day\ 5 - Day\ 1}{Day\ 1} * 100\right)$ and was analyzed using a factorial ANOVA at each age.

3.3.7 Morris Water Maze

The Morris water maze (MWM) is a visually dependent test of spatial learning and memory in which mice must locate a hidden platform in a circular swimming pool. The MWM was performed using a method similar to that described in Wong and Brown (2007). A circular polypropylene pool (Canadian Tire, Toronto, ON) 100 cm in diameter and 20 cm deep was filled to 14 cm with water made opaque by white non-toxic paint (Schola, Marieville, PQ), and an escape platform (13.5 cm high, 9 cm diameter) was used for the acquisition, reversal, and visual trials. Mice were tested in squads of four to six and were released from one of four possible release points equally spaced around the perimeter of the pool (N, S, E, and W). All mice were released from the same point for each trial and the release point was varied semi-randomly over trials, so that mice were never released from the same point twice in a row. Four imaginary quadrants were formed using the four release points and the platform was placed approximately in the

middle of one of these quadrants for the acquisition trials. Mice were trained to find the platform for four trials a day for three days (acquisition), then the platform location was moved to the opposite quadrant for a further three days of four trials per day (reversal); this allows for the study of reversal effects and perseverance. If the mouse did not find the hidden platform in 60 seconds, it was led to the platform with a plastic bucket and allowed to remain on the platform for 30 seconds. The swim path, latency to find the platform, swim speed, distanced travelled, and amount of time spent near the sides of the pool (thigmotaxis) were recorded by an automated tracking system (WaterMaze, Actimetrics, Willamette, IL) using a camera placed 2.1 m above the pool. On day seven a single probe trial was given in which the platform was removed and the mice were allowed to swim in the pool for 60 seconds. The amount of time spent in each of the four quadrants, and the number of crossings of an imaginary annulus drawn around the locations of the platform during reversal, acquisition, and in the two adjacent quadrants was recorded. On day eight, a crude test of vision was performed, using a visible platform with a flag. The mouse was given four trials using the same procedure as in acquisition and reversal. The measures collected were analyzed using a mixed-design ANOVA.

3.3.8 Social Dominance Tube Test

The social dominance tube test is a measure of social behaviour and aggression. This test was performed using a procedure derived from Koh et al (2008) and Lijam et al (1997). Two mice were placed in opposite ends of a tube, and the mouse that forced the other to back out of the tube was the “winner”. The apparatus had two chambers (10 x 10 cm) connected by tube (30 x 3 x 3 cm), all made of clear Plexiglas and mounted to a

wooden board (12.5 x 58 cm) painted white. The end chambers were separated from the connecting tube by a piece of clear Plexiglas that was removed to begin a trial. Each trial lasted a maximum of ten minutes, after which the trial was considered a draw. We tested 3xTg-AD mice against novel B6129S/F2 mice of the same sex, and their relative aggressiveness was determined by how many times they "won" the tube test. Each pair was given two trials. The frequency of winning per genotype was analyzed using a chi-square test and the latency to win was analyzed using a factorial ANOVA between genotypes.

3.3.9 Social Preference/Novelty

The social preference/novelty test measures sociability using a procedure adapted from Moy et al., (2004) and Pearson et al., (2010). The apparatus was an open topped box (69 x 20 x 20 cm) with three chambers (each 23 x 20 x 20 cm) connected by openings (6 x 5.5 cm). The two stimulus mice (Mouse A & B) used in this test were male C57BL/6J (Stock Number: 000664), purchased from the Jackson Laboratories (Bar Harbour, ME). The test had two phases: in the first phase mouse A was placed in a wire cage in one end chamber and a small plastic figurine was placed in a wire cage in the opposite end chamber. The test mouse was placed in the centre chamber and allowed to explore both end chambers for ten minutes. The amount of time spent in each chamber and the amount of time spent interacting with the cage containing the mouse or figurine was recorded. Interaction with the cage was defined as the head of the mouse either touching or being within .5 cm of the wire cage. In the second phase the small plastic figurine was replaced by a novel mouse B, the locations of mice A and B in the chambers were reversed to avoid a side bias, the procedure was repeated, and the same behaviours

were recorded. More social mice will spend more time on the side with the mouse in the first phase and with the novel mouse in the second phase. Mice were tested individually in squads of four, with all four mice completing the first phase before beginning the second phase to create a one hour inter trial interval. The apparatus was cleaned with ethanol between mice. The percentage of time spent with mouse A during the second phase of the test was compared to the amount of time spent with the mouse B to create a preference score $\left(\frac{A-B}{A+B}\right)$ which was analyzed using a factorial ANOVA.

3.3.10 Conditioned Odour Preference Task

The conditioned odour preference task is an olfactory dependent test of learning and memory and was performed as described by Schellinck et al. (2001). Mice were trained to associate one odour (CS+) with a buried sugar reward and another (CS-) with no reward. The odour assigned to be the CS+ varied semi-randomly between cages; all mice in a cage were assigned the same CS+. Before training began the mice were food deprived over three days to between 85% and 90% of their ad lib body weight. The mice were trained in clear plastic cages identical to their home cages, with the bottoms covered in pine chip bedding. In each cage 0.5ml of the odorant was placed on a piece of 55 mm diameter filter paper which was placed in a plastic cup (1.5cm in height, 6.25-cm in diameter), and covered by the top of a plastic Petri dish with 10-12 holes drilled through it. Sugar was placed on top of this for the CS+ odours but not the CS- odours and the odour cups were covered with 2cm of wood chip bedding. Three rooms were used for training, one for each odour, and a third where mice were kept between trials. Training took place over four days with four ten minute trials per day (2 per odour, total 16 trials), alternating between CS+ and CS- odours with a ten minute inter-trial interval.

The mice were tested on day five in the three chambered test apparatus described in section 3.3.8. The mice were given a two minute habituation trial with odour cups without odours in each end chamber. The mice were allowed to explore the apparatus and the amount of time spent in each end chamber was recorded. If there was a side bias (greater than 75% time spent in once chamber) then for the test the CS+ was placed in the non-preferred chamber. Immediately following the habituation trial the mice were tested by placing odour cups containing the CS+ (with no sugar) in one end chamber and the CS- in the other and the amount of time spent digging (displacing the wood chip bedding with the snout or paws) in the odour cups was recorded. If the mouse learned the CS+ sugar association then it should spend more time digging in the CS+ odour cup. The mice were tested 24 hours after the last training trial, and again each time the mice were tested at the next age, to measure long term memory. Therefore mice learned a new odour at two and six months of age and were tested on previous odours learned during the two month tests at six months of age. The odours used at two months were Linalool (lemon) and phenyl acetate (rose) (Sigma-Aldrich, Oakville, ON) diluted using 15% propylene glycol (Caledon Chemicals, Georgetown, ON) and at six months of age the odours were maple and banana extract (McCormick, London, ON) This task allowed us to assess the short term (24 hour) and long term (4 month) memory of these mice in a non-visually dependant task. The percentage of time spent digging in the correct odour was analyzed using a factorial ANOVA.

Chapter 4. Results

4.1 Two Months of Age

Initially 77 mice were tested at two months of age (Table 2.1). One mouse died after the Rotarod before Morris water maze testing (a wildtype male reared by a wildtype foster mother). Two mice (both wildtype males reared by wildtype foster mothers) were not observed for home cage observations as they were singly housed, and two mice (a wildtype female reared by a transgenic foster mother and a transgenic female reared by a wildtype foster mother) did not interact with either mice or the object in the social novelty test and so we were unable to calculate preference scores for those mice. Twelve mice did not dig in either odour cup in the olfactory digging task (four transgenic males reared by transgenic foster mothers, one transgenic female reared by a wildtype foster mother, two transgenic males reared by wildtype foster mothers, two wildtype females reared by transgenic foster mothers, and three wildtype males reared by wildtype foster mothers) the percentage of time digging in the correct cup could not be calculated.

4.1.1 Body Weight

On the first day of the Rotarod test, males weighed significantly more than females ($F(1,69) = 101.939$, $p = < 0.0001$) and there was a sex by pup genotype interaction ($F(1,69) = 5.185$, $p = 0.026$) as wildtype males weighed significantly more than transgenic males ($p < 0.001$) , but there was no genotype difference in the weight of females. Mice reared by wildtype foster mothers weighed significantly more than mice reared by transgenic foster mothers ($F(1,69) = 12.889$, $p = 0.001$) (Figure 1.1).

4.1.2 Home Cage Observations

Mice reared by wildtype foster mothers tended to have more affiliative

behaviours than mice reared by transgenic foster mothers (F(1,67)=3.575, p=0.063) and there was a significant pup genotype by foster mother genotype interaction in number of affiliative behaviours (F(1,67)=4.835, p=0.031) as transgenic mice reared by wildtype foster mothers had significantly more affiliative behaviours than transgenic mice reared by transgenic foster mothers (p=0.040). There was no effect of time on affiliative behaviours (Figure 2.1 and Figure 2.7). Transgenic mice had more agonistic behaviours than wildtype mice (F(1,67)=10.464, p=0.002), males had more agonistic behaviours than females (F(1,67)=4.398, p=0.040), and mice reared by transgenic foster mothers had more agonistic behaviours than mice reared by wildtype foster mothers (F(1,67)=11.029, p=0.001) (Figure 2.2 and Figure 2.8). There were pup genotype by sex (F(1,67)=7.292, p=0.009), pup genotype by foster mother genotype (F(1,67)=3.975, p=0.050), pup genotype by sex by foster mother genotype (1,67)=5.229, p=0.035) interactions on the number of agonistic behaviours as transgenic males reared by transgenic foster mothers had significantly more agonistic behaviours than all other groups of mice (p<0.001). There was a trend toward an effect of time on agonistic behaviours (F(2,134)=2.822, p=0.063), though post-hoc tests revealed no differences between time points, and there was a significant time by foster mother genotype interaction (F(2,134)=5.211, p=0.007) as mice reared by wildtype foster mothers had more affiliative behaviours during the third time point (p=0.003) For non-social behaviours there was a trend toward a pup genotype by sex interaction (F(1,67)=3.920, p=0.052) though post-hoc analyses found no significant differences between groups and there was no effect of pup genotype or sex (Figure 2.3 and Figure 2.9). There was a significant effect of time on the number of non-social behaviours (F(2,134)=4.801, p=0.010), as there were more non-social behaviours

at the second time point than the first time point ($p=0.012$).

4.1.3 Elevated Plus Maze

For the number of line crosses there was no effect of pup genotype, sex, or foster mother genotype, but there was a significant interaction between pup genotype and foster mother genotype ($F(1,69)=4.632, p=0.035$) , though post-hoc analyses indicated there were no significant differences between groups (Figure 3.1). Wildtype mice spent significantly more time (Figure 3.2) ($F(1,69)=72.756, p<0.0001$) and travelled a significantly greater percentage of their total distance (Figure 3.3) ($F(1,69)=45.321, p<0.0001$) in closed arms than transgenic mice. Wildtype mice travelled a significantly greater distance ($F(1,69)=37.703, p<0.0001$) than transgenic mice and there was a trend towards a pup genotype by foster mother genotype interaction ($F(1,69)=3.474, p=0.066$) (Figure 3.4) and a significant sex by foster mother genotype interaction ($F(1,69)=5.209, p=0.026$) as male and female wildtype mice reared by transgenic foster mothers tended to travel a greater distance than male and female transgenic mice reared by transgenic foster mothers ($p<0.01$) (Figure 3.21). Transgenic mice made significantly more head-dips than wildtype mice ($F(1,69)=4.717, p=0.033$) (Figure 3.5). There were no main effects for the number of rears, but there was a significant pup genotype by foster mother genotype interaction ($F(1,69)=7.974, p=0.006$) though post-hoc analyses revealed no significant differences between groups (Figure 3.6). There was a trend for transgenic mice to have more freezing bouts (Figure 3.7) than wildtypes ($F(1,69)=3.467, p=0.067$), and a significant sex by foster mother genotype interaction ($F(1,69)=4.456, p=0.038$) and a trend towards a pup genotype by sex by foster mother genotype interaction ($F(1,69)=10.949, p=0.072$) though post-hoc analyses

revealed no differences between groups in either interaction (Figure 3.22). Transgenic mice had significantly more stretch attend postures (Figure 3.8) than wildtypes ($F(1,69)=9.433$, $p=0.003$) and there was a significant sex by foster mother genotype interaction ($F(1,69)=19.384$, $p=0.008$) though post-hoc analyses found no significant differences between groups (Figure 3.23). For the number of grooming bouts there were no significant differences between groups and no significant interactions (Figure 3.9).There were no significant differences in time spent grooming between any groups and no interactions (Figure 3.10).

4.1.4 Open Field

Females tended to have more line crosses in the open field than males ($F(1,69)=3.289$, $p=0.074$), there was no effect of sex or pup genotype and no significant interactions (Figure 4.1 and Figure 4.19). There were no differences between groups in distance travelled, number of rears (Figure 4.2), or number of center rears (Figure 4.3). Wildtype mice performed more stretch attend postures than transgenic mice ($F(1,69)=4.357$, $p=0.041$) and mice reared by wildtype foster mothers performed more stretch attend postures than mice reared by transgenic foster mothers ($F(1,69)=4.001$, $p=0.049$), there were no interactions (Figure 4.4). There was a significant pup genotype by foster mother genotype interaction on the number of freezing bouts ($F(1,69)=5.287$, $p=0.025$) as transgenic mice reared by wildtype foster mothers had more freezing bouts than transgenic mice reared by transgenic foster mothers ($p=0.008$) (Figure 4.5). There were no differences between groups in the amount of time spent freezing. There was a trend for wildtype mice to have more grooming bouts than transgenic mice ($F(1,69)=3.317$, $p=0.073$) (Figure 4.6), females had significantly more grooming bouts

than males ($F(1,69)=5.800$, $p=0.019$), and there was a significant pup genotype by sex by foster mother interaction ($F(1,69)=8.242$, $p=0.005$) as transgenic males reared by wildtypes had significantly fewer grooming bouts than wildtype females reared by transgenic foster mothers ($p=0.008$) (Figure 4.20). There was a significant pup genotype by sex by foster mother genotype interaction on the time spent grooming ($F(1,69)=3.981$, $p=0.050$)) though post-hoc analyses revealed no differences between groups (Figure 4.7 and Figure 4.21). Transgenic mice and mice reared by transgenic foster mothers had significantly more center entries than wildtype and mice reared by wildtype foster mothers ($F(1,69)=6.426$, $p=0.014$ and $F(1,69)=6.784$, $p=0.11$) (Figure 4.8). Transgenic mice spent more time in the center than wildtype mice ($F(1,69)=5.111$, $p=0.027$) (Figure 4.9).

4.1.5 Auditory Startle and Prepulse Inhibition

Transgenic mice had a larger initial startle response than wildtype mice ($F(1,69)=4.078$, $p=0.047$), females had a larger response than males ($F(1,69)=4.184$, $p=0.025$) and there was a significant pup genotype by sex interaction ($F(1,69)=4.612$, $p=0.035$) as transgenic females tended to have a larger startle response than all other groups ($p>0.06$) (Figure 5.1 and Figure 5.7). There was also a significant sex by foster mother genotype interaction on initial startle response ($F(1,69)=5.236$, $p=0.025$), as female mice reared by transgenic foster mothers had a larger response than male mice reared by transgenic foster mothers ($p=0.050$) (Figure 5.7). Wildtype mice had a significantly larger no-stimulus response than transgenic mice ($F(1,69)=21.126$, $p<0.0001$), there were no differences between sexes of foster mother genotypes (Figure 5.2). The percentage of prepulse inhibition of startle response increased significantly

with increasing prepulse intensity ($F(4,276)=124.539$, $p<0.001$), there were no differences between groups and no interactions (Figure 5.3).

4.1.6 Rotarod

Transgenic mice had a significantly longer latency to fall from the Rotarod than wildtype mice ($F(1,69)=12.734$, $p=0.001$) (Figure 6.1) and females had a significantly longer latency to fall than males ($F(1,69)=19.526$, $p<0.0001$) (Figure 6.6). The latency to fall for all mice increased significantly over days ($F(4,276)=114.107$, $p<0.0001$) and there was a significant day by sex ($F(4,276)=2.547$, $p=0.040$) and day by pup genotype by sex ($F(4,276)=4.408$, $p=0.002$) interaction as males had better performance than females on days 1-3 but not 4-5 ($p>0.05$) and the performance of wildtype females increased across all days but 4 and 5, while the performance of the transgenic females only increased relative to day one across days. There was also a day by foster mother genotype interaction ($F(4,276)=2.817$, $p=0.026$) as there was a trend for mice reared by wildtype foster mothers to have a greater latency to fall than mice reared by transgenic foster mothers on day 3 ($p=0.075$) (Figure 6.6) For the learning score $\left(\frac{Day\ 5 - Day\ 1}{Day\ 1} * 100\right)$ there was a significant pup genotype by sex interaction and post-hoc analyses revealed that wildtype females tended to have a higher learning score than transgenic females ($p=0.051$) (Figure 6.2 and Figure 6.5).

4.1.7 Morris Water Maze

Transgenic mice had significantly greater latency to the hidden platform than wildtype mice ($F(1,68)=12.246$, $p=0.001$) and the latency of all mice decreased significantly over days ($F(5,340)=118.843$, $p<0.0001$) (Figure 7.1). There was a significant day by pup genotype interaction on latency to the platform ($F(5,340)=4.889$,

$p<0.001$), post-hoc tests indicated that transgenic had a greater latency than wildtype mice on only days one and two ($p<0.01$). Transgenic mice and mice reared by wildtype foster mothers had a significantly greater distance to the platform than wildtype mice and mice reared by transgenic foster mothers ($F(1,68)=11.933$, $p=0.00$ and $F(1,68)=7.705$, $p=0.007$) the distance to the platform decreased over days for all mice ($F(5,340)=39.345$, $p<0.0001$) (Figure 7.2). There was a significant day by pup genotype ($F(5,340)=2.629$, $p=0.021$) and day by foster mother genotype interaction ($F(5,340)=2.281$, $p=0.046$) as transgenic mice had a greater distance to the platform than wildtype mice only on day two ($p<0.001$) and mice reared by wildtype foster mothers had a greater distance to the platform only on day one ($p<0.05$). Transgenic mice had a significantly faster swim speed than wildtype mice ($F(1,68)=34.881$, $p<0.0001$) (Figure 7.3) and there was a significant sex by pup genotype interaction as female transgenic mice had a faster swim speed than all other mice ($p<0.05$) (Figure 7.25). The swim speed of all mice increased over days ($F(5,340)=31.584$, $p<0.0001$) and there was a significant day by pup genotype ($F(5,340)=11.452$, $p<0.0001$) and day by foster mother genotype ($F(5,340)=30.723$, $p=0.003$) interaction as transgenic mice had a greater swim speed on days two to six ($p<0.01$) and wildtype mice had a greater swim speed only on day one ($p<0.01$). Mice reared by wildtype foster mothers spent more time in thigmotaxis than mice reared by transgenic foster mothers and the time spent in thigmotaxis decreased over days for all mice ($F(5,340)=33.154$, $p<0.001$) and there was a day by pup genotype ($F(5,340)=3.589$, $p=0.004$) and day by foster mother genotype ($F(5,340)=3.027$, $p=0.011$) interaction as mice reared by wildtype foster mothers had more thigmotaxis than mice reared by transgenic foster mothers on day one only ($p<0.01$), post-hoc analyses revealed no

significant differences between pup genotypes across days (Figure 7.4).

