CHARACTERIZATION OF SOCIAL DEFICITS IN THE 5XFAD MOUSE MODEL OF ALZHEIMER'S DISEASE

by

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Dedication

This work is dedicated to AMK, AEK, KK, and WZK for their boundless support and encouragement over the years.

This work is also dedicated to my past and future self as proof that hard work and perseverance can pay off, although it's important to take a break once in a while.

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Abstract

The 5xFAD mouse strain is a double-transgenic Alzheimer's disease (AD) model containing mutant APP (K670N/M671L, I716V, and V717I) and PS1 (M146L and L286V) genes. Mice expressing these mutations have rapid accumulation of beta-amyloid in the central nervous system, subsequent neurodegeneration, and impaired synaptic signaling. While previous work with the strain has focused on cognitive, sensory, and motor function, few studies have investigated if this strain models the social deficits commonly present in AD patients. This work examined responses to social odour cues, social approach, social novelty preference, social recognition memory, and reciprocal social interactions in 3- to 9-month old females, and aggression and dominance in 6-month-old males. Results indicate that 5xFAD transgenic male and female mice engage in reduced social interaction and 5xFAD transgenic male mice exhibit increased aggression leading to injury. This study has implications for future research on neurological underpinnings of social deficits in human AD patients.

List of Abbreviations Used

Aβ: beta-amyloid protein

$A\beta_{42}$: 42-amino acid beta-amyloid protein

A/A: agitation/aggression

AICD: APP intracellular domain

AD: Alzheimer's disease

APP: amyloid precursor protein

CNS: central nervous system

EPM: elevated plus maze

FAD: Familial Alzheimer's disease

NPI: Neuropsychiatric Inventory

NPS: neuropsychiatric symptoms

PSEN1: Presenilin-1

PSEN2: Presenilin-2

sAPPα: secreted APP-alpha

sAPPβ: secreted APP-beta

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Chapter 1: Introduction

1.1 Alzheimer's Disease in Humans

1.1.1 A Brief Overview

Dementias are a group of progressive neurodegenerative disorders affecting cognition and behaviour. They are currently estimated to affect nearly 600,000 Canadians, with Alzheimer's disease (AD) being the most common form of dementia (Alzheimer Society of Canada, 2016; Dementia Strategy Advisory Committee and Project Team, 2015). Originally characterized by Alois Alzheimer in 1907, the hallmarks of AD are the presence of amyloid-beta (Aβ) plaques and neurofibrillary tangles concomitant with memory impairments, although there are additional behavioural deficits associated with the disease (Jarvik & Greenson, 1987). While the patient in Alzheimer's original study was 51 years old, AD is considered a disease of the elderly, with fewer than 5% of patients in Canada exhibiting the *early-onset* (diagnosis prior to 65 years of age) form of the disease (Alzheimer Society of Canada, 2016); the *sporadic* form of AD accounts for approximately 95% of patients and typically develops after 65 years of age. However, although a number of risk factors have been identified—including smoking, high blood pressure, poor diet and low levels of exercise (Sabayan & Sorond, 2017)—the complete etiology of the sporadic form is unknown.

1.1.2 Neuropathology in AD

1.1.2.1 Causes of AD

Early work investigating the mechanisms of AD led to the *cholinergic hypothesis,* suggesting that memory deficits arise from impaired cholinergic signaling (Bartus, Dean,

Beer, & Lippa, 1982). However, the sequencing of the Aβ protein (Glenner & Wong, 1984) and subsequent identification of its neurotoxicity (Yankner et al, 1989) provided an explanation for the relationship between amyloid plaques and neurodegeneration. This led to the *amyloid hypothesis*, which suggested that an accumulation of Aβ proteins results in neurotoxicity, tau hyperphosphorylation, and subsequent neurofibrillary tangles characteristic of AD (Hardy & Higgins, 1992). Shortly afterwards, the Swedish K670N/M671L amyloid precursor protein (APP) double-mutation associated with familial AD (FAD) was identified (Mullan et al, 1992). FAD is an autosomal dominant disorder that leads to an accumulation of Aβ in the brains of affected individuals, primarily through mutations in presenilin (including presenilin-1; PSEN1) and APP, resulting in an early-onset form of AD (Bilkei-Gorzo, 2014; Claeysen et al., 2012). The identification of the Swedish mutation, and the identification that Aβ accumulation affects cholinergic signaling (Auld, Kar, & Quirion, 1998) made the amyloid hypothesis an attractive theory. However, continued work suggests that Aβ accumulation may be a downstream result of some other neurodegenerative mechanisms, rather than the driving factor behind AD (Hardy, 2006; Sorrentino, Iuliano, Polverino, Jacini, & Sorrentino, 2014). One alternative is the *early tau hypothesis* (Maccioni, Farías, Morales, & Navarrete, 2010), which suggests that microglial activation through various cascades could lead to tau hyperphosphorylation, leading to alterations in neuronal cytoskeletons and subsequent neurodegeneration. This neurodegeneration would release hyperphosphorylated tau into the extracellular environment, driving activation of microglia, and initiating a cascade that results in rapid and progressive neurodegeneration. More recent work has also suggested that altered presenilin function

(particularly PSEN1) or erroneous cleavage of APP may be initial triggers for neurodegeneration (Sorrentino et al, 2014); both PSEN1 (a component of the γ-secratase complex) and APP are upstream factors in the development of Aβ and are involved in FAD (Bilkei-Gorzo, 2014; Claeysen et al., 2012).

1.1.2.2 Aβ Accumulation in AD

Despite the debate on the causes of AD, Aβ is neurotoxic and remains a hallmark of the disease (Sorrentino et al, 2014; Wisniewski & Goñi, 2015). Aβ is a 40- to 42-amino acid protein formed by abnormal processing of APP at the cell membrane (Bruce, Clinton, Gentleman, Roberts, & Royston, 1992; Claeysen et al., 2012). Typically, APP is sequentially cleaved by α- and γ-secretases, resulting in the production of secreted APP-alpha (sAPPα), P3, and an APP intracellular domain (AICD); however, alternative cleavage of APP by β- and γ-secretases leads to the formation of secreted APP-β (sAPPβ), AICD, and Aβ (Claeysen et al., 2012). While Aβ is neurotoxic, Sorrentino *et al* (2014) indicated that sAPPα has neuroprotective and neuroproliferative effects; as a result, this shift in APP cleavage not only increases levels of neurotoxic Aβ, it also decreases levels of protective sAPPα. Additionally, previous work has suggested that Aβ may act as a prion (Wisniewski & Goñi, 2015), thereby driving the formation of additional Aβ proteins and aggregation of amyloid *plaques* within the CNS, leading to localized neurodegeneration.

The noradrenergic locus coeruleus and cholinergic basal forebrain neurons are particularly susceptible to Aβ toxicity at early stages of the disease (Bilkei-Gorzo, 2014). The loss of noradrenergic neurons in the locus coeruleus (Sara, 2015) or cholinergic

neurons in the basal forebrain and contralateral inferior temporal cortex (Easton, Ridley, Baker, & Gaffan, 2002) have both been linked with memory deficits, consistent with effects seen during the early stages of AD. Noradrenaline also acts as an endogenous anti-inflammatory agent (Heneka et al, 2006), and the subsequent impaired noradrenergic signaling can compound neuroinflammation at later stages of the disease, ultimately leading to widespread neurodegeneration in the cortex and hippocampus (Bilkei-Gorzo, 2014). Overall, while Aβ may not be a driving factor in AD, its accumulation is related to a number of factors that promote neurodegeneration and subsequent cognitive and behavioural deficits in AD patients.

1.1.3 Cognitive and Behavioural Effects of AD

1.1.3.1 Cognitive Deficits in AD

While the course of disease progression can vary between patients, impaired episodic memory is a common early sign of AD (Lambon Ralph, Graham, Dawson, & Hodges, 2003; Perry & Hodges, 1999). This impairment appears to be specifically related to memory consolidation, as patients seem to retain pre-existing memories but have difficulty in forming new ones. As the disease progresses, patients also exhibit impaired working memory and delayed recall, as well as cognitive slowing, impaired concentration, attention, and perception (Artero, Tierney, Touchon, & Ritchie, 2003; Kluger et al, 1997; Perry & Hodges, 1999). Disease progression is also associated with language difficulties, particularly with respect to spelling and comprehension of complex sentences (Lambon Ralph et al, 2003).

1.1.3.2 Neuropsychiatric Symptoms of AD

In addition to the characteristic cognitive deficits, AD patients also exhibit a number of neuropsychiatric symptoms (NPS). While the specific presentation of these symptoms varies between patients, the Neuropsychiatric Inventory (NPI) broadly categorizes them into 10 behavioural domains (delusions, hallucinations, agitation/aggression, anxiety, elation/euphoria, apathy/indifference, disinhibition, irritability, and aberrant motor behavior) and 2 neurovegetative domains (sleep and night-time behaviour disorders, and appetite and eating disorders; Cummings, 1997; Cummings et al., 1994). Alzheimer's original patient exhibited behaviours characteristic of three major NPS—delusions, hallucinations, and agitation (Jarvik & Greenson, 1987)—and many patients are likely to exhibit NPS over the course of the disease. In one study, 80% of community-dwelling patients with dementia exhibited at least one NPS since the onset of cognitive symptoms, with 75% of them exhibiting at least one NPS in the month prior to testing (Lyketsos, Lopez, Jones, & Fitzpatrick, 2002). Of these symptoms, agitation/aggression (A/A), irritability, depression, anxiety, and apathy are the most common, with each symptom occurring in approximately 36-49% of all patients world-wide (Zhao et al, 2016). Some of these symptoms, including apathy, A/A, irritability, and depression, are also correlated with age, disease progression, or cognitive impairment (Zhao et al, 2016). A/A is also related to sex, with aggressive behaviours being more common in males than females (Lovheim, Sandman, Karlsson, & Gustafson, 2009).

1.1.3.3 Effects of NPS on Social Interactions

While the cognitive effects of AD can be frustrating and confusing for patients and families alike (MacRae, 2011), NPS can have a major impact on quality of life for AD

patients, families, and caregivers. Apathy, including social withdrawal, is highly prevalent in patients with AD, and many patients exhibit social withdrawal even prior to diagnosis (Chow et al, 2002; Chung & Cummings, 2000; Frisoni et al, 1999; Jost & Grossberg, 1996; Lyketsos et al, 2002; Steffens, Maytan, Helms, & Plassman, 2005; Zhao et al, 2016). The diagnosis itself can further isolate patients and their caregivers (Chenoweth & Spencer, 1986; Katsuno, 2005), although some patients indicate a voluntary withdrawal from distant relationships (Sorensen, Waldorff, & Waldemar, 2008). The frequency and severity of NPS also affects caregiver distress, with A/A being "severely distressing" for caregivers (Chow et al., 2002; Craig, Mirakhur, Hart, McIlroy, & Passmore, 2005). While remaining in the community can be beneficial for patients by allowing them to remain active (Phinney, Chaudhury, & O'Connor, 2007), behavioural disturbances, including A/A, are a leading factor in institutionalization (Chenoweth & Spencer, 1986; Cohen et al., 1993; Steele, Rovner, Chase, & Folstein, 1990). As a result, patients in care facilities exhibit a higher prevalence of behavioural disturbances compared to matched community-dwelling individuals with AD, with 67-84% of institutionalized patients exhibiting A/A, depression, irritability, and apathy, and approximately 50% exhibiting anxiety (Steele et al., 1990; Wood et al., 2000). As a result, placement in a care facility can not only affect AD patients by reducing meaningful social interactions, but also has the potential to cause infantilization of the patient by care staff at the facility (Beard & Fox, 2008; Harman & Clare, 2006; MacRae, 2011).