Mice reared by wildtype foster mothers had a significantly greater latency reversal score than mice reared by transgenic foster mothers ($F(1,68)=4.537$, $p=0.037$) (Figure 7.5). There were no differences between groups on reversal distance effect (Figure 7.6), swim speed reversal effect or thigmotaxis reversal effect, and no interactions.

All mice spent more time in the correct quadrant than the other quadrants ($F(3,204)=77.776$, $p<0.001$) and there was a significant quadrant by sex by foster mother genotype interaction ($F(2,204)=3.095$, $p=0.028$) as there was no difference between time spent in the correct quadrant and the first adjacent quadrant by female mice reared by transgenic foster mothers and male mice reared by wildtype foster mothers (Figure 7.7 and Figure 7.26). There was a significant sex by foster mother genotype interaction on the amount of time spent in the correct quadrant ($F(1,68)=6.645$, $p=0.012$)) though post-hoc analyses revealed no differences between groups. All mice crossed the correct annulus significantly more than the annulus in all other quadrants ($F(3,204)=92.622$, $p<0.0001$), there were no differences between groups on number of annulus crossings across all quadrants (Figure 7.8). There were no significant differences between any groups in the number of correct annulus crossings.

There were no differences between groups in the latency to the visual platform (Figure 7.9). There was a trend for transgenic mice to take a greater distance to reach the visual platform than wildtype mice ($F(1,68)=3.545$, $p=0.064$) (Figure 7.10). Transgenic mice had a significantly greater swim speed than wildtype mice during the visual platform trials ($F(1,68)=10.702$, $p=0.002$) (Figure 7.11). There were no differences

between pup genotypes, sexes, or foster mother genotypes on amount of thigmotaxis in the visual platform (Figure 7.12).

4.1.8 Social Dominance Tube Test

There were no differences between genotypes in the proportion of wins in the tube test (χ^2=0.120, p=0.729) (Figure 8.1).

4.1.9 Social Preference / Novelty

There was a trend for mice reared by transgenic foster mothers to have a greater mouse/object time preference score than mice reared by wildtype foster mothers ($F(1,68)=3.318$, $p=0.073$) (Figure 9.1). Transgenic mice had a significantly greater mouse/object interaction preference score than wildtype mice ($F(1,68)=5.511$, $p=0.022$) (Figure 9.2). There was a trend for mice reared by transgenic foster mothers to have a significantly lower familiar/novel time preference score than mice reared by wildtype mice ($F(1,68)=3.685$, $p=0.059$) (Figure 9.3). There were no differences between groups in the familiar/novel interactions preference score (Figure 9.4).

4.1.10 Conditioned Odour Preference Task

Wildtype mice spent a significantly greater percentage of time digging in the CS+ than transgenic mice ($F(1,56)=6.815$, $p=0.012$) and there was a significant pup genotype by foster mother genotype interaction as transgenic mice reared by wildtypes had a higher percentage of time spent digging in the CS+ than transgenic mice reared by wildtype mice ($p>0.01$) (Figure 10.1).

4.2 Six Months of Age

Seventy-six mice were tested at six months of age (Table 2.2). One mouse was not observed for home cage observations (a wildtype male reared by a wildtype foster

mother) as it was singly housed. Two mice did not interact with either the object of mouse in the social novelty/ social preference test (a transgenic male reared by a wildtype foster mother and a transgenic female reared by a transgenic foster mother) and five mice did not dig in the six month odour test in the conditioned odour preference task (one transgenic male reared by a wildtype foster mother, two transgenic females reared by transgenic foster mothers, one wildtype female reared by a transgenic foster mother and one wildtype female reared by a wildtype foster mother). For the re-testing of the two month odour thirty mice did not dig in either odour cup in the conditioned odour preference score (four wildtype female and six males mice reared by wildtype foster mothers, three wildtype females and one male reared by transgenic foster mothers, nine transgenic females and two males reared by wildtype foster mothers, three transgenic females and two males reared by transgenic foster mothers).

4.2.1 Body Weight

On the first day of the Rotarod test, males weighed significantly more than females ($F(1,68)=22.040$, $p<0.001$) and there was a trend towards a pup genotype by sex interaction ($F(1,68)=3.857$, $p=0.054$) as transgenic females weighed significantly more than wildtype females ($p<0.05$) (Figure 1.2).

4.2.2 Home Cage Observations

For the number of affiliative behaviours there was a significant time by sex interaction ($F(2,134)=3.548$, $p=0.032$) though post-hoc analyses revealed no significant differences(Figure 2.4 and Figure 2.10). Males had significantly more agonistic behaviours than females ($F(1,67)=4.585$, $p=0.036$) and there was a significant time by sex interaction ($F(2,134)=3.209$, $p=0.044$) as males only exhibited more agonistic

behaviours during the first time point (Figure 2.5 and Figure 2.11). Transgenic mice exhibited significantly more non-social behaviours than wildtype mice ($F(1,67)=4.058$, $p=0.048$) and there was a significant time by pup genotype ($F(2,134)=3.604$, $p=0.030$) interaction though post-hoc analyses revealed no differences between groups, and a time by sex ($F(2,134)=6.390$, $p=0.002$) interaction as males exhibited more non-social behaviours than females during the first time point only ($p<0.05$) (Figure 2.6 and Figure 2.12).

4.2.3 Elevated Plus Maze

There was a trend for wildtype mice to have more line crosses (Figure 3.11) than transgenic mice ($F(1,68)=3.674$, $p=0.060$) and transgenic mice spent less time in the closed arms than wildtype mice ($F(1,68)=20.208$, $p<0.0001$) (Figure 3.12). Transgenic mice and males travelled a lower percentage of their total distance in the closed arms ($F(1,68)=16.813$, $p<0.0001$ and $F(1,68)=4.567$, $p=0.036$) and there was a significant pup genotype by sex interaction as transgenic females travelled a greater percentage of their total distance in the closed arms than transgenic males ($p<0.001$) (Figure 3.13 and Figure 3.25). Wildtype mice travelled a greater overall distance than transgenic mice ($F(1,68)=5.297$, $p=0.024$) and mice reared by wildtype mice travelled a greater distance than mice reared by transgenic mice ($F(1,68)=4.144$, $p=0.046$) (Figure 3.14). Transgenic mice performed significantly more head dips (Figure 3.15) ($F(1,68)=4.061$, $p=0.048$) than wildtype mice. There were no significant differences between groups in number of rears (Figure 3.16), stretch attend postures (Figure 3.18), and time spent freezing. Transgenic mice had significantly more freezing bouts (Figure 3.17) ($F(1,68)=4.857$, $p=0.031$) than wildtype mice. Wildtype mice had significantly more grooming bouts

(Figure 3.19) (F(1,68)=5.138, p=0.027) and more time spent grooming (F(1,68)=4.029, p=0.049) than transgenic mice (Figure 3.20).

4.2.4 Open Field

There were no significant differences between groups in the number of line crosses (Figure 4.10) or distance travelled in the open field. Wildtype mice reared significantly more than transgenic mice (F(1,68)=4.023, p=0.049) (Figure 4.11). Transgenic mice tended to have more center rears than wildtype mice (F(1,68)=3.944, p=0.051) and there was a significant pup genotype by sex by foster mother genotype interaction (F(1,68)=4.361, p=0.041), though post-hoc tests determined there were no significant differences between groups (Figure 4.12 and Figure 4.22). For the number of stretch attend postures there were no significant differences between groups (Figure 4.13). There was a trend towards a sex by foster mother genotype interaction for the number of freezing bouts (F(1,68)=3.543, p=0.064), though post-hoc analyses found no significant differences between groups (Figure 4.14 and Figure 4.23). There were no significant differences between groups on the amount of time spent freezing. There were no differences between groups on the number of grooming bouts (Figure 4.15). Wildtype mice spent significantly more time grooming than transgenic mice (Figure 4.16) (F(1,68)=3.976, p=0.050) and there was a trend towards a sex by foster mother genotype interaction (Figure 4.24) (F(1,68)=3.499, p=0.068) as there was a trend for males reared by wildtype foster mothers to groom more than females reared by wildtype foster mothers (p=0.051). There were no differences between groups on the number of entries into the center square (Figure 4.17). There was a trend for transgenic mice to spend more time in the center square (F(1,68)=3.844, p=0.053) than wildtype mice (Figure 4.18).

4.2.5 Auditory Startle and Prepulse Inhibition

Transgenic mice had a larger acoustic startle response than wildtypes ($F(1,67)=17.904$, $p<0.0001$) (Figure 5.4), females had a larger response than males ($F(1,67)=6.862$, $p=0.011$) (Figure 5.8), and there was a significant sex by pup genotype interaction ($F(1,67)=5.476$, $p=0.022$) as transgenic females had a larger response than all other groups ($p<0.01$). For the no-stimulus trials males had a larger response than females ($F(1,68)=12.669$, $p=0.001$) and there was a trend towards a pup genotype by sex interaction ($F(1,68)=3.627$, $p=0.061$) as transgenic males had a larger response than transgenic females ($p<0.005$) (Figure 5.5 and Figure 5.9). The percentage of prepulse inhibition of acoustic startle increased significantly as the intensity of the prepulse stimulus increased ($F(4,268)=118.467$, $p<0.0001$), and there was a trend for a stimulus intensity by genotype interaction ($F(4,268)=2.363$, $p=0.054$), but post-hoc analyses found no significant differences (Figure 5.6). There was a significant prepulse intensity by pup genotype by foster mother genotype ($F(4,268)=5.579$, $p<0.0001$) as there was a difference in response between 74 and 78 dB in wildtype mice reared by transgenic foster mothers and not in any other groups.

4.2.6 Rotarod

Transgenic mice had a significantly longer latency to fall than wildtype mice ($F(1,68)=29.490$, $p<0.0001$), the latency to fall for all mice increased over days ($F(4,272)=118.859$, $p<0.0001$) and there was a significant day by genotype interaction ($F(4,272)=7.782$, $p<0.0001$), as there was no increase from day three to four in wildtype mice, though there was in transgenic mice ($p<0.05$) (Figure 6.3). There was a significant sex by foster mother genotype interaction on the learning score ($F(1,68)=4.455$,

p=0.038), though post-hoc analyses found no significant differences between groups and there were no main effects of pup genotype, sex, or foster mother genotype (Figure 6.4 and Figure 6.7).

4.2.7 Morris Water Maze

There was a significant interaction between pup genotype and sex on the latency to find the hidden platform ($F(1,68)=8.454$, $p=0.005$) though post-hoc analyses indicated no significant differences between groups (Figure 7.27). The latency to the platform decreased over time for all mice ($F(5,340)=47.787$, $p<0.0001$), and there was a trend for a day by foster mother genotype interaction (Figure 7.13) ($F(5,340)=2.092$, $p=0.066$) though post-hoc analyses found no significant differences between groups over days. Transgenic mice took a significantly longer distance to reach the hidden platform than wildtype mice ($F(1,68)=6.669$, $p=0.012$), and the distance to the platform decreased significantly across days for all mice ($F(5,340)=43.844$, $p<0.0001$) (Figure 7.14). Transgenic mice swam significantly faster than the wildtype mice ($F(1,68)=15.541$, $p<0.0001$) and there was a significant sex by pup genotype interaction ($F(1,68)=7.758$, $p=0.007$) as transgenic females swam significantly faster than all other groups ($p>0.05$) (Figure 7.15 and Figure 7.28). The swim speed of all mice increased over time ($F(5,340)=5.710$, $p<0.001$). Transgenic mice spent significantly more time in thigmotaxis (Figure 7.16) than wildtype mice ($F(1,68)=122.784$, $p=0.007$) and there was a significant pup genotype by sex interaction (Figure 7.29) ($F(1,68)=4.271$, $p=0.043$) as wildtype males had significantly less thigmotaxis than transgenic males and transgenic females $p>0.05$) (Figure). The amount of thigmotaxis decreased with time for all mice ($F(5,340)=17.277$, $p<0.0001$) and there was a significant day by pup genotype interaction

($F(5, 340)=3.170$, $p=0.008$) as there was a trend for transgenic mice to spend more time in thigmotaxis than wildtype mice on days one and five ($p=0.072$), and there trend for a day by foster mother interaction ($F(5,304)=2.036$, $p=0.073$), though post-hoc analyses revealed no significant differences between foster mother genotypes across days.

Mice reared by wildtype mothers had a significantly larger latency reversal effect than mice reared by transgenic mice ($F(1,68)=4.537$, $p=0.037$) (Figure 7.17). There were no differences between groups on the distance reversal effect (Figure 7.18). There were no differences between groups on the swim speed reversal effect or the thigmotaxis reversal effect.

All mice spent significantly more time in the correct quadrant than any other quadrant ($F(3,204)=161.632$, $p<0.0001$) and there was a significant genotype by sex by foster mother interaction ($F(1,68) =6.710$, $p=0.012$), though post-hoc analyses revealed no significant differences between groups (Figure 7.19 and Figure 7.30). All mice made significantly more annulus crossings in the correct quadrant than any other ($F(3,204)=95.545$, $p<0.0001$), and there was a significant quadrant by genotype by sex interaction ($F(3,204)=3.723$, $p=0.012$), though post-hoc analyses revealed no significant differences between groups across quadrants (Figure 7.20).

There was no effect of pup genotype, sex, or foster mother genotype on latency to the visual platform (Figure 7.21). Transgenic took a significantly longer distance to reach the visual platform than wildtype mice ($F(1,68)=4.736$, $p=0.033$) (Figure 7.22). Transgenic mice had a faster swim speed than wildtype mice ($F(1,68)=6.630$, $p=0.012$) and there was a significant genotype by sex interaction ($F(1,68)=5.756$, $p=0.019$) as female transgenic mice swam significantly faster than wildtype mice of both sexes

($p<0.01$) and tended to swim faster than male transgenic mice ($p=0.051$) (Figure 7.23 and Figure 7.31). Transgenic mice spent more time in thigmotaxis than wildtype mice during the visual trial ($F(1,68)=4.454$, $p=0.038$) and females spent more time in thigmotaxis than males ($F(1,68)=5.426$, $p=0.023$) (Figure 7.24 and Figure 7.32).

4.2.8 Social Dominance Tube Test

There was no significant difference in the proportion of wins between wildtype and transgenic mice ($\chi^2=0.053$, $p=0.819$) (Figure 8.2).

4.2.9 Social Preference / Novelty

There were no significant differences between groups in mouse/objet time (Figure 9.5), mouse/object interaction (Figure 9.6), or familiar/novel time preference scores (Figure 9.7). There was a significant sex by foster mother genotype interaction on the familiar/novel interactions preference score ($F(1,66)=4.391$, $p=0.040$), though post-hoc analyses determined there were no significant differences between groups (Figure 9.8 and Figure 9.9).

4.2.10 Conditioned Odour Preference Task

In the memory test for the odours learned at two months of age six months of age there were no significant differences between any groups, all mice who dug remembered the odours (Figure 10.2). Mice reared by transgenic foster mothers spent a significantly greater percentage of time digging in the CS+ of the six months odours in the conditioned odour preference task ($F(1,63)=4.590$, $p=0.036$), there was no effect of pup genotype or sex and no interactions (Figure 10.3).

Chapter 5. Discussion

5.1. Evaluation of Hypotheses and Comparison to the Literature

The main effects of genotype, foster mother genotype and sex for the main aspects of the test battery at all ages tested are summarized in Tables 3.1, 3.2, and 3.3, respectively.

5.1.1 Body Weight

As we hypothesized, males weighed more than females at two and six months of age. We predicted that transgenic mice would weigh more than wildtype mice, but at two months of age male wildtype mice weighed more than male transgenic mice, and at six months of age transgenic females weighed more than wildtype females. This differs from previous studies which have reported that transgenic mice weigh more than wildtype mice of both sexes (Gimenez-Llort et al., 2010; Julien et al., 2010). This difference may be the result of differences in early life environment, as all our mice were cross fostered, or differences in other environmental factors, such as diet.

5.1.2 Home Cage Observations

We expected that transgenic mice and males would exhibit more agonistic and less affiliative behaviour than wildtype mice and females, and that the difference between transgenic mice and wildtypes would increase with age. This prediction was partially upheld, as transgenic mice had more agonistic behaviours at two months , and males had more agonistic behaviours than females at two and six months of age. This pattern of results suggests that, overall, transgenic mice and males tended to be more agonistic than wildtype mice and females at two and six months of age. This is, to the authors' knowledge, the only study to examine social behaviour in these mice.

5.1.3 Elevated Plus Maze

We expected that transgenic mice might exhibit less anxiety on the elevated plus maze, which our results supported. Transgenic mice spent less time in the closed arms than wildtype mice at all ages tested and generally had higher levels of exploratory behaviours. We expected that transgenic mice would display increased locomotor behaviour; however we found decreased locomotion at all ages tested, as transgenic mice travelled a shorter distance than wildtypes. Mice reared by wildtype foster mothers tended to have increased locomotion at two and six months of age, and increased anxiety at two months of age, and there were very few differences between sexes in the elevated plus maze. The finding that transgenic mice display less anxiety-like behaviour than wildtype mice is similar to the findings of Sterniczuk et al. (2010), but differs from others who found no differences between genotypes. Previous studies found that transgenic mice displayed increased locomotor behaviour, while we found the opposite (Peitropaolo, Feldon, and Yee, 2008; Gimenez-Llort et al., 2007). The testing procedures and apparatus, and age at testing of those who found differing results are similar to ours, so the reason for this discrepancy remains unclear.