1.2 Development and Neuropathology of Animal Models of AD

1.2.1 A Brief History of Animal Models in AD Research

Based on the cholinergic hypothesis, early animal models examined cholinergic signaling through the use of lesions and injectable agonists and antagonists (Bartus, 2000). However, continued exploration into Aβ suggested that memory deficits arising from impaired cholinergic signaling could be exacerbated in the presence of Aβ (Bartus, 2000). Early studies utilized injections of exogenous Aβ into the brains of animals, but the identification of gene mutations related to early-onset AD led to the development of transgenic models of AD that allowed for the progressive development of endogenous Aβ (Bartus, 2000; LaFerla & Green, 2012; Puzzo, Lee, Palmeri, Calabrese, & Arancio, 2014). In particular, transgenic mouse models based on altered APP have been shown to recapitulate the pattern of Aβ accumulation, neurodegeneration, and concomitant memory deficits observed in human AD patients.

1.2.2 Current Transgenic Mouse Models of AD

There are five transgenic models commonly used today: Tg2576, APP23, APP/PS1, 3xTg-AD, and 5xFAD (Tg6799; Bilkei-Gorzo, 2014). Both the Tg2576 and APP23 models use human APP with the Swedish double mutation (K670N/M671L), although APP in the Tg2576 strain is encoded in a modified hamster prion protein that drives APP production throughout the brain, whereas the APP23 strain uses a Thy-1 promoter that restricts APP overexpression to neurons (Bilkei-Gorzo, 2014; Hsiao et al, 1996; Sturchler-Pierrat et al, 1997). Both of these strains exhibit amyloid plaque development, as well as concomitant deficits in learning and memory and altered social interactions,

with amyloid plaques evident in Tg2576 mice by 11 months of age and in APP23 mice by 6 months of age (Hsiao et al, 1996; Sturchler-Pierrat et al, 1997).

Neither the Tg2576 nor the APP23 strains exhibit the same level of cognitive deficits or neurodegeneration characteristic of human AD, with cell loss restricted to cholinergic and adrenergic neurons (Bilkei-Gorzo, 2014). In order to accelerate Aβ accumulation and neurodegeneration, Swedish APP overexpression was coupled with a mutated PS1 gene. A number of early models were formed by crossing Tg2576 mice with models expressing the PS1 M146L mutation (Holcomb et al, 1998; Kurt et al, 2001), although the most current model was created by inserting mutant APP K670N/M671L and PS1 L166P transgenes onto the same Thy-1 promoter into a C57BL/6J background, thereby driving neuron-specific APP and PS1 production (Radde et al, 2006). Like the Tg2576 and APP23 strains, the APP/PS1 model exhibits cognitive deficits in learning and memory and altered social interactions (Bilkei-Gorzo, 2014). This model also exhibits progressive neurodegeneration, as well as tau hyperphosphorylation in later stages, although the neurodegeneration is akin to early stages of human AD rather than the widespread effects seen in later disease progression (Bilkei-Gorzo, 2014; Jackson et al, 2016; Koffie et al, 2009).

In order to accelerate the neurofibrillary tangles seen in human AD while retaining the aggressive Aβ accumulation of the APP/PS1 model, Oddo *et al* (2003) introduced the 3xTg-AD model. This model was created by combining mutated Swedish APP and tau P301L genes in a PS1 M146V strain under a Thy1.2 promoter, thereby leading to APP and tau expression within neurons. The resulting model exhibits extracellular Aβ accumulation from around 6 months of age, as well as tau immunoreactivity at 12 months

of age. This strain exhibits loss of noradrenergic and cholinergic neurons, as well as increased microglial activity, concomitant with progressive deficits in cognitive function and social interaction (Bilkei-Gorzo, 2014). However, despite the presence of both hyperphosphorylated tau and aggressive Aβ accumulation, this strain does not exhibit the same level of neurodegeneration seen in humans, particularly in the hippocampus, suggesting that the memory deficits observed in this strain are due to different neural pathways than those observed in AD (Bilkei-Gorzo, 2014; Janelsins et al, 2008; Manaye et al, 2013).

1.2.3 The 5xFAD Mouse Model of AD

Though these models have been successfully used to examine cognitive and behavioural effects of AD, and the time-course of disease progression exhibits high face validity compared to human AD, the delayed accumulation of Aβ requires these animals to be raised to advanced ages before testing (Bilkei-Gorzo, 2014). In addition to the costs and delays involved in raising these animals, as well as the increased likelihood of natural death with advanced age, the limited neurodegeneration relative to human AD means that examination of effects related to disease progression can be difficult due to the need for repeated testing at increasing ages. In order to provide a model that exhibited a much more rapid accumulation of Aβ and associated deficits, Oakley *et al* (2006) used C57 B6SJL mice to develop the 5xFAD model—alternatively named Tg6799—which exhibits five gene mutations previously related to early-onset FAD, including three APP mutations (Swedish K670N/M671L, Florida I716V, and London V717I) and two PSEN1 mutations (M146L and L286V). Of these, the Swedish APP mutation (K670N/M671L) and PS1 M146L are the same as those previously used by Kurt *et al* (2001) to develop

their APP/PS1 model. Oakley *et al* (2006) also indicated that each pairing of APP I716V with V717I and PS1 M146L with L286V resulted in a two-fold increase in Aβ accumulation compared to any single mutation alone; as a result, transgenic 5xFAD mice exhibit a very high rate of fibrillogenic 42-amino acid Aβ accumulation with age, with whole-brain levels at ~9 – 21 ng/mg of protein by two months of age, compared to ~10 ng/mg protein in the Tg2576 strain at 16 months of age (Oakley et al, 2006).

As a result of this accelerated Aβ pathology, transgenic 5xFAD mice exhibit deficits in axon myelination (specifically in the prelimbic area, entorhinal cortex, and CA1 region of the hippocampus, later followed by the retrosplenial granular cortex) by 1 month of age, amyloid plaques and gliosis throughout the brain by 2 months of age, reduced glucose metabolism in the olfactory bulb by 3 months of age, and a reduction in whole-brain synaptophysin at 4 months of age (Gu et al, 2018; Oakley et al, 2006; Xiao et al, 2015). By 6 months of age, neurodegeneration of noradrenergic and cholinergic neurons, as well as pyramidal neurons in Layer V of the cerebral cortex, synaptic loss in the ventral horn of the spinal cord, and reduced basal synaptic transmission levels in the somatosensory cortex and hippocampus is evident, and by 9 months of age transgenic 5xFAD mice exhibit a 25% reduction in whole-brain synaptophysin levels, as well as reduced syntaxin and PSD-95 (synaptic membrane-associated proteins), indicating significant synaptic degeneration and impaired signaling (Crouzin et al., 2013; Crowe & Ellis-Davies, 2014; Devi & Ohno, 2010; Eimer & Vassar, 2013; Jawhar, Trawicka, Jenneckens, Bayer, & Wirths, 2012; Li et al., 2013; Oakley et al, 2006). Overall, this suggests numerous effects including impaired communication between cortical and subcortical structures, impaired motor control, altered tactile perception, and impaired

memory. Additionally, the rapid neurodegeneration exhibited by this strain results in memory deficits by four months of age, with sensory and motor deficits arising at later stages. As a result, the 5xFAD strain has proven popular in examining the neurological causes of cognitive and behavioural deficits observed in AD (Bilkei-Gorzo, 2014).

1.3 Cognitive and Physiological Effects in the 5xFAD Strain

1.3.1 Working and Short-Term Memory

Memory impairment is a hallmark of AD, and models of the disease exhibit deficits in learning and memory concomitant with neurodegeneration (Bilkei-Gorzo, 2014). An important factor in learning and memory is working memory, which is a temporary "store" for information whether for ongoing processing or eventual long-term storage (Baddeley, 2010); as a result, impaired working memory can impact both short-term (e.g., decision making) and long-term (e.g., learning) processes. Due to a tendency for animals to alternate choices during exploration, the Y-maze (and, by extension, the plus-maze) provide a simple method for assessing working memory (Lalonde, 2002; Olton, 1979). In brief, the Y-maze consists of three equally-spaced corridors (four in the case of the plus maze) extending from a central hub. The animal is placed into the apparatus and allowed to explore, and the number of times the animal picks a "new" arm over a previously entered arm (*spontaneous alternation*) is tracked, either over successive trials (Lalonde, 2002; Olton, 1979) or over one extended trial (Oakley et al, 2006). The consistent selection of a new arm indicates no working memory deficits, whereas reduced rates of alternation suggest memory impairments.

While the 5xFAD strain exhibits spontaneous alternations (and therefore evidence of normal working memory) at 2 months of age, transgenic mice exhibited reduced

spontaneous alternations compared to wild-type controls in the Y-maze from 4 – 18 months of age (Devi & Ohno, 2010; Oakley et al, 2006; Ohno et al, 2007) and in the plus maze from 6 months of age (Jawhar et al, 2012; Jawhar et al, 2011). In all cases, this difference was not due to decreased activity, as there was no difference in the number of arm entries between transgenic mice and wild-type controls.

Four- to six-month-old transgenic mice are also impaired on the trace fear conditioning task, exhibiting lower levels of freezing in response to a conditioned stimulus presented 30 sec before a foot shock (Ohno et al, 2006). This indicates that transgenic mice were unable to relate the two stimuli (a foot shock preceded by a tone), suggesting an impairment in working memory.

The novel object recognition task is used to examine memory for previously encountered objects. Mice are allowed to first interact with two objects in an open field, after which subjects are removed, one of the objects is exchanged, and the mice are reintroduced after a delay; mice exhibiting normal memory will explore this novel object more, whereas mice with memory impairments cannot identify which object was previously encountered (Joyashiki, Matsuya, & Tohda, 2011). Novel object recognition was impaired in 4- to 7-month-old transgenic males after a delay of 30 minutes (Tohda, Nakada, Urano, Okonogi, and Kuboyama, 2011), although the researchers suggested that these same effects are not seen with a 10-minute delay between object presentations. Similarly, Braun and Feinstein (2017) indicate that 8-month-old transgenic males exhibit no deficits in novel object recognition after a 1-minute delay. This indicates that object memory can persist for approximately 10 minutes following initial exposure, although this memory is lost within 30 minutes following initial exposure.

Roddick, Schellinck, & Brown (2014) also examined working memory using an olfactory delayed matching to sample task. In this task, mice are trained to respond to an odour cue (in this case, licking a spout for a water reward) that follows an identical odour cue; i.e., a response to two vanilla scents in a row results in a reward, whereas a response to a banana scent following a vanilla cent does not. The inter-stimulus delay (time between scent presentation) was progressively increased from 2 sec to 30 sec. Transgenic mice from 6 – 7 months of age were not impaired on the task compared to wild-type controls, suggesting no impairments in working memory or olfactory perception (Roddick et al, 2014). Females also performed better than males, with more females than males reaching each inter-stimulus delay.

Overall, these results indicate that transgenic 5xFAD mice exhibit impaired working and short-term memory, with deficits arising by four months of age, but only for sensory modalities other than olfaction. This suggests impairments in learning and decision-making, provided that the tasks are not primarily based on olfaction.

1.3.2 Spatial Memory

The Morris water maze is commonly used to assess spatial learning and memory in mice, and a number of studies have indicated an age-related deficit in the 5xFAD strain. This apparatus consists of a shallow pool of opaque water with a small platform submerged just below the surface at a fixed location (Gallagher, Burwell, & Burchinal, 2015; Schoenfeld, Schiffelholz, Beyer, Leplow, & Foreman, 2017). Mice are aversive to swimming, and therefore the goal is to locate the escape platform as soon as possible (Gallagher et al, 2015; Schoenfeld et al, 2017). However, tinting the water (using powdered milk or non-toxic white paint) prevents visualization of the platform during

swimming, so the mice need to rely on spatial extra-maze cues to determine its location (Gallagher et al, 2015; Schoenfeld et al, 2017).