5.1.4 Open Field

In the open field we expected that transgenic mice would have an increased level of anxiety which would increase with age, though the literatures provides conflicting reports, and we expected increased locomotion in transgenic mice. We found that transgenic mice tended to have lower anxiety at two and six months of age, and there were no differences in locomotion. At two months of age mice reared by transgenic foster mothers tended to display lowered anxiety than mice reared by wildtype foster

mothers, as they made fewer centre square entries, but had few differences on other measures. Overall there were few differences between the sexes in the open field. The finding of lowered anxiety is consistent with our findings in the elevated plus maze, but again discrepant with the literature on the subject, though the procedures and apparatus used are roughly similar (Peitropaolo, at al., 2008; Sterniczuk et al., 2010, Gimenez-Llort et al., 2007; Gimenez-Llort et al., 2010).

5.1.5 Auditory startle and prepulse inhibition

We expected that transgenic mice would have a deficit in prepulse inhibition that would increase with age, and that transgenic mice would have a larger acoustic startle response than wildtypes, and that males would have a larger no-stimulus response than females. Our results did not support our prediction that transgenic mice would have a deficit in PPI, and there were few differences between groups in PPI. As hypothesized, transgenic mice had a larger acoustic startle response than wildtype mice at all ages. Interestingly, females had a larger acoustic startle response than males at two and six months of age. Unfortunately the only study to assess acoustic startle in this strain used only male mice (Peitropaolo et al., 2008). Our hypothesis that males would consistently have a larger no-stimulus response than females was not supported; only at six months of age did males have a larger response. Our finding that transgenic mice have a lager acoustic startle response than wildtype mice is similar to the findings of Peitropaolo et al., (2008). The transgenic mice may have no deficit in PPI because their neuropathology and genetics differ from those transgenic models of AD reported to develop deficits in PPI, or they may have not yet suffered enough neurodegeneration to cause deficits in this test (McCool et al., 2003).

5.1.6 Rotarod

For the Rotarod we hypothesized that transgenic mice may have better motor performance than wildtype mice at two months, but that any differences would decrease with age. Our hypothesis was partially supported, as transgenic mice had a longer latency to fall than wildtype mice at all ages tested. There were few differences between foster mother genotypes, and at two months of age, female mice had a greater latency to fall off the Rotarod than male mice. This increased performance on the Rotarod at two and six months of age is different from the study by Sterniczuk et al. (2010) who found no difference at seven to eleven months of age. This difference may be the result of the substantially different testing procedures, as Sterniczuk et al. (2010) tested mice on only three trials by placing them on an already rotating rod, while our procedure begins with a stationary rod and consists of six trials per day over five days, or the different ages tested, therefore comparing performance between studies even on day one may not be appropriate.

5.1.7 Morris Water Maze

We predicted that transgenic mice would be similar to wildtype mice at two months of age, but develop deficits in learning and memory by six months of age. We found a deficit in learning, but not memory at both two and six months of age in transgenic mice, as demonstrated by transgenic mice taking greater distances to reach the platform and spending more time in thigmotaxis than wildtype mice. However by the end of the training phase of the MWM, mice of both genotypes performed similarly. These results differ from those reported in the literature. There have been no reported differences in learning in the MWM at two months of age, while we found differences in

learning at two and six months of age, and others have reported a deficit in memory at four months of age, but no deficit in learning, though we found no deficit in memory at two or six months of age (Billings et al., 2005; Clinton et al., 2007). The difference in learning that we observed in the 3xTg-AD relative to the wildtype controls is that transgenic mice have a greater latency to the platform at two months of age, a greater distance travelled to the hidden platform and greater thigmotaxis at six months of age. Previous studies have only measured latency to the platform, which is confounded by swim speed, and we found that transgenic mice swam significantly faster than wildtype mice throughout the test which explains the lack of a difference in latency at six months of age (Billings et al., 2005; Billings et al., 2007; Clinton et al., 2007; Gimenez-Llort et al., 2007; Gimenez-Llort et al., 2010; Pietropaolo et al., 2008). It is possible that the mice in previous experiments had a deficit in acquisition, but that it was simply not detected with the measures used.

The lack of a memory deficit at six months of age may be the result of practice, though a study on the effect of previous experience in the MWM found that while previous experience in the MWM improves performance, it did not mask the differences between genotypes at nine months of age (Billings et al., 2007). Because Billings et al. (2007) used mice that were nine months of age, it is possible that the deficit at six months is small enough to be masked by training effects.

Transgenic mice had a faster swim speed than wildtype mice, and transgenic females tended to swim faster than all other mice, which demonstrate increased motor performance, supporting the results in the Rotarod.

For the reversal effects, mice reared by wildtype mothers had a larger latency

reversal effect at two and six months of age, which may represent difficulty in learning a new platform location or performance.

In the visual trials transgenic mice took a longer distance to reach the platform than wildtype mice. This may be a result of transgenic mice having a greater difficulty in switching tasks from locating the hidden platform to locating a visible platform. Previous studies have found that transgenic mice either performed no differently than wildtypes or had improved performance. The discrepancy between our findings and the literature may be due to differences in testing procedure, as other experiments had the visible platform test before the hidden platform test and so mice would be naïve to the apparatus and have not yet learned the location of another escape platform (Billings et al., 2005; Billings et al., 2007; Clinton et al., 2007; Gimenez-Llort et al., 2007; Peitropaolo et al., 2008).

5.1.8 Social Dominance Tube Test

We expected that transgenic mice would win more of the tube test trials than wildtype mice, as we expected them to be more aggressive and thus socially dominant, but there were no differences at any age. These results suggest that social dominance, as measured by this test, appears to remain unaffected by the transgenes at two and six months of age.

5.1.9 Social Novelty/Preference

We hypothesized that transgenic mice would have a decreased preference for social novelty at six months of age as they may develop impairments in memory or social recognition. Our results did not support this hypothesis. We found that at two months of age transgenic mice showed more interactions with the mouse than the object, but not more time spent interacting and no differences in the novel/familiar test. At six

months of age there were no differences between groups. Mice reared by transgenic mothers tended to have a greater preference for interacting with the mouse than the object at two months. Overall, mice tended to prefer interacting with the mouse rather than the object, but there seemed to be little difference in time spent interacting with the novel or familiar mouse. This may be because of the strain chosen as demonstrator mice, C57BL/6J, as studies have shown that mice may have difficulty in identifying different individuals in this strain (Pearson et al., 2010). Overall this test does not seem to have been an effective measure of social behaviour.

5.1.10 Conditioned Odour Preference Task

Our hypothesis was that the transgenic mice would develop a deficit in memory as displayed by a decrease in percentage of time spent digging in the CS+, in the conditioned odour preference task with age. We found little evidence to support this hypothesis. At two months of age transgenic mice spent a lower percentage of their time than wildtype mice digging in the in the CS+, but there were no differences at six months and no differences in the 4 month memory test of the odour learned at 2 months of age, an assessment of long term memory. At six months of age mice reared by transgenic mothers spent more time digging in the CS+ in the short-term (24 hour) memory test than mice reared by wildtype mothers, and there were no differences between sexes in this test. The results from this test support our finding of no memory deficits in the MWM.

5.2 Neurodevelopment and Later Life Performance

Pup genotype had a significant effect on neurodevelopment in these mice from 2-24 days of age (Blaney et al., 2012; Table 1). The 3xTg-AD mice developed motor

reflexes earlier than wildtype pups, but had delayed sensory reflexes and decreased activity in the open field, and less habituation to the novel object in the object recognition test. The genotype of the foster mother also had a significant effect on development; several reflexes were affected, though there was no overall trend for delayed or accelerated development. The results of the neurodevelopmental test battery relates to later life performance in several ways. The decreased activity in the 3xTg-AD mice as measured the automated open field continued post-weaning, as transgenic mice had decreased levels of activity compared to wildtypes at two and six months of age (Tables 1 and 3). The earlier development of motor reflexes in transgenic mice may be related to the increased motor performance in transgenic mice at two and six months of age in the Rotarod and the increased swim speed in the MWM. The poorer habituation to the novel object in transgenic mice may be a sign of an early cognitive deficit, which is similar to our finding of impaired learning in the MWM at two and six months of age. While there were several differences between mice reared by wildtype and transgenic mice, there was little consistency in the findings, and this pattern of results continued at two and six months of age. Overall it appears that many of the differences between transgenic and wildtype pups begin before the development of any neuropathology during the early postnatal period, and are stable into early adulthood.

5.3 General Discussion

Overall, transgenic mice appear to have an increased preference for socialization at two months of age, decreased anxiety-like behaviours and locomotion in the elevated plus maze and open field, increased motor performance on the Rotarod and MWM, and impaired learning, but not memory, in the MWM, and few differences in others

behaviour between two and six months of age (Table 3.1).

The effects of maternal genotype on behaviour do not appear to follow a general pattern. Though there were several differences between maternal genotypes, they were often conflicting or did not appear on other measures of the same trait. Overall there were fewer differences between maternal genotypes at six months of age than at two months of age (Table 3.2).

There were several effects of sex during testing at two and six months of age. Males consistently weighed more than females, females had a larger acoustic startle response, and males exhibited more agonistic behaviours than females. In humans, women have a greater risk to develop AD than men (Andersen et al., 1999). Though there were several tests in which sex modulated the effect of genotype, there was no overall trend for sex modulating the effect of genotype or foster mother genotype in one direction (Table 3.3).

Genotype and sex effects appeared to be generally stable from two to six months of age, while the effect of foster mothers genotype appears to be decreasing with age (Tables 3.1, 3.2, and 3.3). Body weight increased from two to six months of age (Figure 1), and behaviours in home cage observations, elevated plus maze, and open field remained relatively consistent, from two to six months of age (Figures 2, 3 and 4). The initial startle response appears to have increased from two to six months of age, though the no-stimulus trials and overall PPI effect appear consistent (Figure 5), as does performance on the Rotarod (Figure 6). Performance on the MWM, especially day one performance, appears to generally improve from two to six months of age, though probe trial performance appears consistent (Figure 7). The tube test performance was very

similar at two and six months of age (Figure 8), though the genotype and foster mother genotype effects that were present at two months have decreased by six months of age in the social novelty/preference task (Figure 9). In the conditioned odour preference task the deficit in short term memory that was present at two months of age in transgenic mice is not present at six months of age (Figure 10).

As previously discussed, the literature on the behaviour of the 3xTg-AD strain provides conflicting reports on most aspects of behaviour, and the results outlined here do little to remedy this situation. We have found some results that are consistent with the literature, for example the deficit in learning in the MWM at six months of age, or the increased acoustic startle in transgenic mice, but many of our other findings differ from the findings of others. There are several general factors that may be responsible for the discrepancy in findings between researchers using this strain. Testing procedure and apparatus design varied to differing degrees between the researchers; the consistent findings within laboratories (Billings et al., 2005 and Billings et al., 2007; Gimenez-Llort et al., 2007 and Gimenez-Llort et al., 2010) may be the result of consistency in procedure and environment. Another factor that could affect results is the sex of the mice used, as many of the studies that have examined behaviour in these mice have used only one sex, or both sexes but not analyzed the effect of sex, which could impact the results. The sexes used and whether the effect of sex was analyzed for the studies cited are summarized in Table 4. Another factor is the strain of mice used as a control. The majority of studies on this strain have used transgenic and control mice obtained directly from Dr. LaFerla (University of California at Irvine), which are a hybrid strain (C7BL/6;129X1/SvJ;129S1/Sv). Sterniczuk et al. (2010) used C57BL/6J mice. The

3xTg-AD mice are now available commercially through the Mutant Mouse Regional Resource Center (stock # 034830-JAX) and the strain recommended as a control is the B6129S/F2, which is an approximate control to the original. Original control mice from Dr. LaFerla, and the control mice commercially available, as well as C57B/6J mice, may behave differently on different tests, as even sub-strains in the 129 line can differ in behaviour (Cook et al., 2002). Finally, there is the point of origin; mice from different sources may have different aspects in behaviour, as there have been differences in neuropathology reported (Hirata-Fikae et al, 2008).

Studying the behaviour of mouse models of AD early in development is important for evaluating the suitability of the strain as a model of AD, as well as for studying new treatments or diagnostic techniques. The ideal model of Alzheimer's disease would have normal early development, as AD is an adult disease, and develop progressive deficits at some point in adulthood, and so it is important to study behaviour throughout development. Early behavioural testing also provides baseline behaviour that can be used for comparison when testing novel treatments for AD. On the other hand, there is now evidence that AD may begin much earlier than originally thought (de Waal et al., 2012), this the study of early development in AD model mice may give some insight into the neurodevelopmental origins of AD (Simmons, 2009; Miller and O'Callaghan, 2008) and some of these effects may be mediated by maternal effects (Champagne and Curley, 2009).

5.4. Suitability of this Strain as a Model of Alzheimer's Disease

Though we found many differences between wildtype and transgenic mice, the only consistent effects that we observed which would be expected in a mouse model of

AD are a deficit in learning in the MWM, and enhanced acoustic startle response, which has been suggested to represent hippocampal hypofunctionality (Pietropaolo et al., 2008). The finding that these mice have differences in behaviour as early as two months of age is interesting as it demonstrates there are differences in behaviour before any overt neuropathology is present, which may indicate that these transgenes are influencing behaviour through other mechanisms (Oddo et al., 2003; Mastrangelo and Bowers, 2008). Overall it appears that at two and six months of age this strain is beginning to demonstrate some aspects that would be expected in a mouse model of AD, though a complete assessment of its suitability will require behavioural assessment at more advanced ages. These mice have been tested using the same procedures at twelve months of age and are currently be tested at eighteen months of age. After behavioural testing is complete at eighteen months of age the mice will be sacrificed and their brains will be analyzed for the presence of amyloid beta plaques and neurofibrillary tangles using immunohistochemistry to confirm the presence of pathology, and compare the levels of pathology between mice reared by transgenic and wildtype foster mothers.

When interpreting the results of this, or any study, it is important to take into consideration the replicability of the findings. As discussed in section 1.4.2, some measures of mouse behaviour, mainly those involving emotionality, can vary significantly even when all experimental protocols are controlled. This may provide an explanation for some of the discrepancies between our research and previous studies. However, the research on variability in mouse testing has found that some measures, for example locomotor activity, performance on the Morris water maze, and differences with large effect sizes, are stable between laboratories (Crabbe et al., 1998; Wahlsten et al.,

2003; Whalsten et al., 2006). Overall, it is likely that our findings in the tests of memory, and the large differences found in motor performance, are more likely to be replicable differences between transgenic mice and wildtype controls.

Another important consideration when interpreting the results of this study is that the 3xTg-AD may suffer from circadian rhythm disruptions, as do human patients with AD (Vitiello et al, 1992). The timing of testing during the dark:light cycle is an important consideration in mouse behavioural testing as it can affect performance on many tasks. If the 3xTg-AD mice are suffering from circadian rhythm disruptions then this could add variability to the results of our behavioural studies. A study measuring the activity levels of these mice over time would be useful to determine if they are suffering from disruptions compared to control mice, and this should be taken into account in future behavioural testing.

5.5. Conclusions

We have demonstrated that the 3xTg-AD mouse model of AD has decreased anxiety-like behaviours, decreased locomotion, increased startle response, and deficits in visually-dependent learning in the MWM at two and six months of age, and that foster mother genotype can affect the behaviour of these mice. Further behavioural analysis at more advanced ages along with an assessment of the neuropathology in these mice will provide a more complete assessment of the effect of the transgenes, maternal genotype and the early life environment, and age on these mice.

References

Alzheimer's Society of Canada (2010) Rising tide: The impact of dementia on Canadian society. Online. http://www.alzheimer.ca/en/Get-involved/Raise-your-voice/Rising-Tide

Andersen K, Launer LJ, Dewey ME, Letenneur L, Ott A, Copeland JRM, Dartigues JF, Kragh-Sorensen P, Baldereschi M, Brayne C, Lobo A, Martinez-Lage JM, Stijnen T, Hofman, A (1999) Gender differences in the incidence of AD and vascular dementia: The EURODEM Studies. Neurology, 53(9):1992-1997

Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT (1992) Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology, 42:631-639

Bierer LM, Hof PR, Purohit DP, Carlin L, Schmeidler J, Davis KL, Perl DP (1995) Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. Archives of Neurology 52:81-88

Bilbul M and Schipper HM (2011) Risk profiles of Alzheimer disease. Canadian Journal of Neurological Science, 38:580-592

Billings LM, Green KN, McGaugh JL, LaFerla FM (2007) Learning decreases A beta*56 and tau pathology and ameliorates behavioral decline in 3xTg-AD mice. The Journal of Neuroscience 27:751-761

Billings LM, Oddo S, Green KN, McGaugh JL, LaFerla FM (2005) Intraneuronal Abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. Neuron 45:675-688

Blaney C, Gunn R, Brown R (2012) Maternal genotype influences behavioral development of 3xTg-AD mouse pups. Submitted to Genes, Brain, and Behaviour 16/06/2012.