Similar to the tasks of working memory, transgenic 5xFAD mice began to show deficits from 4 – 6 months of age, with increased latencies to locate the escape platform and higher cumulative search error (a measure of the actual distance traveled relative to the shortest possible distance; Gallagher et al, 2015) compared to wild-type controls (O'Leary, 2013; Ohno et al, 2006; Xiao et al, 2015). O'Leary (2013) also reported an increase in distance travelled to locate the platform in transgenic 5xFAD mice compared to wild-type controls at 9 months of age. However, although these deficits progress with age, the results vary between experiments. During a probe trial (which does not include an escape platform, and is used to examine search strategy; Gallagher et al, 2015), Ohno *et al* (2006) indicated that 4- to 6-month-old transgenic mice spent less time than wild-type controls in the quadrant previously containing the platform, whereas O'Leary only reported genotype effects at 15 months of age. Similarly, Urano and Tohda (2010) indicated no improvement in females over successive trials (suggesting impaired learning) at 7 – 9 months of age, whereas O'Leary (2013) indicated improvement over successive trials even at 9 months of age. As a result, although the rate of progression is unclear, transgenic 5xFAD mice exhibit deficits in spatial memory by 4 months of age, and these deficits progress with time.

1.3.3 Social Recognition Memory

Flanigan, Xue, Kishan Rao, Dhanushkodi, and McDonald (2014) indicated that transgenic mice between 9 – 14 months of age exhibited decreased social recognition memory compared to wild-type controls. Following a delay of 90 minutes and 24 hours

after an initial encounter with a novel conspecific, transgenic subjects exhibited similar interaction durations with both novel and familiar conspecifics, although there were no deficits in discrimination tests performed immediately after initial exposure. This suggests that transgenic 5xFAD mice exhibit impaired social recognition memory; while they can identify a previously encountered conspecific immediately after exposure, they are unable to recall the interaction at periods of 90 minutes or more.

1.3.4 Sensory Perception

Deficits in the sensory systems of the 5xFAD strain are largely dependent on modality. Although transgenic 5xFAD mice exhibit reduced glucose metabolism in the olfactory bulb by 3 months of age (Xiao et al, 2015), no overall impairment in olfactory processes has been observed in 5xFAD transgenic mice from 3 – 15 months of age. However, both audition and vision are susceptible to Aβ-related pathology (Hart, Koronyo, Black, & Koronyo-Hamaoui, 2016; O'Leary et al, 2017; Park et al, 2014; Pogue, Dua, Hill, & Lukiw, 2015). Additionally, the 5xFAD strain is also susceptible to vision loss caused by a mutant gene ($Pde6b^{rd1}$), present in mice on the C57 B6SJL background, that leads to a progressive loss of photoreceptors independent of Aβ accumulation (Yassine et al, 2013).

To examine olfactory sensitivity, Roddick, Roberts, Schellinck, & Brown (2016) trained mice with a positive and negative odour stimulus; in this case, licking a water spout was considered a positive response, whereas no licks were considered a negative response. Based on the stimulus (either positive or negative), the mouse was either required to lick or not in order to receive a reward. In order to test sensitivity, odourant bottles were prepared, each containing between 1 to 10^{-5} ppm, and mice were tested at

progressively decreasing concentrations. As in the delayed matching to sample task, transgenic 5xFAD mice were not impaired on odour sensitivity, and females performed better than males (Roddick et al, 2016).

To examine olfactory memory and olfactory discrimination, O'Leary (2013) conditioned mice to associate one stimulus odour with sugar reward, compared to a second stimulus odour with no reward. Twenty-four hours following training, mice underwent a test trial in which both odours were present, but with no reward associated with either; time spent investigating each stimulus for the reward was calculated. An inability to recall the correct rewarded stimulus, or an inability to differentiate between either stimulus, would be represented by no difference in investigation of either stimulus. However, O'Leary indicated that transgenic 5xFAD mice had no impairments in discriminating between the odours or remembering which odour was associated with the reward from 3 to 15 months of age, with both wild-type and transgenic mice investigating the rewarded stimulus over 85% of the time. O'Leary also indicated that this preference for the rewarded stimulus persisted for 90 days (between 3 to 6 months of age), suggesting that olfactory memory is robustly maintained despite other memory deficits.

While these results indicate no deficits in olfactory memory in the 5xFAD strain with age, transgenic 5xFAD males at six months of age had longer latencies compared to wild-type controls in finding a buried or hidden food pellet, suggesting olfactory dysfunction concomitant with the reduced glucose metabolism (Xiao et al, 2015). Though the authors indicate that the olfactory discrimination task requires training and requires higher cortical processing (Xiao et al, 2015), the lack of deficits indicated in the previous tasks (O'Leary, 2013; Roddick et al, 2014, 2016) suggests that these differences may arise due

to motivation for the reward. Although Roddick *et al* (2014; 2016) provided a water reward for correct responses for both studies, mice were placed on water restriction for 10 days prior to testing and were highly motivated to receive the reward. Additionally, O'Leary (2013) used sugar as a reward in the positive condition stimulus, whereas Xiao *et al* (2015) used a standard food pellet; although both studies used three days of food restriction, this indicates that the reward itself affects motivation to complete the task. As a result, this suggests that transgenic 5xFAD mice do not exhibit olfactory deficits in sensitivity, memory, or odour discrimination, but that the choice of reward influences the motivation to complete the task.

Although transgenic 5xFAD mice exhibit no deficits in olfactory perception, they do have deficits in acoustic processing. O'Leary *et al* (2017) indicated reduced acoustic startle response in transgenic mice as early as 4 months of age, increased auditory brainstem threshold at 13 months, and a greater loss of hair cells in the cochlea at 15 months of age, compared to wild-type controls. While Aβ-related pathology was indicated as the cause of peripheral hearing loss, the reduced startle response and increased auditory brainstem threshold present prior to peripheral hearing loss indicate that other factors (such as impaired motor control or Aβ-related pathology in the brainstem) are also likely involved (O'Leary et al, 2017), suggesting that auditory deficits result from an accumulation of impairments throughout the auditory system.

In addition to auditory deficits, visual system alterations are also present in transgenic 5xFAD mice. Transgenic 5xFAD mice have been shown to exhibit Aβ deposits in the retinal epithelium (Hart et al, 2016; Park et al, 2014; Pogue et al, 2015). These deposits are similar to those seen in age-related macular degeneration and can affect the structure

of the retinal epithelium by reducing the integrity of tight junctions (Park et al, 2014), thereby increasing the permeability of the blood-retinal barrier. By three months of age, transgenic 5xFAD mice also exhibit an increase in the pro-inflammatory biomarkers cyclooxygenase-2 and C-reactive protein in the brain and retina compared to wild-type controls (Pogue et al, 2015). These proteins are associated with inflammatory neuropathology in AD, suggesting possible neurodegeneration of the retina. In addition, mice based on the C57 B6SJL background—like the 5xFAD strain—exhibit a recessive $Pde6b^{rd1}$ allele associated with *retinitis pigmentosa* (Yassine et al, 2013). This mutation causes extensive neurodegeneration of rods within 7 weeks of age, as well as progressive degeneration of cones over lifespan, resulting in progressive vision impairments.

Overall, while performance on olfactory tasks is unlikely to be affected by deficits in perception or discrimination, tasks involving the acoustic startle response could be affected past 4 months of age, whereas tasks involving general audition should be interpreted carefully after 12 months of age for possible effects of hearing impairments. Additionally, though screening for the mutant Pde6b gene is possible and can be done simultaneously with genotyping of animals, it is important to note that Aβ deposits in the retinal epithelium are associated with inflammatory neuropathology and reduced integrity of the blood-retinal barrier, and may lead to vision impairments, particularly in later stages of disease progression.

1.3.5 Motor Deficits

There have been conflicting reports as to the onset of motor deficits exhibited by transgenic 5xFAD mice. With the exception of reduced locomotor speed at 4 months of age on a balance-beam task (O'Leary, 2013), transgenic mice exhibit no reduction in

locomotor speed or distance travelled up to 9 months of age (Braun & Feinstein, 2017; Jawhar et al, 2012; O'Leary, 2013). However, at 12 months of age, Jawhar *et al* (2012) indicated no deficits in locomotor speed or distance travelled in the open field, whereas O'Leary (2013) reported that transgenic mice covered less distance in the open field than wild-type controls. While the reasons for this discrepancy were unclear, O'Leary suggested that the background strain (C57 B6SJL, compared to the C57 B6J strain used by Jawhar *et al* [2012]) or differences in methodology are likely involved. O'Leary (2013) further reported that 12- to 15-month-old 5xFAD transgenic mice had reduced swim speed in the Morris water maze compared to wild-type controls, and that 16-month-old 5xFAD transgenic mice had reduced locomotor speed compared to wild-type controls. This indicates that locomotor function exhibits an age-related decline in transgenic mice, although this impairment does not appear until approximately 12 months of age.

To test motor coordination and motor learning, the balance beam or rota-rod tasks can be used. Both of these tasks examine motor coordination by testing the latency to fall off a narrow rod, with the rota-rod also testing motor learning by inducing a progressive increase in rotation of the rod (O'Leary, 2013). Transgenic mice exhibit a decreased latency to fall off the balance beam compared to wild-type controls at 9 – 10 and 12 – 13 months of age (Jawhar et al, 2012; O'Leary, 2013). O'Leary also indicated that transgenic mice had a decreased latency to fall and exhibited less improvement over successive trials on the rota-rod compared to wild-type controls at 9, 12, and 15 months of age. This indicates that transgenic mice exhibit impaired motor coordination and motor learning prior to obvious deficits in locomotor speed or distance travelled.

The string suspension task (in which mice are suspended from a string or wire by grasping it with their forepaws) and grid-suspension task (in which mice are placed on a grid that is then inverted) are both used to test motor strength. Jawhar *et al* (2012) indicated that transgenic mice exhibited decreased latency to fall during the string suspension task compared to wild-type controls at 9 and 12 months of age, although O'Leary (2013) indicated no differences until 16 months of age. Again, this discrepancy may have been due to differences in background strain or methodology (O'Leary, 2013). However, O'Leary also indicated that latency to fall on the grid hang task was lower in transgenic mice than wild-type controls at 7, 13, and 16 months of age, indicating that the results seen in the string suspension task were likely affected by the protocol. In addition to impaired strength on the suspension task, O'Leary (2013) indicated that transgenic mice exhibited fewer rears (which require hind limb strength) in the open field compared to wild-type controls at 9, 12, and 15 months of age.

Overall, these results indicate that transgenic mice exhibit deficits in locomotor activity, motor coordination and learning, and strength arising between 9 – 12 months of age, concomitant with Aβ pathology in the cortex and spinal cord (Jawhar et al, 2012). As a result, care must be taken when interpreting results of tasks performed by mice 9 months of age or older if the tasks may be affected by changes in motor behaviour.