Borenstein AR, Copenhaver CI, Mortimer JA (2006) Early-life risk factors for Alzheimer disease. Alzheimer Disease and Associated Disorders 20(1):63-72

Braak H, and Braak E (2001) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathologia, 82:239-259

Brown RE, Corey SC, Moore AK (1999) Differences in measures of exploration and fear in MHC-congenic C57BL/6J and B6-H-2K mice. Behavior Genetics 29:263–271

Brown RE, Mathieson WB, Stapleton J, Neumann PE (1999) Maternal behavior in female C57BL/6J and DBA/2J inbred mice. Physiology & Behavior, 67(4):599-605

Brown RE, Wong AA (2007) The influence of visual ability on learning and memory performance in 13 strains of mice. Learning & Memory 14:134-144

Crabbe JC, Wahlsten D, Dudek BC (1999) Genetics of mouse behavior: Interactions with laboratory environment. Science, 284:1670-1672

Champagne FA, and Curley JP (2009) Epigenetic mechanisms mediating the long-term effects of maternal care on development. Neuroscience and Biobehavioral Reviews 33:593-600

Chemerinski E, Petracca G, Manes F, Leiguarda R, Starkstein SE (1998) prevalence and correlates of anxiety in Alzheimer’s disease. Depression and Anxiety 7:166–170

Chouliaras L, Sierksma ASR, Kenis G, Prickaerts J, Lemmens MAM, Brasnjevic I, van Donkelaar EL, Martinez-Martinez P, Losen M, De Baets MH, Kholod N, van Leeuwen F, van Os J, Steinbusch HWM, van den Hove DLA, Rutten BPF (2010) Gene-environment interaction research and transgenic mouse models of Alzheimer’s disease. International Journal of Alzheimer’s Disease, 2010:1-27

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

Clinton LK, Billings LM, Green KN, Caccamo A, Ngo J, Oddo S, McGaugh JL, LaFerla FM (2007) Age-dependent sexual dimorphism in cognition and stress response in the 3xTg-AD mice. Neurobiology of disease 28:76-82

Cook MN, Bolivar VJ, McFadyen MP, Flaherty L (2002) Behavioral differences among 129 substrains: Implications for knockout and transgenic mice

Cummings JL (2003) Frequency and characteristics of anxiety among patients with Alzheimer’s disease and related dementias. The Journal of Neuropsychiatry and Clinical Neurosciences 15:180-186

D’Andrea I, Alleva E, Branchi I (2007) Communal nesting, an early social enrichment, affects social competences but not learning and memory abilities at adulthood. Behavioural Brain Research 183:60-66

de Waal H, Stam CJ, de Haan W, van Straaten ECW, Scheltens P, van der Flier W (2012) Young Alzheimer patients show distinct regional changes of oscillatory brain dynamics. Neurobiology of Aging, 33: 1008.e25 -1008.e31

Desai MK, Sudol KL, Janelsins MC, Mastrangelo M a, Frazer ME, Bowers WJ (2009) Triple-transgenic Alzheimer’s disease mice exhibit region-specific abnormalities in brain myelination patterns prior to appearance of amyloid and tau pathology. Glia 57:54-65

Devanand DP, Michaels-Marston KS, Liu X, Pelton GH, Padilla M, Marder K, Bell K, Stern Y, Mayeux R (2000) Olfactory deficits in patients with mild cognitive impairment predict Alzheimer’s disease at follow-up. American Journal of Psychiatry 157:1399-1405

Dick MB, Nielson KA, Beth RE, Shankle WR, Cotman CW (1995) Acquisition and long term retention of a fine motor skill in Alzheimer’s disease. Brain and Cognition, 29:294-306

Doty RL, Reyes PF, Gregor T (1987) Presence of both odor identification and detection deficits in Alzheimer's disease. Brain Research Bulletin 18(5):597-600

Eslinger PJ, Damasio AR (1986) Preserved motor learning in Alzheimer's disease: implications for anatomy and behavior. The Journal of Neuroscience, 6(10):3006-3009

Ferretti L, McCurry SM, Logsdon E, Gibbons L, Teri L (2001) Anxiety and Alzheimer's disease. Journal of Geriatric Psychiatry and Neurology 14:52-58

Giménez-Llort L, Blázquez G, Cañete T, Johansson B, Oddo S, Tobeña A, LaFerla FM, Fernández-Teruel A (2007) Modeling behavioral and neuronal symptoms of Alzheimer's disease in mice: A role for intraneuronal amyloid. Neuroscience and Biobehavioral Reviews 31:125-147

Giménez-Llort L, García Y, Buccieri K, Revilla S, Suñol C, Cristofol R, Sanfeliu C (2010) Gender-specific neuroimmunoendocrine response to treadmill exercise in 3xTg-AD mice. International Journal of Alzheimer's Disease, 2010:1-17

Grant EC, Mackintosh JH (1963) A comparsion of social postures of some common laboratory rodents. Behaviour 23:246-259

Haroutunian V, Purohit DP, Perl DP, Marin D, Khan K, Lantz M, Davis KL, Mohs RC (1999) Neurofibrillary tangles in nondemented elderly subjects and mild Alzheimer disease. Archives of Neurololgy 56(6):713-718

Harper DG, Volicer L, Stopa EG, McKee AC, Nitta M, Satlin A (2005) Disturbance of endogenous circadian rhythm in aging and Alzheimer disease. American Journal of Geriatric Psychiatry,13:359-368

Hock C, Konietzko U, Streffer JR, Tracy J, Signorell A, Muller-Tillmanns B, Lemke U, Henke K, Moritz E, Garcia E, Wollmer MA, Umbricht D, de Quervain DJF, Hofmann M, Maddalena A, Papassotiropoulos A, Nitsch RM (2003) Antibodies against β-Amyloid slow cognitive decline in Alzheimer's disease. Neuron, 38:547-554

Julien C, Tremblay C, Phivilay A, Berthiaume L, Émonda V, Julien P, Calon F (2010) High-fat diet aggravates amyloid-beta and tau pathologies in the 3xTg-AD mouse model. Neurobiology of Aging 31:1516-1531

Kluger A, Gianutsos JG, Golomb J, Ferris SH, Reisberg B (1997) Motor/psychomotor dysfunction in normal aging, mild cognitive decline, and early Alzheimer's disease: Diagnostic and differential diagnostic features. International Psychogeriatrics, 9:307-316

Koh H-Y, Kim D, Lee J, Lee S, Shin H-S (2008) Deficits in social behavior and sensorimotor gating in mice lacking phospholipase Cbeta1. Genes, Brain, and Behavior 7:120-128

Koss E, Weiffenbach JM, Haxby JV, Friedland RP (1988) Olfactory detection and identification performance are dissociated in early Alzheimer's disease. Neurology 38:1228-1232

Kovacs T, Cairns NJ, Lantos PL (1999) β-Amyloid deposition and neurofibrillary tangle formation in the olfactory bulb in ageing and Alzheimer's disease. Neuropathology and Applied Neurobiology 25:481–491

Landes AM, Sperry SD, Strauss ME, Geldmacher DS (2001) Apathy in Alzheimer's disease. Journal of the American Geriatrics Society 49:1700-1707

Lewis J, Mcgowan E, Rockwood J, Melrose H, Nacharaju P, Van M, Gwinn-hardy K, Murphy MP, Baker M, Yu X, Duff K, Hardy J, Corral A, Lin W-lang, Yen S-hui, Dickson DW, Davies P, Hutton M (2000) Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. Nature Genetics 26:402-405

Lijam N, Paylor R, McDonald MP, Crawley JN, Deng CX, Herrup K, Stevens KE, Maccaferri G, McBain CJ, Sussman DJ, Wynshaw-Boris a (1997) Social interaction and sensorimotor gating abnormalities in mice lacking Dvl1. Cell 90:895-905

Luo Y, Bolon B, Kahn S, Bennett BD, Babu-Khan S, Denis P, Fan W, Kha H, Zhang J, Gong Y, Martin L, Louis JC, Yan Q, Richards WG, Citron M, Vassar R (2001) Mice deficient in BACE1, the Alzheimer's β-secretase, have normal phenotype and abolished β-amyloid generation. Nature Neuroscience 4(3):231-232

Martin, AL, & Brown, RE (2010) The lonely mouse: Verification of a separation-induced model of depression in female mice. Behavioural Brain Reseach, 207:196-207.

Mastrangelo MA, Bowers WJ (2008) Detailed immunohistochemical characterization of temporal and spatial progression of Alzheimer's disease-related pathologies in male triple-transgenic mice. BMC Neuroscience 9:81-116

McCool MF, Varty GB, Del Vecchio RA, Kazdoba TM, Parker EM, Hunter JC, Hyde LA (2003) Increased auditory startle response and reduced prepulse inhibition of startle in transgenic mice expressing a double mutant form of amyloid precursor protein. Brain Research 994:99-106

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease. Neurology 34:939-944

Miller DB, and O'Callaghan JP (2008) Do early-life insults contribute to the late-life development of Parkinson and Alzheimer diseases? Metabolism Clinical and Experimental 57(Suppl 2):S44–S49

Moy S, Nadler J, Perez A, Barbaro R, Johns J, Magnuson T, Piven J, Crawley J (2004) Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. Genes, Brain and Behavior 3:287–302

Murray ME, Graff-Radford NR, Ross OA, Petersen RC, Duara R, Dickson DW (2011) Neuropathologically defined subtypes of Alzheimer's disease with distinct clinical characteristics: a retrospective study. Lancet Neurology 10:785-796

Murphy C, Gilmore MM, Seery CS, Salmon DP, Lasker BR (1990) Olfactory thresholds are associated with degree of dementia in Alzheimer's disease. Neurobiology of Ageing, 11(4):465-469

Oddo, S. (2003). Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. Neurobiology of Aging, 24(8):1063-1070.

Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: Intracellular Aβ and synaptic dysfunction. Neuron 39:409-421

Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, Jouanny P, Dubois B, Eisner L, Flitman S, Michel BF, Boada M, Frank A, Hock C (2003) Subacute meningoencephalitis in a subset of patients with AD after Aβ42 immunization. Neurology, 61:46-54

Pearson BL, Defensor EB, Blanchard DC, Blanchard RJ (2010) C57BL/6J mice fail to exhibit preference for social novelty in the three-chamber apparatus. Behavioural Brain Research 213:189-194

Pettersson AF, Olsson E, Wahlund LO (2005) Motor function in subjects with mild cognitive impairment and early Alzheimer's disease. Dementia and Geriatric Cognitive Disorders, 19:299-304

Pietropaolo S, Feldon J, and Yee BK (2008) Age-dependent phenotypic characteristics of a triple transgenic mouse model of Alzheimer disease. Behavioral Neuroscience 122(4)733-747

Porter VR, Buxton WG, Fairbanks LA, Strickland T, O'Connor SM, Rosenberg-Thompson S, Cummings JL (2003) Frequency and characteristics of anxiety among patients with Alzheimer's disease and related dementias. The Journal of Neuropsychiatry and Clinical Neurosciences 15:180–186

Price DL, Sisodia SS (1998) Mutant genes in familial Alzheimer's disease and transgenic models. Annual Review of Neuroscience 21:479-505

Rodríguez JJ, Jones VC, Tabuchi M, Allan SM, Knight EM, LaFerla FM, Oddo S, Verkhratsky A (2008) Impaired adult neurogenesis in the dentate gyrus of a triple transgenic mouse model of Alzheimer's disease. PloS one 3:e2935

Schellinck HM, Forestell CA, LoLordo VM (2001) A simple and reliable test of olfactory learning and memory in mice. Chemical Senses 26:663-672

Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad L, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S, (1996) Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nature Medicine, 2:864-870

Selkoe DJ (2011) Alzheimer's disease: Genes, proteins, and therapy. Physiological Reviews 81(2):742-760

Shoghi-Jadid K, Small GW, Agdeppa ED, Kepe V, Ercoli LM, Siddarth P, Read S, Satyamurthy N, Petric A, Huang SC, others (2002) Localization of neurofibrillary tangles and beta-amyloid plaques in the brains of living patients with Alzheimer disease. American Journal of Geriatric Psychiatry 10:24-35

Simmons RA (2009) Developmental origins of adult disease. Pediatric Clinics of North America, 56:449-466

Sterniczuk R, Antle MC, Laferla FM, Dyck RH (2010) Characterization of the 3xTg-AD mouse model of Alzheimer's disease: part 2. Behavioral and cognitive changes. Brain Research 1348:149-155

Szyf M, Weaver I, Meaney M (2007) Maternal care, the epigenome and phenotypic differences in behavior. Reproductive Toxicology 24:9-19

Takasugi N, Tomita T, Hayashi I, Tsuruoka M, Niimura M, Takahashi Y, Thinakaran G, Iwatsubo T (2004) The role of presenilin cofactors in the γ-secretase complex. Nature, 422(27):438-441

Terranova ML, Laviola G, Alleva E (1993) Ontogeny of amicable social behavior in the mouse: Gender differences and ongoing isolation outcomes. Developmental Psychobiology, 26(8):467-481

Teri L, Ferretti EL, Gibbons LE, Logsdon RG, McCurry SM, Kukull WA, McCormick WX, Bowen JD, Larson EB (1999) Anxiety in Alzheimer's disease: Prevalence and comorbidity. Journal of Gerontology: Medical Sciences 54A(7):M348-M352

Theuns J, Marjaux E, Vandenbulcke M, Van Laere K, Kumar-Singh S, Bormans G, Brouwers N, Van den Broeck M, Vennekens K, Corsmit E, Cruts M, De Strooper B,Van Broeckhoven C, Vandenberghe R (2008) Alzheimer dementia caused by a novel mutation located in the app c-terminal intracytosolic fragment. Human Mutation, 27(9):888-896

Tiraboschi P, Hansen LA, Thal LJ, Corey-Bloom J (2004) The importance of neuritic plaques and tangles to the development and evolution of AD. Neurology, 62(11):1984-1989

van Someren EJW, Hagebeuk EEO, Lijzenga C, Scheltens P, de Rooij SEAJ, Jonker C, Pot A, Mirmiran M, Swaab DF (1996) Circadian rest-activity rhythm disturbances in Alzheimer' s disease. Biological Psychiatry, 40:259-270

Vitiello MV, Bliwise DL, Prinz PN (1992) Sleep in Alzheimer's disease and the sundown syndrome. Neurology 42(supplement 6):83-94

Wahlsten D, Metten P, Phillips TJ, Boehm SL, Burkhart-Kasch S, Dorow J, Doerksen S, Downing C, Fogarty J, Rodd-Henricks K, Hen R, McKinnon CS, Merrill CM, Nolte C, Schalomon M, Schlumbohm JP, Sibert JR,Wenger CD, Dudek BC, Crabbe JC (2003) Different data from different labs: Lessons from studies of gene–environment interaction. Journal of Neurobiology 54(1): 283:311

Wahlsten D, Bachmanov A, Finn DA, Crabbe JC (2006) Stability of inbred mouse strain differences in behavior and brain size between laboratories and across decades. Proceedings of the national academy of sciences, 103(44): 16364–16369

Wong AA, Brown RE (2007) Age-related changes in visual acuity, learning and memory in C57BL/6J and DBA/2J mice. Neurobiology of Aging 28:1577-1593

Wu J, Basha R, Zawia NH (2008) The environment, epigenetics and amyloidogenesis. Journal of Molecular Neuroscience, 34:1-7

Wulff K, Gatti S, Wettstein JG, Foster RG (2010) Sleep and circadian rhythm disruption in psychiatric and neurodegenerative disease. Nature Reviews Neurosceince 11:1-11

Appendix 1. Tables

Table 1. Neurodevelopmental Test Battery Results. Main effects of pup genotype, foster mother genotype, and sex. WT= B6129S/F2, TG = 3xTg-AD, — = no difference, all results at least p<0.05.

Behavior	Pup Genotype	Foster Mother Genotype	Sex
Body Weight	WT > TG (PND 20)	WT > TG (PND 18)	M > F (PND 20)
Day of Appearance			
Eye Opening	—	—	F > M
Pinnae Detachment	TG > WT	—	—
Forelimb Grasp Reflex	—	—	—
Hindlimb Grasp Reflex	WT > TG	WT > TG	—
Tactile Orientation Reflex	WT > TG	—	—
Vibrissae Response	—	—	—
Auditory Startle Response	WT > TG	—	—
Day of Loss of Reflex			
Crossed Extensor Reflex	—	WT > TG	—
Activity			
Open Field: Horizontal	WT > TG	—	—
Open Field: Vertical	WT > TG	WT > TG	F > M
Other Measures			
Righting Reflex	TG > WT (PND 8&10)	—	—
Negative Geotaxis	TG > WT (PND 8&12)	—	—
Homing Test	—	—	—
Object Investigation Ratio	TG > WT	—	—
Grip Strength	WT > TG	—	F > M (PND 10-14)

Table 2.1 Distribution of Mice by Pup Genotype and Maternal Genotype at Two Months of Age.

Pup Genotype	Maternal Genotype		Total
	Wildtype	Transgenic	
Wildtype	11M, 7F	8M, 12F	38
Transgenic	8M, 14F	9M, 9F	40
Total	40	38	78

Table 2.2 Distribution of Mice by Pup Genotype and Maternal Genotype at Six Months of Age.

Pup Genotype	Maternal Genotype		Total
	Wildtype	Transgenic	
Wildtype	11M, 7F	8M, 12F	38
Transgenic	7M, 14F	9M, 8F	38
Total	39	37	76

Table 3.1 Mouse Genotype Effects**.** Differences between 3xTg-AD and wildtype controls. The — represents no difference between genotypes. In tests with multiple measures, those shown here are interpretations of the overall findings for that test.

Measure	**Test**	**Age (months)**	
		2	6
Growth	Body Weight	WT > TG (in males)	TG > WT (in females)
Anxiety	Elevated Plus Maze	WT > TG	WT > TG
	Open Field	WT > TG	WT > TG (trend)
Locomotion	Elevated Plus Maze	WT > TG	WT > TG
	Open Field	—	—
PPI and Acoustic Startle	Prepulse Inhibition	—	—
	Acoustic Startle	TG > WT	TG > WT
Motor Ability/Learning	Rotarod	TG > WT	TG > WT
	MWM Swim Speed	TG > WT	TG > WT
Learning and Memory	Conditioned odour preference	WT > TG	—
	Morris Water Maze	WT > TG (learning)	WT > TG (learning)
Social Behaviour	Home Cage Observations	TG > WT (agonistic)	TG > WT (non-social)
	Social Novelty/Preference	TG > WT	—
	Tube Test	—	—

Table 3.2 Maternal Genotype Effects. Differences between mice reared by 3xTg-AD mice and mice reared by wildtype controls. The — represents no difference between mice reared by 3xTg-AD and mice reared by wildtype controls. In tests with multiple results, those shown here are interpretations of the overall findings for that test.

Measure	**Test**	**Age (in months)**	
		2	6
Growth	Body Weight	WT > TG	—
Anxiety	Elevated Plus Maze	—	—
	Open Field	WT > TG	—
Locomotion	Elevated Plus Maze	WT > TG (trend)	WT > TG
	Open Field	—	—
PPI and Acoustic Startle	Prepulse Inhibition	—	—
	Acoustic Startle	—	—
Motor Ability/Learning	Rotarod	—	—
	MWM Swim Speed	—	—
Learning and Memory	Conditioned Odour Preference	—	TG > WT
	Morris Water Maze	TG > WT (learning)	—
Social Behaviour	Home Cage Observations	WT > TG (affiliative) TG > WT (agonistic)	—
	Social Preference/Novelty	TG > WT (trend)	—

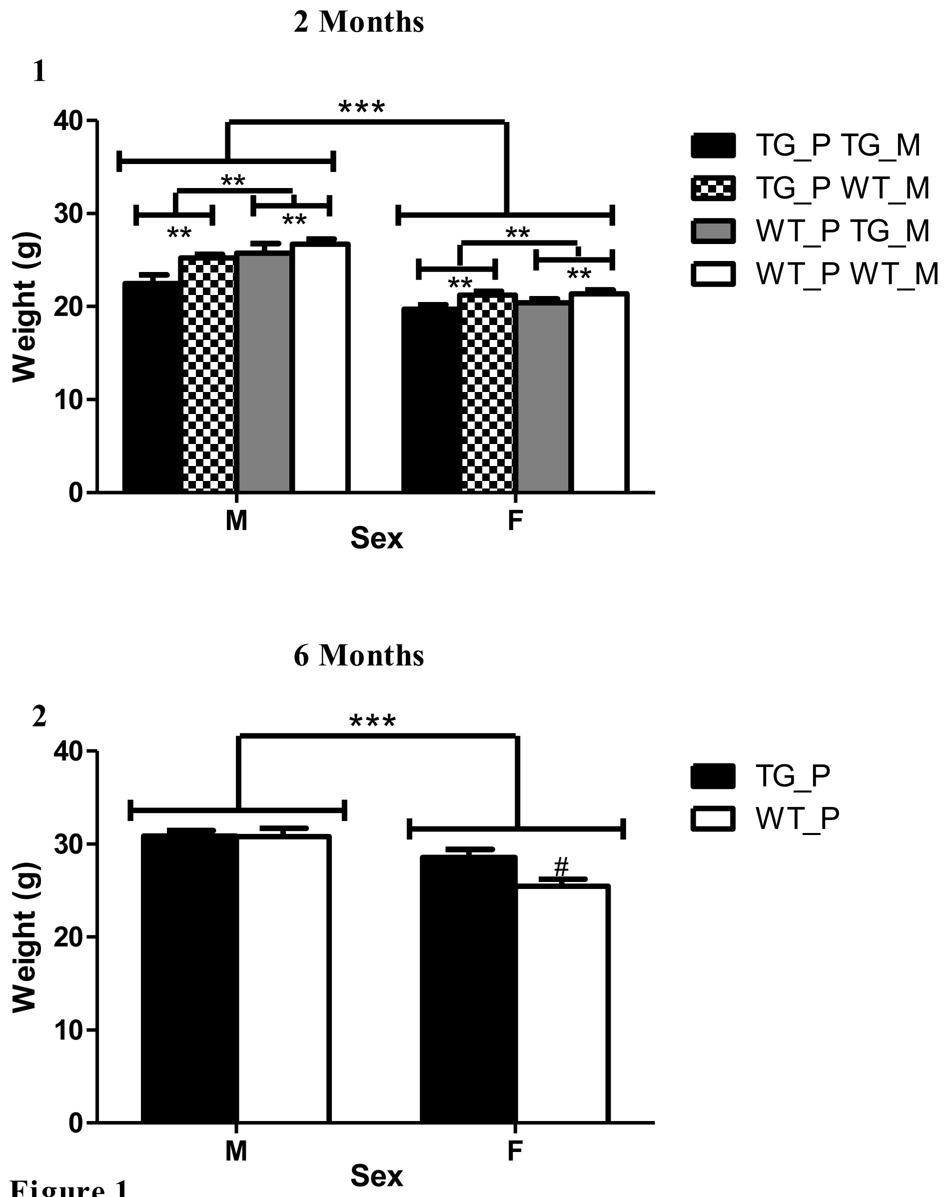
Table 3.3 Sex Effects. Differences between males in females. — = no difference between sexes. In tests with multiple results, those shown here are interpretations of the overall findings for that test. M = male, F = female.

Measure	Test	Age (in months)	
		2	6
Growth	Body Weight	M > F	M > F
Anxiety	Elevated Plus Maze	—	F > M
	Open Field	—	—
Locomotion	Elevated Plus Maze	—	—
	Open Field	—	—
PPI and Acoustic Startle	Prepulse Inhibition	—	—
	Acoustic Startle	F > M	F > M
Motor Ability/Learning	Rotarod	F > M	—
	MWM Swim Speed	—	—
Learning and Memory	Conditioned Odour Preference	—	—
	Morris Water Maze	—	—
Social Behaviour	Home Cage Observations	M > F (agnostic)	M > F (agnostic)
	Social Preference/Novelty	—	—

Table 4. Summary of Animals Used in Previous Experiments. Original = control mice provided by Dr. Frank LaFerla and are C7BL/6;129X1/SvJ;129S1/Sv, M = male, F= female, N = no analysis of sex effects, Y = an analysis of sex effects was included, N/A = only one sex was tested.

Experiment	Sex Tested	Sex Analysis	Control Strain
Behaviour			
Billings et al. (2005)	M & F	N	Original
Billings et al. (2007)	M & F	N	Original
Clinton et al. (2007)	M & F	Y	Original
Gimenez-Llort et al. (2007)	M	N/A	Original
Gimenez-Llort et al. (2010)	M & F	Y	Original
Sterniczuk et al. (2010)	F	N/A	C57B/6J (Novartis)
Pietropaolo et al. (2008)	M	N/A	Original
Neuropathology			
Oddo et al. (2003)	?	N	Original
Mastrangelo and Bowers (2008)	M	N/A	Original
Rodriguez et al. (2008)	M & F	Y	Original

Appendix 2. Figures



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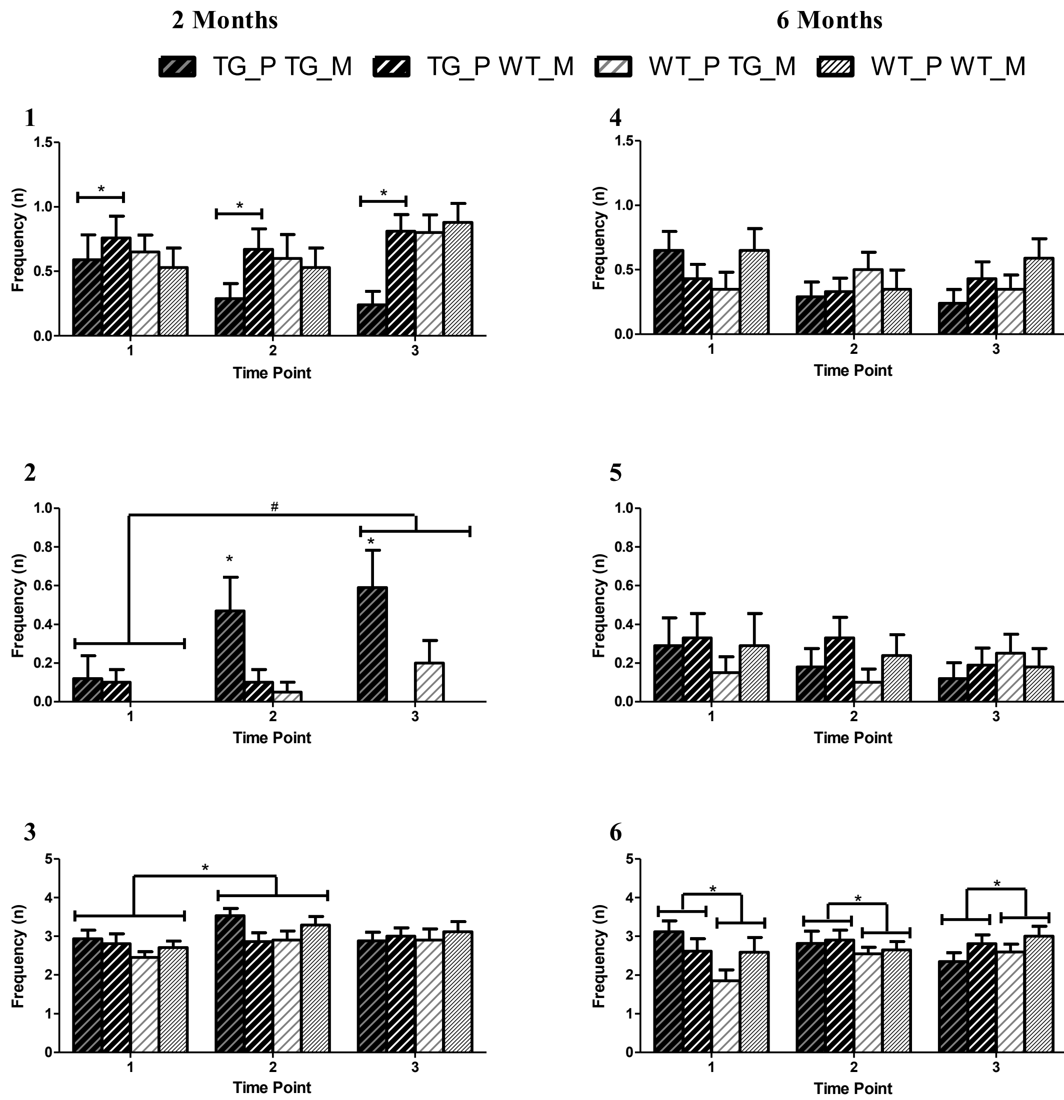

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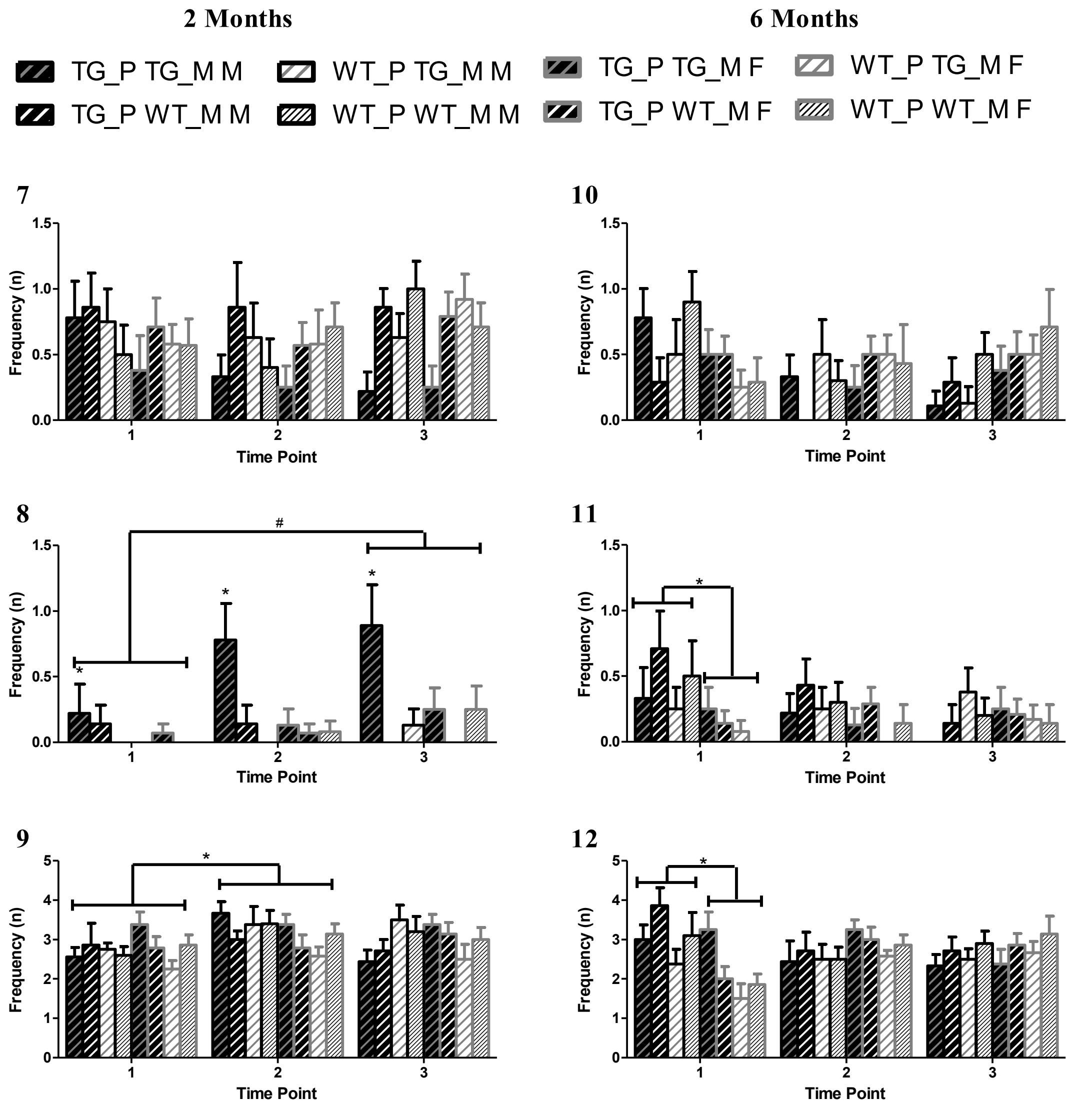

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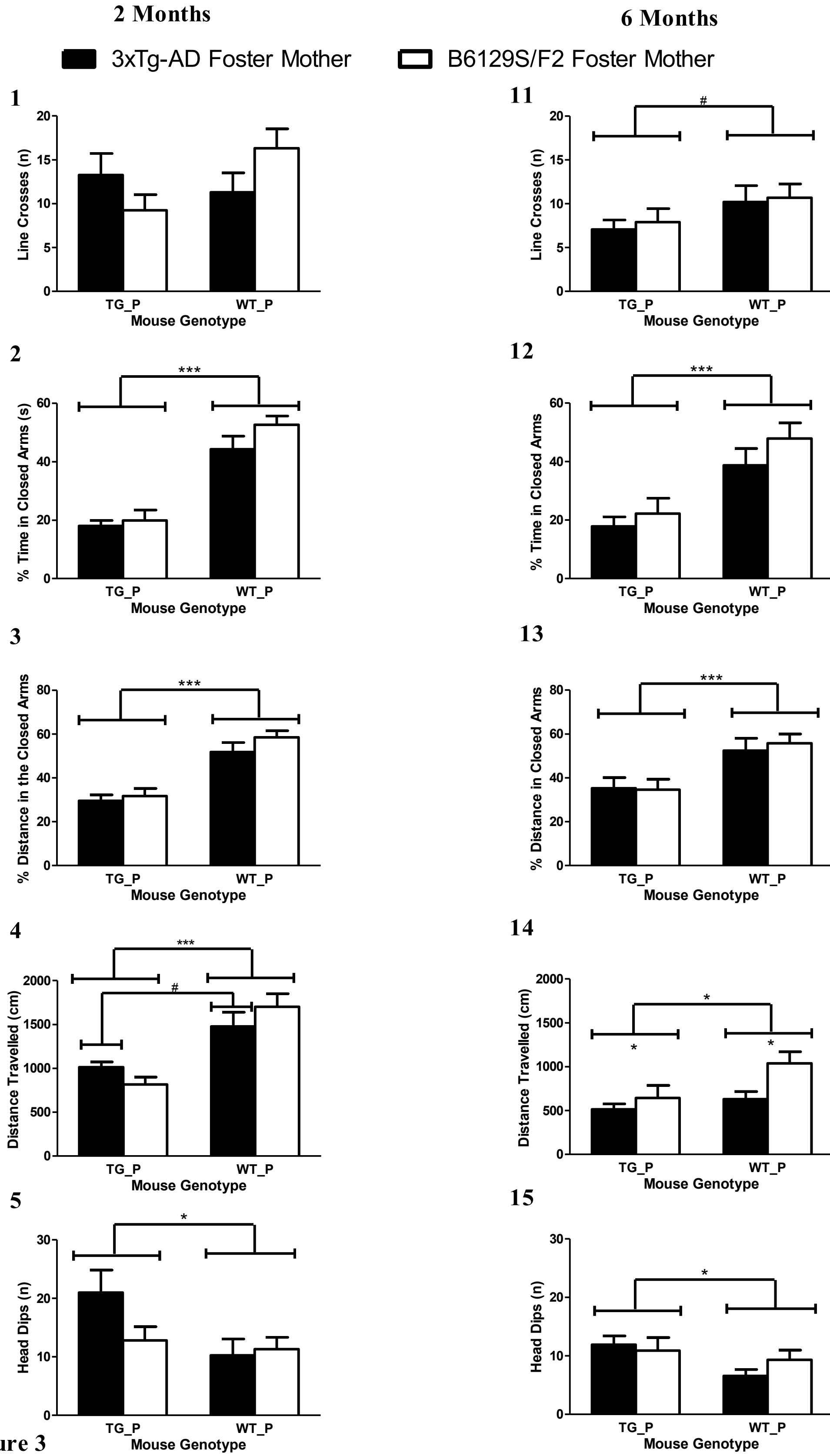

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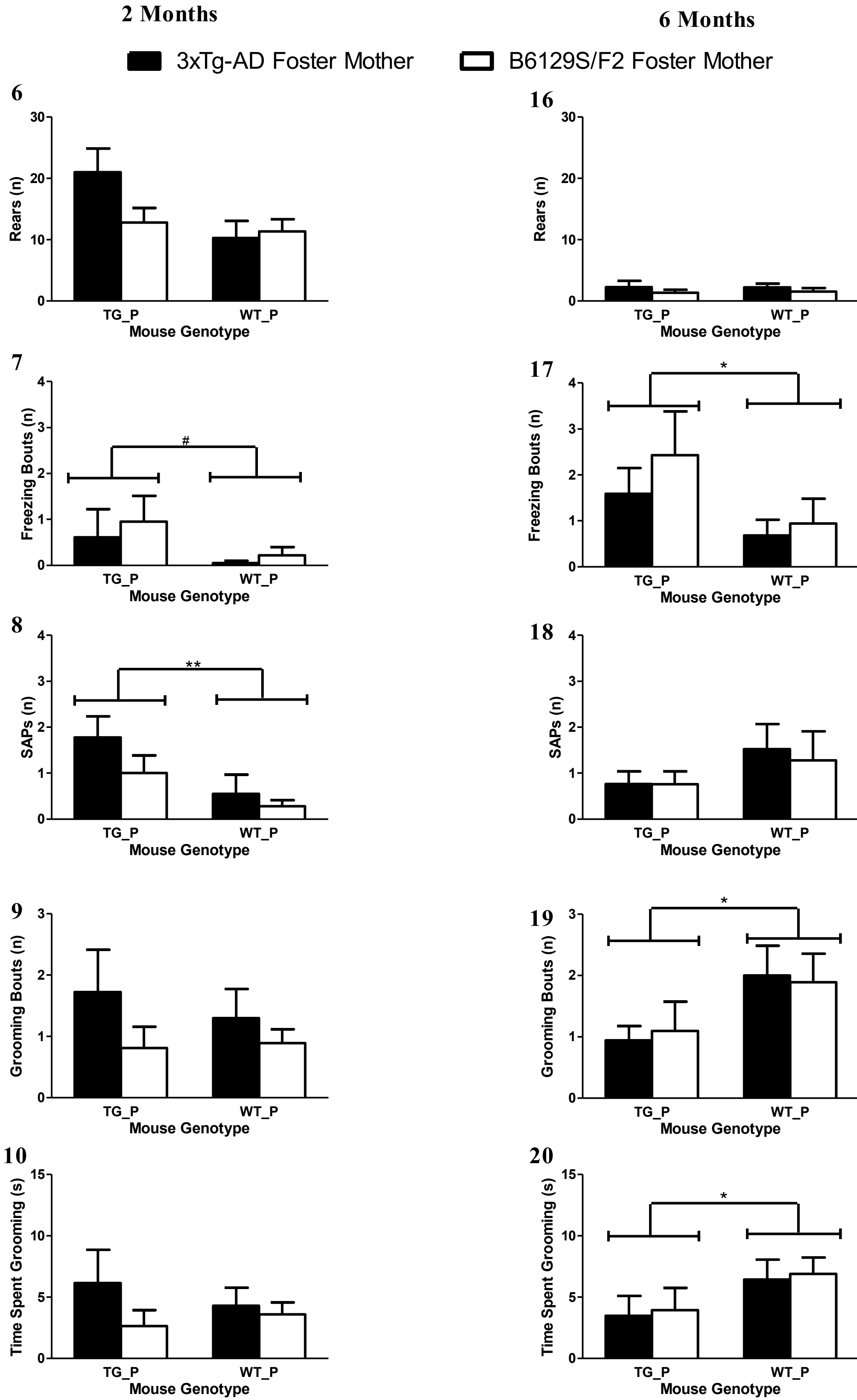