1.3.6 Life Expectancy

It has been reported that 5xFAD mice have a shortened life expectancy compared to wild-type control mice (Rae & Brown, 2015); transgenic 5xFAD males had a median life expectancy of 738 days, compared to a median of over 880 days in wild-type B6SJL mice. No sex differences in survivorship in transgenic 5xFAD mice or wild-type controls

were noted when only spontaneous deaths were examined. Increased frailty, an accumulation of physiological deficits and increased vulnerability to insult, was associated with older age in mice (Whitehead et al, 2014) and increased AD pathology in humans (Buchman, Schneider, Leurgans, & Bennett, 2008), and level of frailty was predictive of hospitalization and mortality in AD patients (Kelaiditi, Andrieu, Cantet, Vellas, & Cesari, 2016), suggesting that the decreased life expectancy may be due to an accumulation of AD-related deficits in transgenic 5xFAD mice.

1.4 Behaviours Associated with NPS in Mouse Models of AD

1.4.1 Anxiety

Evidence suggests that anxiety in mouse models of AD is strain-dependent, with anxiolytic, anxiogenic, or no effects seen in different models (Deacon, Koros, Bornemann, & Rawlings, 2009; Filali, Lalonde, & Rivest, 2011a; Ognibene et al, 2005; Sterniczuk, Antle, LaFerla, & Dyck, 2010a). Transgenic 5xFAD mice appear to generally exhibit no difference in anxiety compared to wild-type controls (Braun & Feinstein, 2017; Flanigan et al, 2014; Jawhar et al, 2012; O'Leary, 2013).

Though a number of tests have been developed, two common ones are the *open field* and the *elevated plus maze* (EPM). Both of these tests are based the aversion of mice to open areas and light, and are typically scored for time spent in an exposed vs protected area, number of entries into an exposed or protected area, overall exploration (whether by distance travelled or number of line crosses), and number of fecal boli (an indication of anxiety; Carola, D'Olimpio, Brunamonti, Mangia, & Renzi, 2002; Lister, 1987). Though details vary by lab, the open field consists of a square box (approximately 60 x 60 cm) with high opaque sides and grid lines marked on the floor every 10 cm (Carola et al,

2002). The peripheral grid squares (adjacent to the walls) are considered a protected area, whereas the centre squares represent an exposed area. The EPM consists of four arms mounted at right angles to each other, with each pair of opposing arms being either "closed" (shielded on three sides by high walls) or "open" (edge marked by a short raised lip around the entire arm; Carola et al, 2002; Lister, 1987). This apparatus is then mounted on a pedestal in order to raise it above the ground.

Overall, transgenic 5xFAD mice do not appear to exhibit any difference in anxiety relative to wild-type controls, although there is contradictory evidence. Transgenic 5xFAD males at 8 months of age exhibited reduced latency to approach a novel object, suggesting reduced neophobia, and increased time spent in the centre of the open field, suggesting decreased anxiety (Braun & Feinstein, 2017). However, O'Leary (2013) indicated no genotype effects of anxiety in the 5xFAD strain in the open field, and Flanigan *et al* (2014) also indicated no genotype effects in the open field or light/dark apparatus (an apparatus containing one darkened, enclosed chamber and one lit, exposed chamber, similar in concept to the EPM) for transgenic 5xFAD mice at ~9 months of age. While previous evidence suggested that transgenic 5xFAD mice spent less time in the closed arms of the EPM (Flanigan et al, 2014; Jawhar et al, 2012), this was shown to be due to hypersensitivity of the vibrissae in transgenic 5xFAD mice due to decreased inhibitory interneurons, and trimming of the whiskers abolished this effect at 14 months of age (Flanigan et al, 2014), indicating no difference in anxiety between transgenic mice and wild-type controls.

Evidence for anxiety in other strains is also mixed. Transgenic APP/PS1 males did not differ from wild-type controls on anxiety in the open field or EPM at 6 months of age,

but spent more time in the open arms of the EPM at 8 months of age, which may be an indication of reduced anxiety-like behaviour (Filali et al, 2011a). Transgenic Tg2576 mice exhibited no differences from wild-type controls on the EPM between 7 – 12 months of age (Ognibene et al, 2005), although transgenic males at 21 months of age exhibited reduced anxiety in the open field (Deacon et al, 2009); though they do not report the values, Deacon *et al* also indicate no genotype differences in anxiety in Tg2576 females near the same age. Transgenic 3xTg-AD females, however, exhibited increased anxiety on the open field and EPM, and increased defecation during testing, between 7.5 – 11 months of age (Sterniczuk et al, 2010a).

Overall, this evidence suggests that anxiety is dependent on strain, age, and sex, which may reflect differences in background strain or deficit accumulation. However, the majority of evidence indicates that the 5xFAD strain does not exhibit a change in anxiety, suggesting that investigative behaviours are unlikely to be affected by anxiety.

1.4.2 Exploratory Behaviour

Much like anxiety, exploratory behaviour varies by strain, and is often used to examine whether differences in behaviour are due to a particular stimulus itself or an overall change in activity; in particular, exploratory behaviour is often measured on tasks of anxiety. Although evidence in the 5xFAD strain varies, transgenic mice do not appear to differ on exploratory behaviour compared to wild-type controls. From the results discussed in Section 1.3.5, transgenic 5xFAD mice exhibited no difference relative to wild-type controls in distance travelled in the open field up to 9 months of age, and no difference in EPM arm entries at 9 months of age, suggesting no changes in exploration compared to wild-type controls, although results were conflicting at 12 months of age

(Braun & Feinstein, 2017; Jawhar et al, 2012; O'Leary, 2013). However, Braun and Feinstein (2017) also indicate that 8-month-old transgenic males exhibited increased exploration time compared to wild-type controls in response to a novel object, which may suggest neophilia.

Exploratory behaviour also differs across AD mouse models. Transgenic APP/PS1 males at 6 months of age exhibited increased exploratory behaviour compared to wild-type controls (Filali et al, 2011a). Similarly, transgenic Tg2576 males exhibited increased exploration in the Y-maze between 7 – 12 months of age (Ognibene et al, 2005) as well as increased exploration in the open field at 12 months of age (Deacon et al, 2009). In contrast, transgenic 3xTg-AD females between 7.5 – 11 months of age exhibited reduced exploratory behaviour compared to wild-type controls in a battery of tests (Sterniczuk et al, 2010a).