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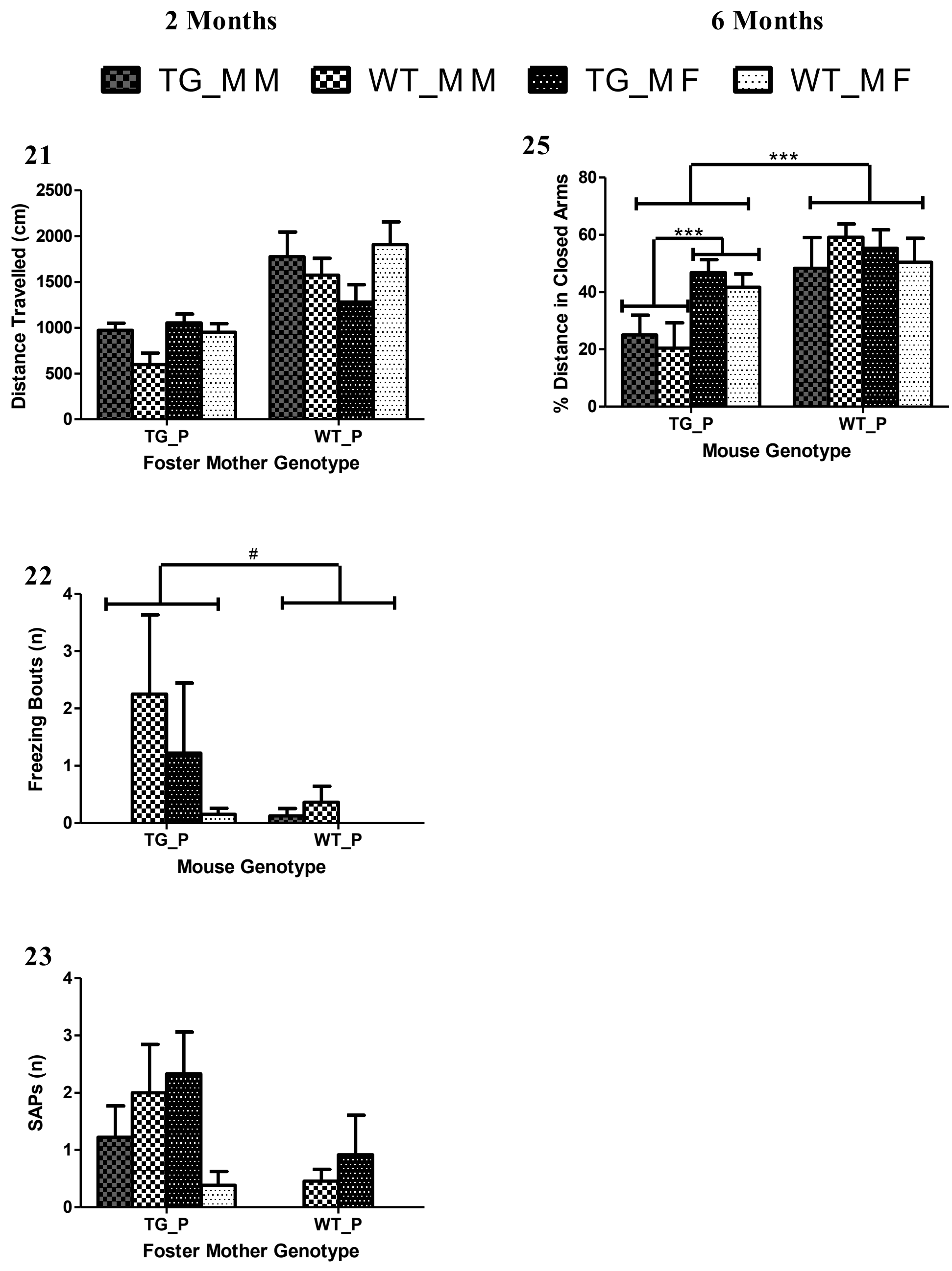

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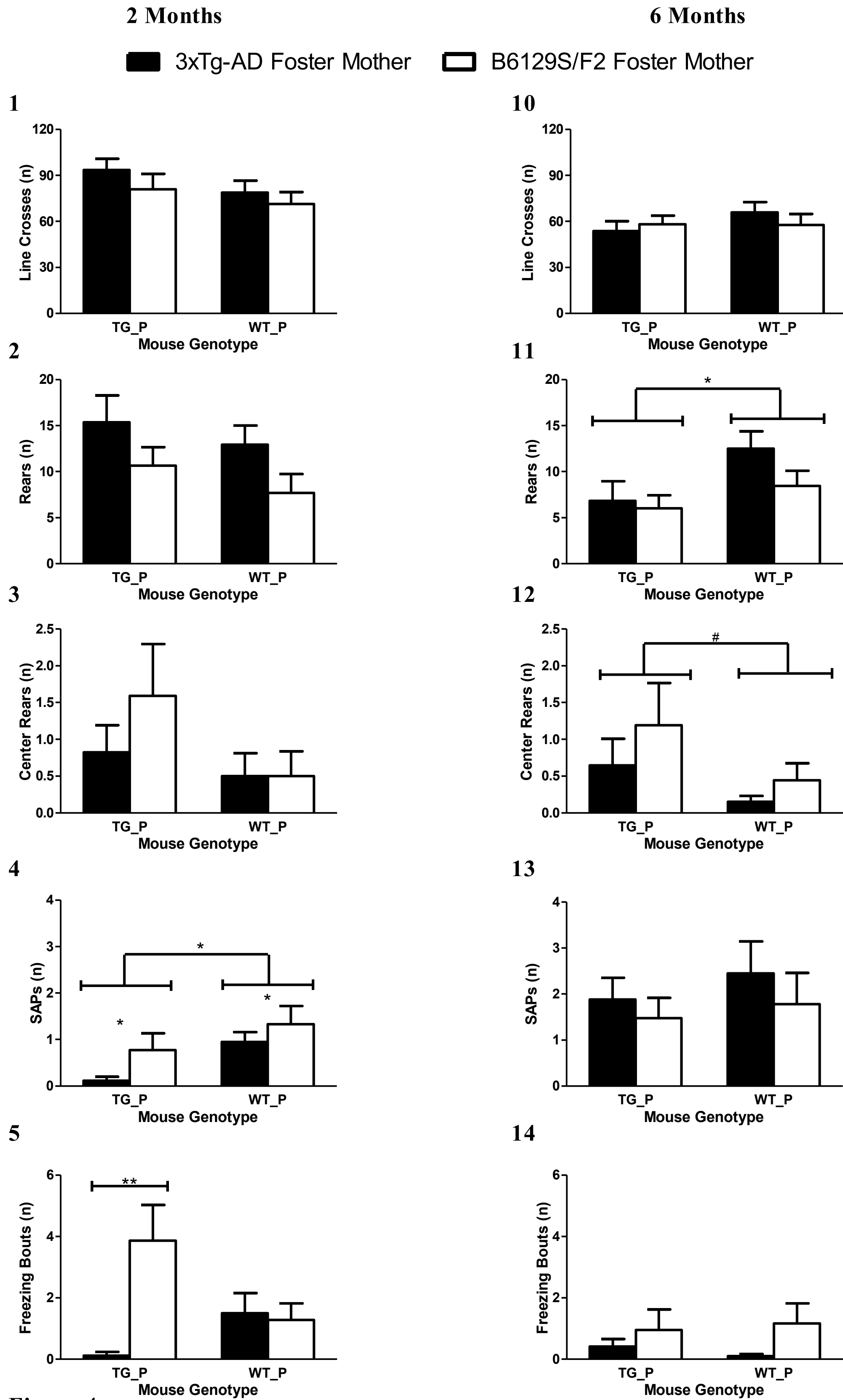

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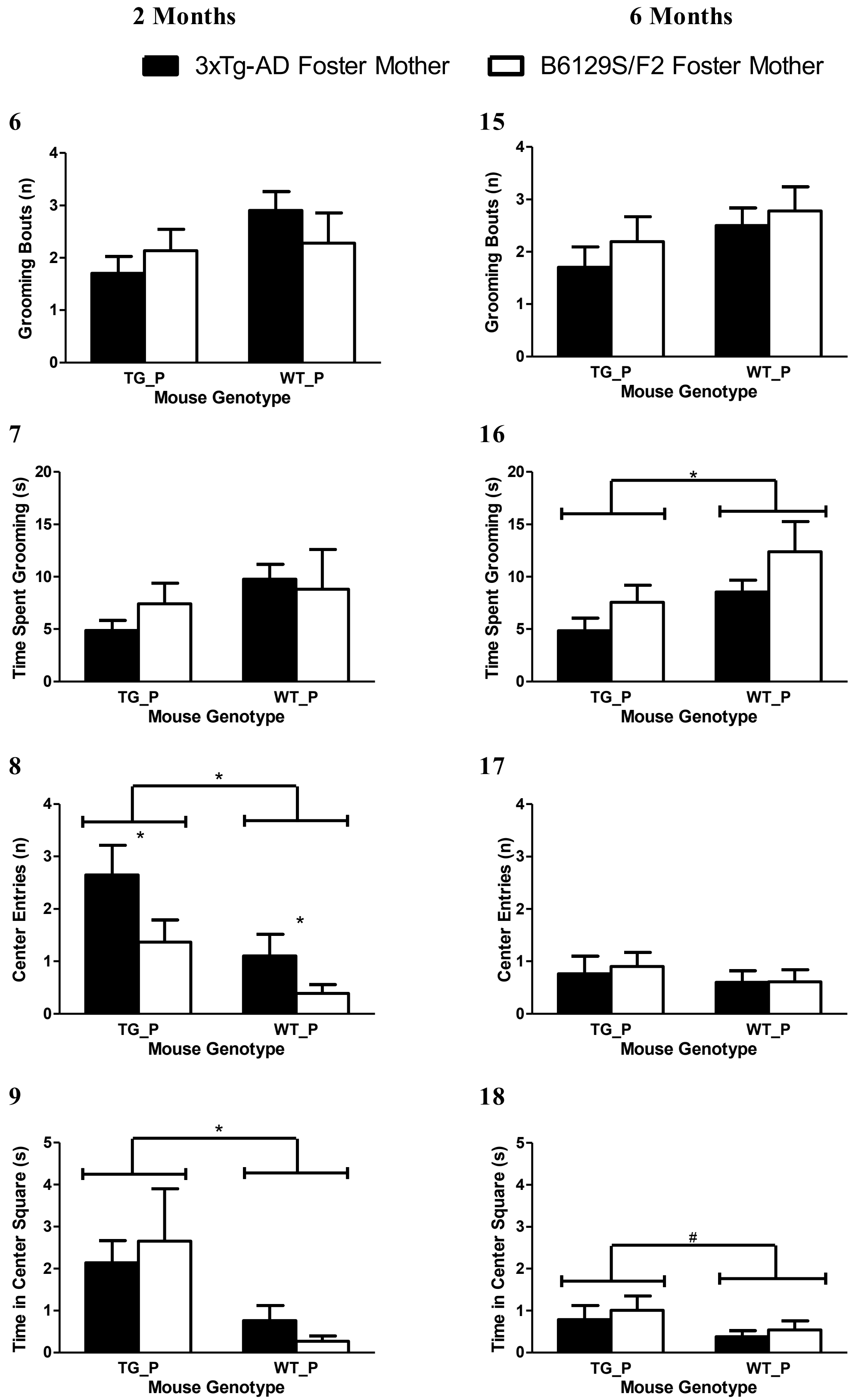

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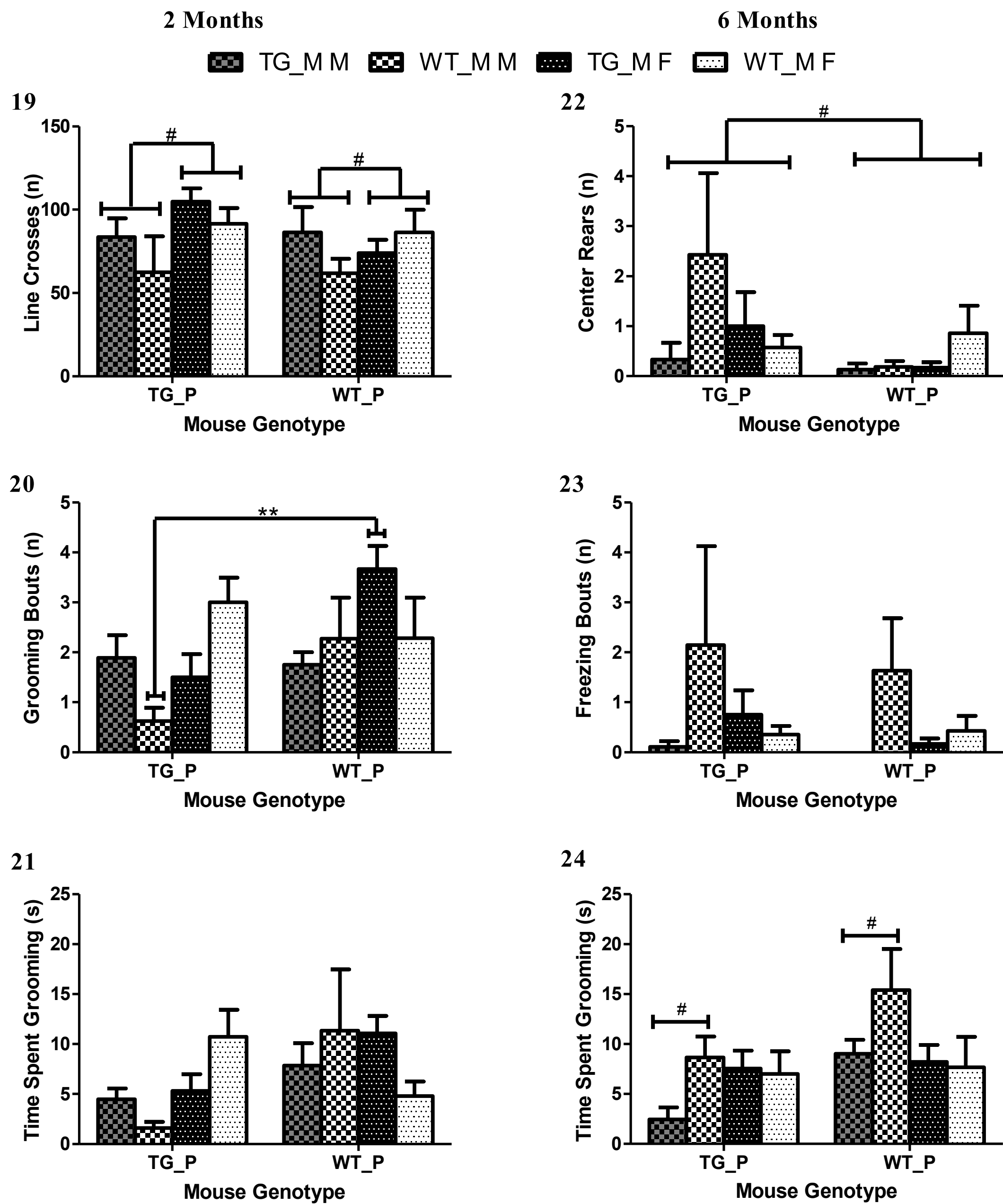

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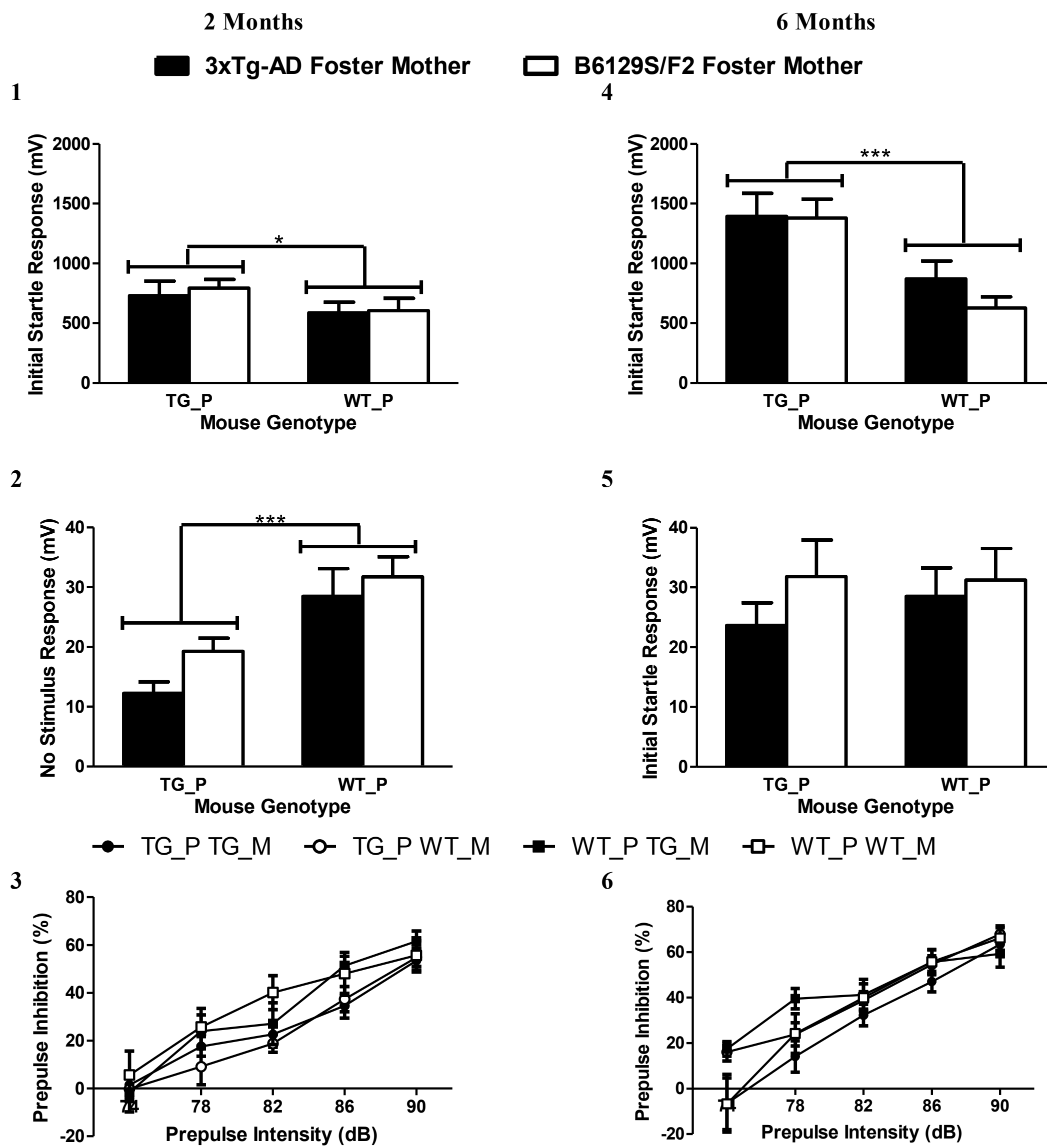

Figure 5

2 Months **6 Months**

TG_M M WT_M M TG_M F WT_M F

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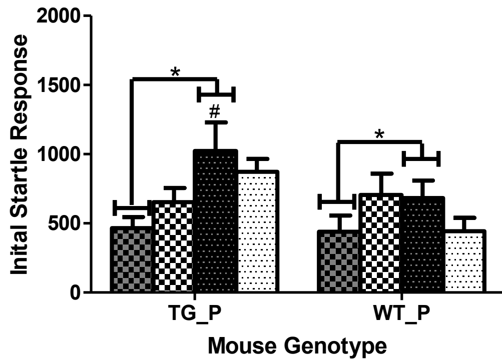

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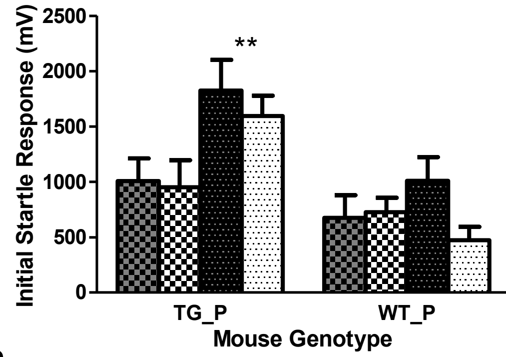

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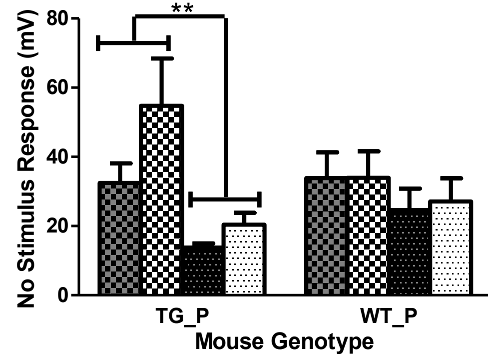

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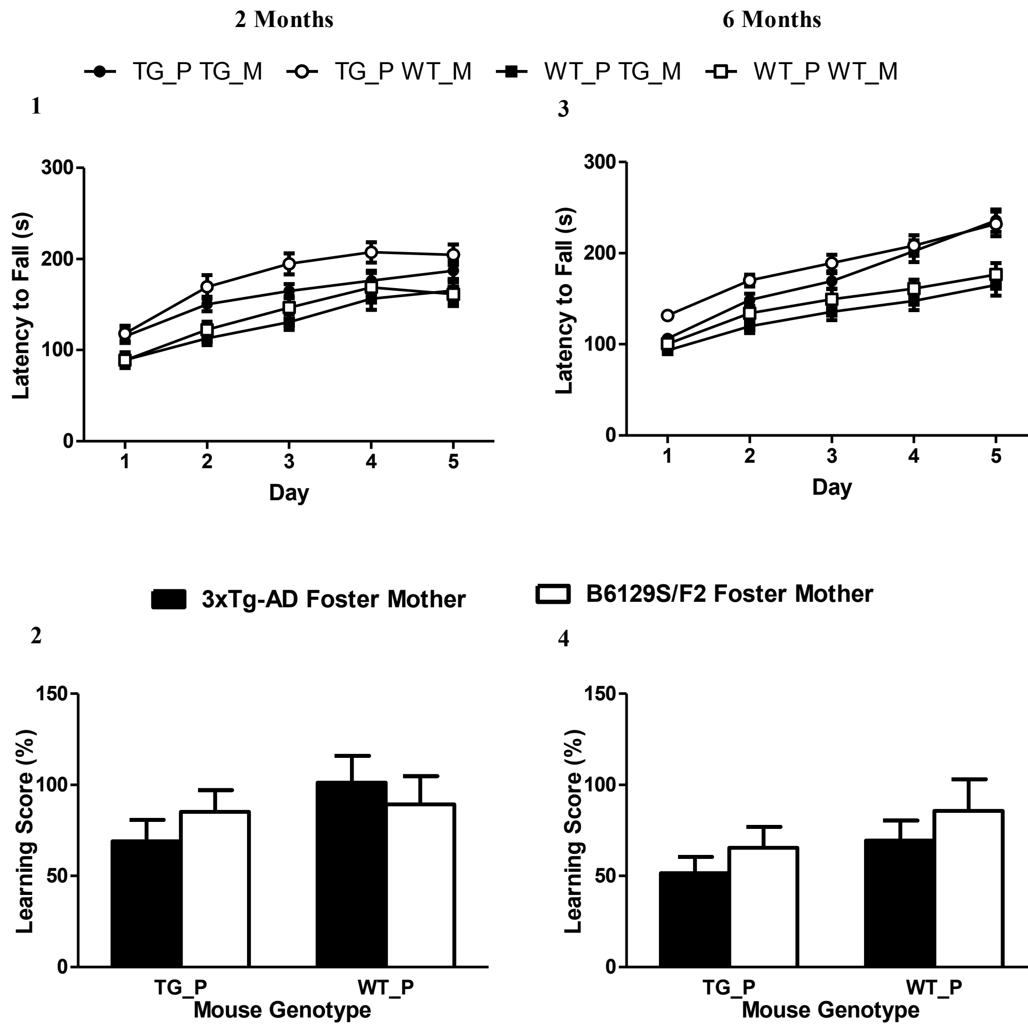

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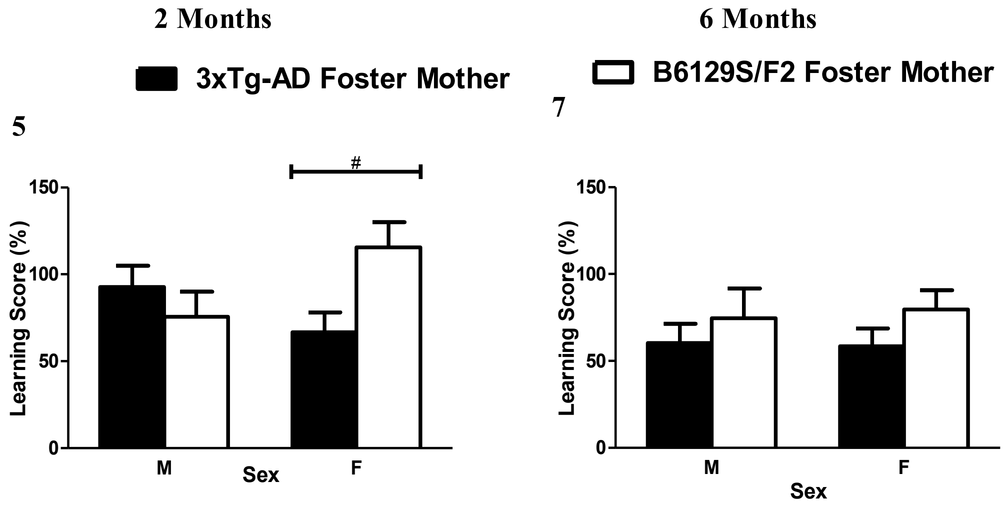

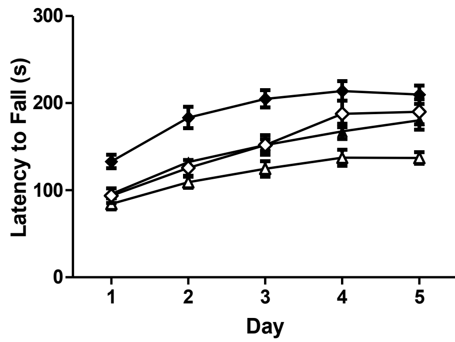

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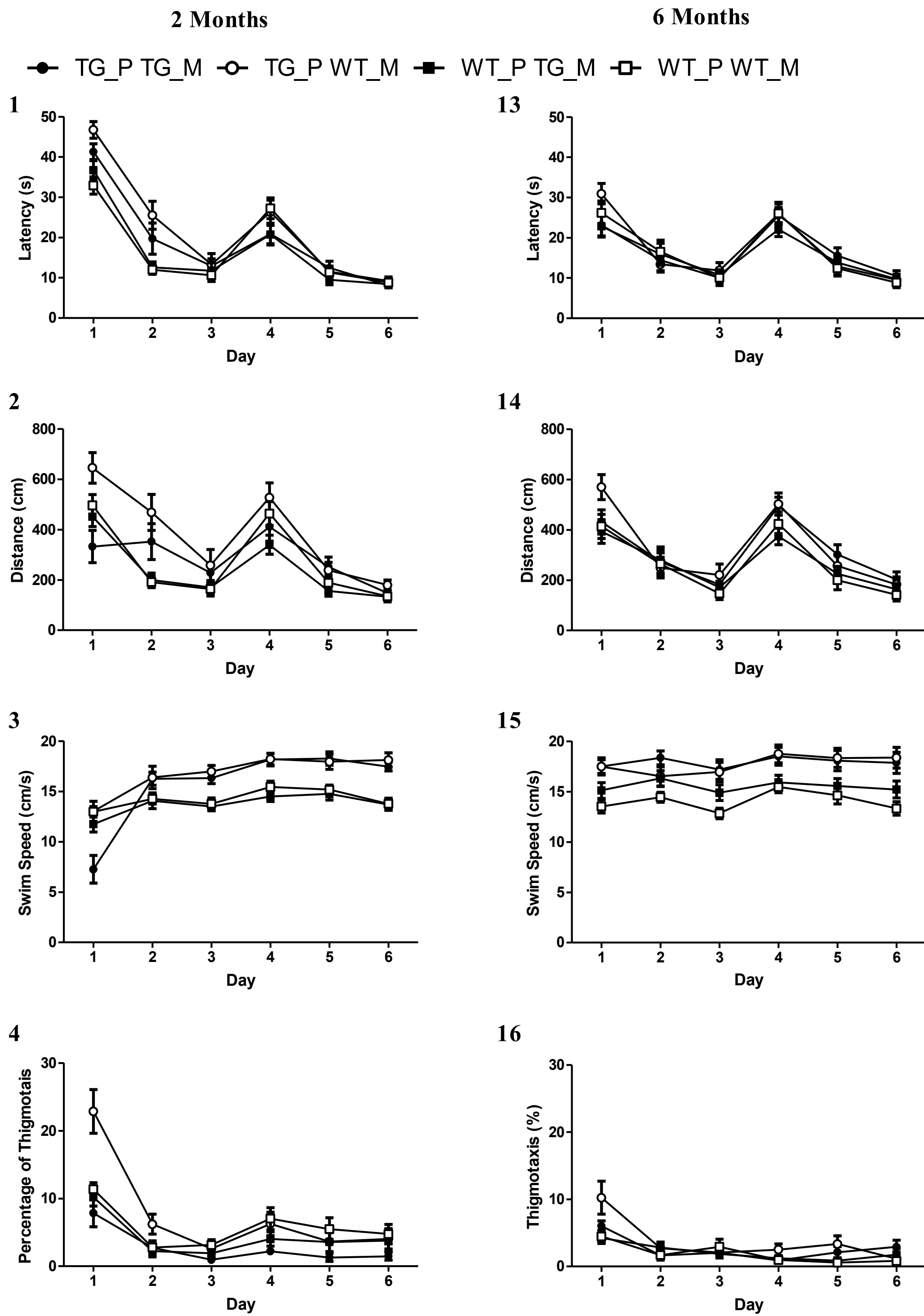

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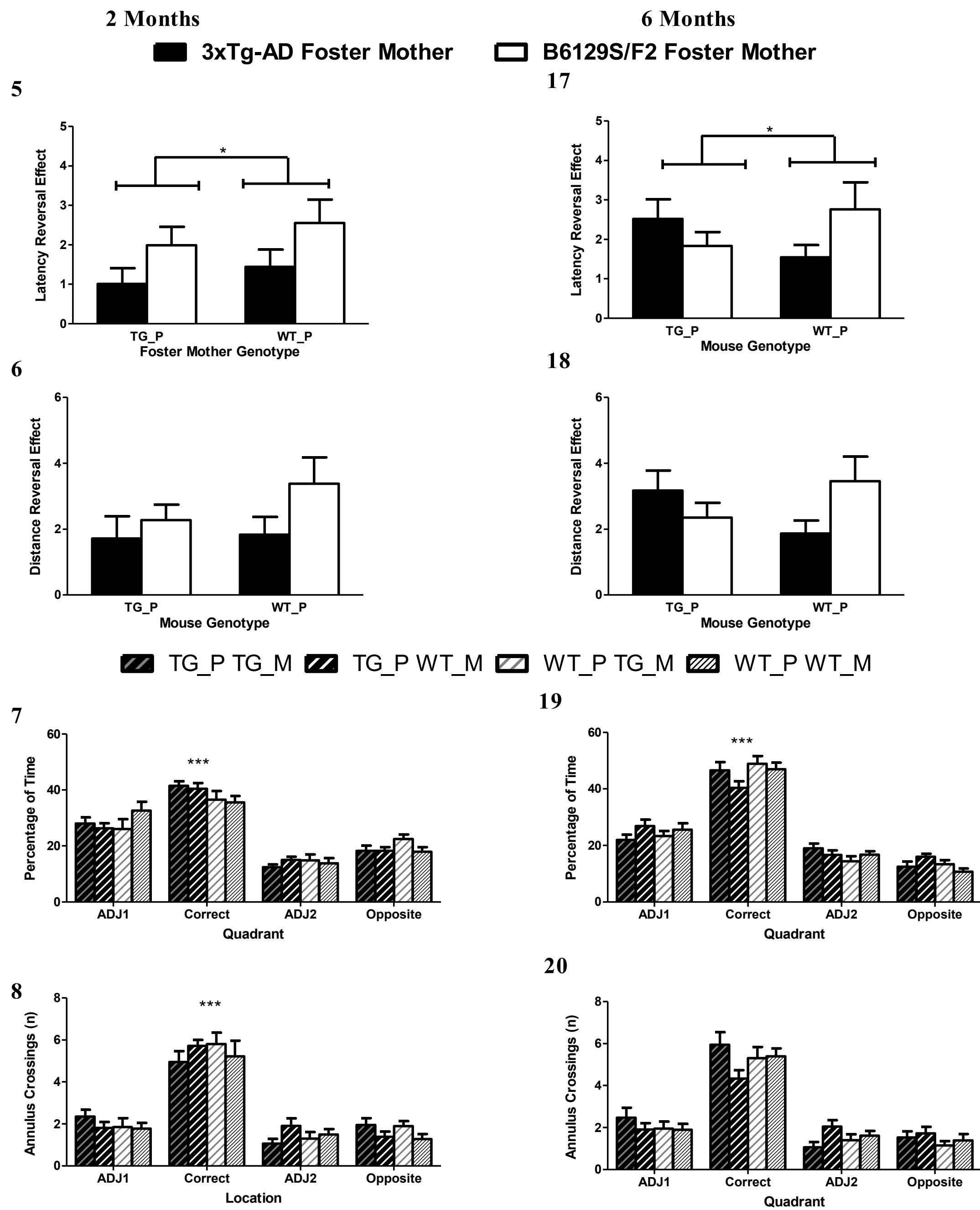

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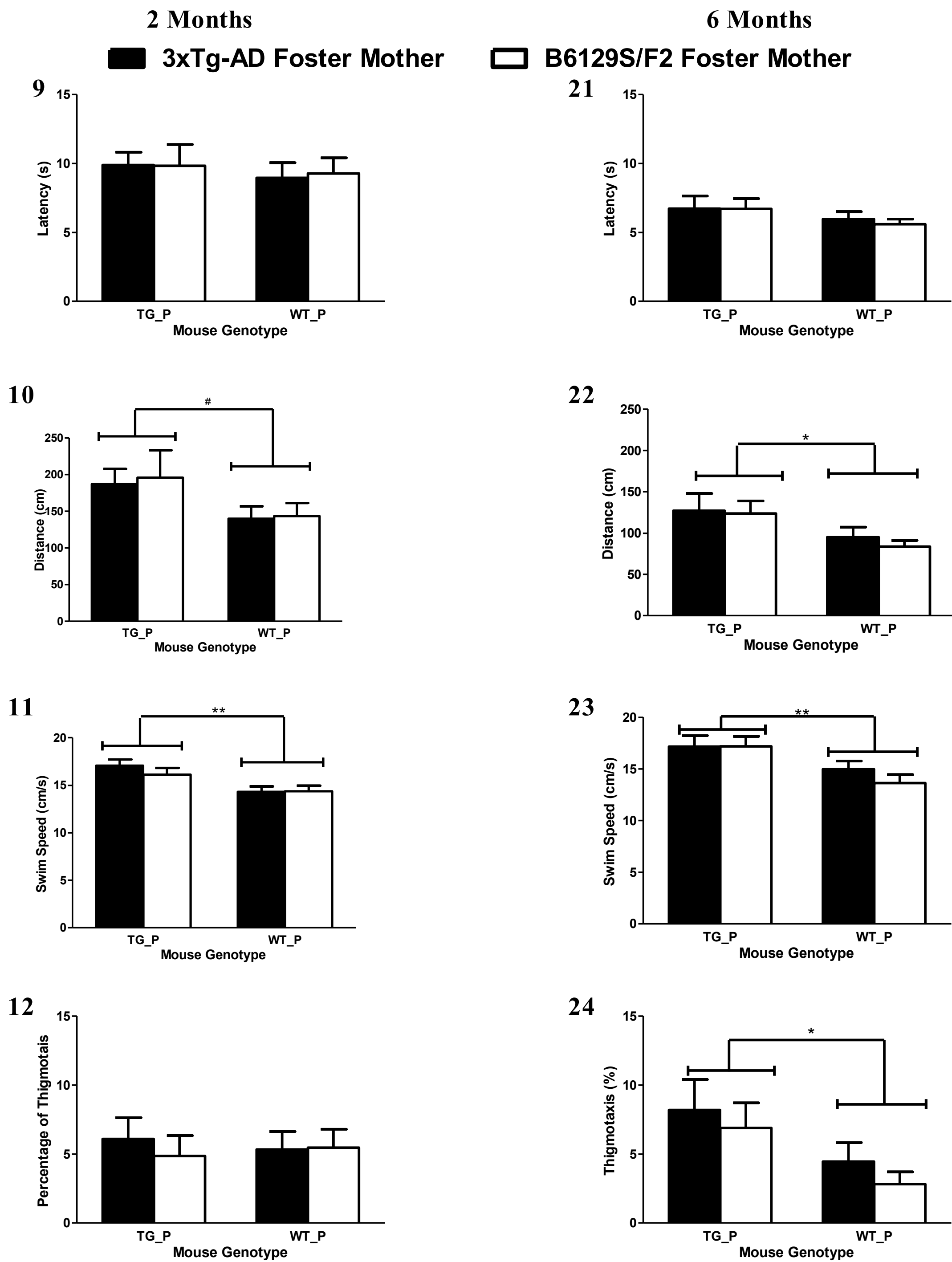

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2 Months | **6 Months**

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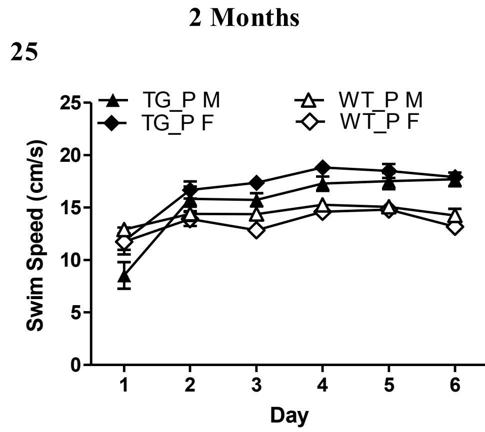

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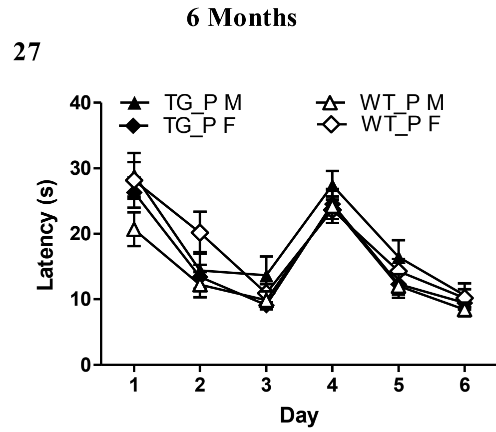

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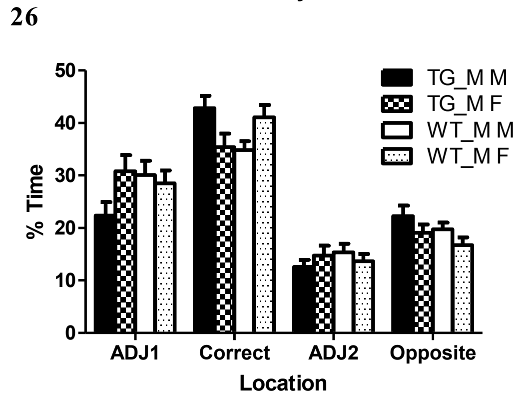

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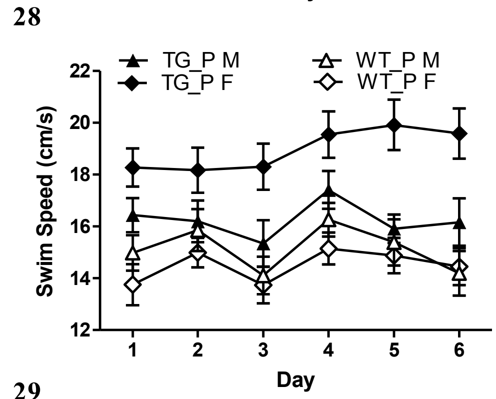

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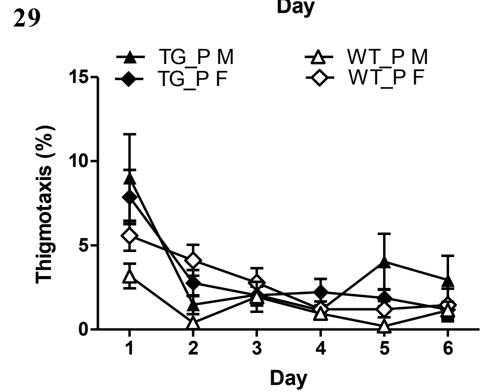

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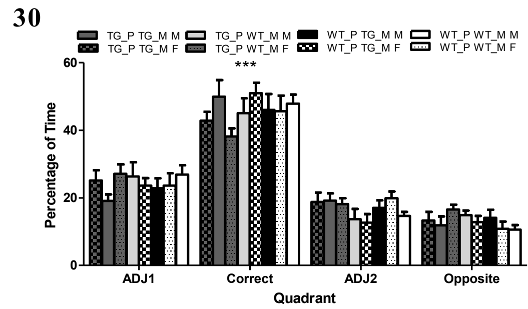

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6 Months

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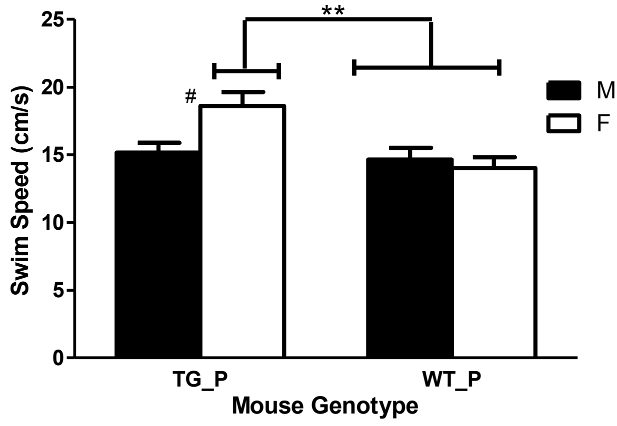

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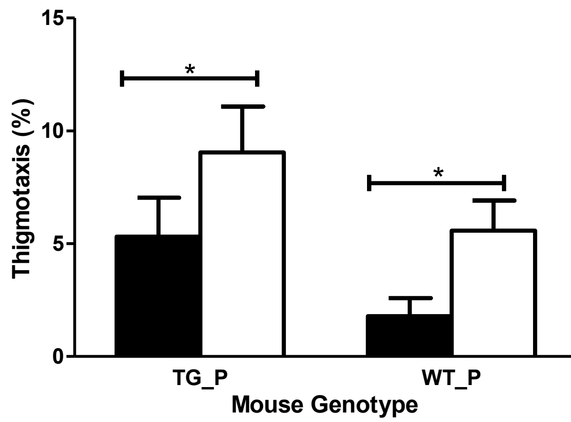

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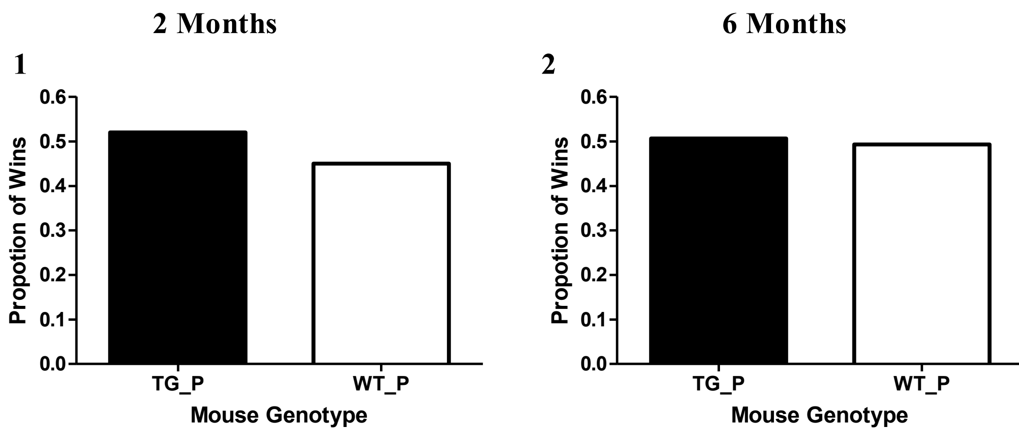

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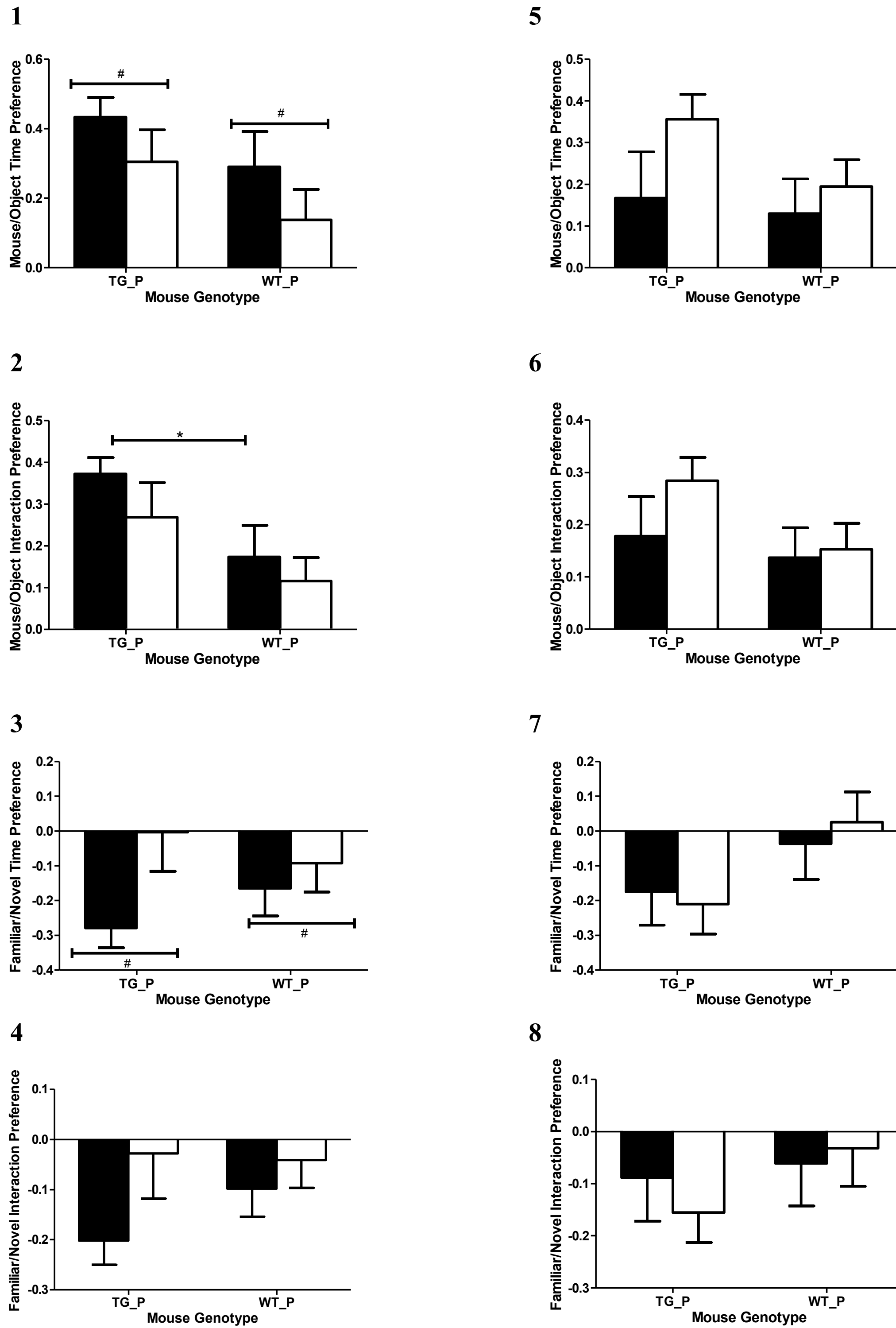
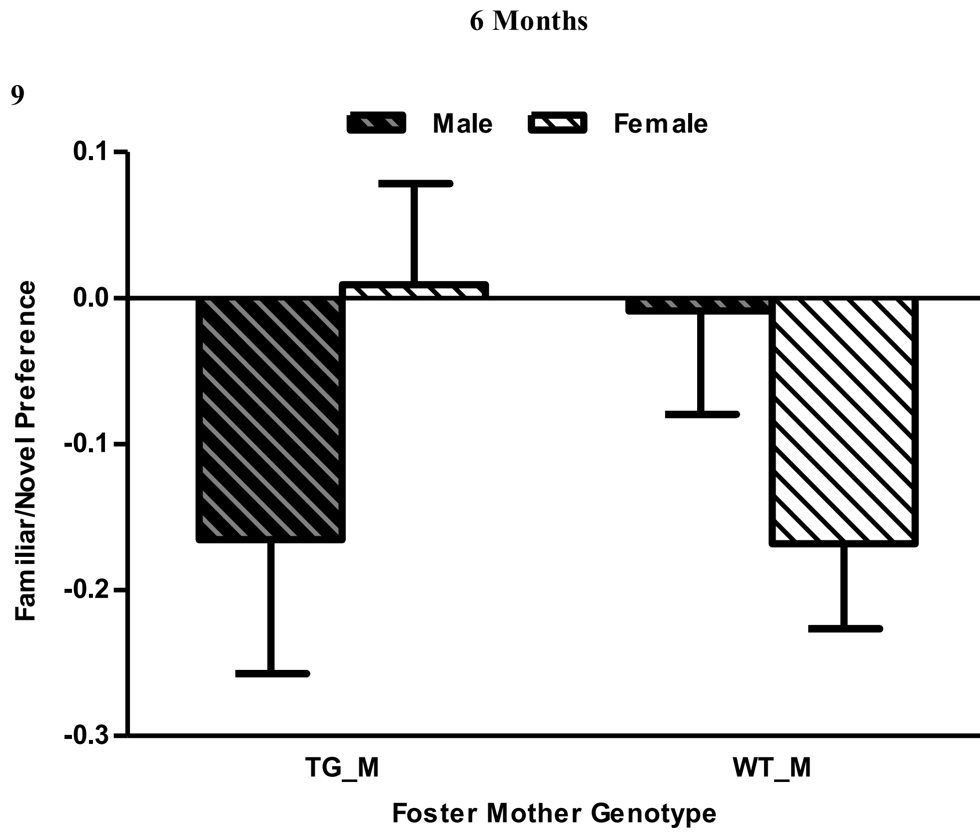


Figure 9

6 Months



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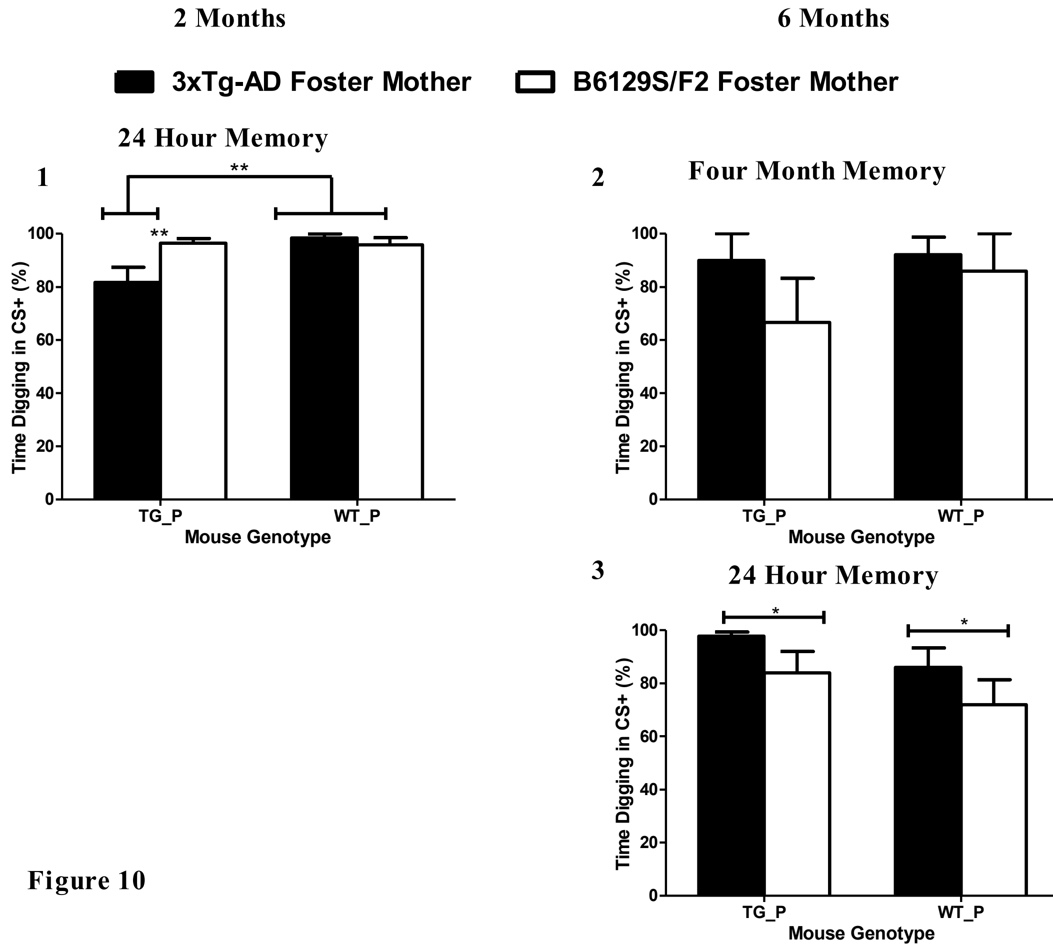

Figure 10