Overall, exploratory behaviour varies by strain, although appears to be largely related to level of anxiety, with highly anxious strains exhibiting decreased exploration. Aside from possible neophilia, transgenic 5xFAD mice do not exhibit differences in exploration compared to wild-type controls, suggesting that changes in investigative behaviour are not due to an overall apathy. However, it is important to note that exploratory behaviour is tied to overall level of activity, which itself is tied to locomotor activity, and locomotor deficits may partially account for variations in exploratory behaviour.

1.4.3 Sociability

Sociability is a measure of willingness to engage in social interaction (Moy et al, 2004). While labs may simply score the number of social behaviours or time spent interacting with a free roaming conspecific (e.g., Bories et al, 2012; Deacon et al, 2009;

Flanigan et al, 2014), the three-chamber apparatus provides an accurate measure of sociability by requiring all approach behaviour to be initiated by the subject (Moy et al, 2004). Briefly, this apparatus involves a rectangular box (approximately 60 x 40 x 20 cm) partitioned into three equal-sized chambers by internal walls, with access to each chamber provided by floor-level openings. A plastic or metal cage is placed in each of the two outer chambers, allowing for a different stimulus to be contained in each; by containing the stimuli within these cages, approach behaviour can only be initiated by the subject, allowing for an accurate measure of preference for one stimulus over the other. The tested subject is placed in the centre chamber and allowed to roam freely for a pre-defined length of time, during which the exploration of each stimulus is measured. By varying the stimuli between a novel conspecific vs novel toy, or novel vs familiar conspecific, it is possible to examine sociability (preference for social vs non-social interactions), social novelty preference, and social recognition memory (Moy et al, 2004).

Previous evidence suggests that transgenic 5xFAD mice exhibit increased sociability compared to wild-type controls. In our own lab, using the three-chamber apparatus described above, MacGowan, Brown, and Franklin (2016) indicated an increased exploration of a novel conspecific by transgenic 5xFAD males at 6 – 8 months of age compared to wild-type controls, albeit with small sample groups. By measuring instances of social behaviours in a home-cage, Flanigan *et al* (2014) also indicated increased sociability in transgenic 5xFAD males and females at ~ 9 months of age.

Evidence from other strains suggests that the level of sociability (preference for social over non-social interactions) varies based on strain, sex, and age, but most strains do not avoid a social stimulus. Transgenic APP/PS1 males at 6 months of age exhibited no

preference for social over non-social interactions, although this was due to an increased investigation the non-social stimulus compared to wild-type controls (Filali et al, 2011a). These males also exhibited altered social novelty preference, spending more time with a familiar conspecific compared to a novel one, suggesting that these mice exhibit a reduced interest in social interactions (Filali et al, 2011a).

At 12 months of age, transgenic 3xTg-AD females initiated more social behaviours than wild-type controls, although no difference was seen in transgenic males (Bories et al, 2012). However, by 18 months of age, transgenic males exhibited more social behaviours than wild-type controls, whereas transgenic females exhibited less; this suggests that females exhibit a more rapid disease progression than males, and that this change in sociability (increase, followed by a decrease) is representative of different stages of the disease (Bories et al, 2012).

Transgenic Tg2576 females at 21 months of age exhibited no differences in social interaction time compared to wild-type controls in response to a juvenile conspecific, as well as no decrease in social interaction duration in response to a second encounter with the same conspecific (Deacon et al, 2009); while this indicates impaired social recognition memory, it also indicates that transgenic Tg2576 females do not exhibit a decrease in sociability at 21 months of age.

Overall, AD models exhibit either similar or higher levels of sociability compared to wild-type controls, with the 5xFAD strain exhibiting increased sociability between 8 – 10 months of age. However, transgenic 3xTg-AD females exhibit an increase, followed by a decrease, in social behaviours from 12 to 18 months of age, suggesting that social

interaction may be dependent on disease stage, with early stages characterized by increased social interaction, and later stages characterized by social withdrawal.

1.4.4 Affiliative Behaviours

Affiliative behaviours are behaviours that promote the formation of relationships (Cole & Young, 2009). In mice, nest building is an important behaviour in both males and females, and is involved in the development of reproductive relationships, raising young, warmth, and protection from predators (Deacon, 2006; Wesson & Wilson, 2011). As a result, impairments in nest building can impact survival of the animal as well as development of relationships with conspecifics.

Overall, mouse models of AD exhibit deficits in nest building with age. Transgenic 5xFAD males at 9 months of age (Schneider, Baldauf, Wetzel, & Reymann, 2014) and transgenic males and females at 12 months of age (Devi & Ohno, 2015) exhibit impaired nest building. However, Schneider *et al* also note that impaired motor behaviour could have an effect on nesting, which is in line with motor deficits discussed previously. Transgenic APP/PS1 mice also exhibit deficits in nest building compared to wild-type controls at 8 months of age (Filali, Lalonde, & Rivest, 2011b). Additionally, transgenic Tg2576 males and females exhibit impaired nest quality at 2 – 3 months of age, with progressive age-related declines at 10 – 12 months and 18 – 20 months of age (Wesson & Wilson, 2011). Overall, mouse models of AD consistently exhibit deficits in nest building that are evident by 9 months of age in the 5xFAD strain, and as early as 2 months in the Tg2576 strain, suggesting an impairment in affiliative behaviours.

1.4.5 Depressive-like Behaviour

There is mixed evidence for depressive-like behaviour in mouse models of AD. Patel *et al* (2014) indicated the presence of depressive-like behaviours in the 5xFAD strain, with transgenic mice at 4.5 – 5 months of age exhibiting longer periods of immobility compared to wild-type controls during a forced swim task. However, Yamazaki, Jin, Tsuchiya, Kanno, & Nishizaki (2015) indicated that 6-month-old transgenic 5xFAD mice exhibited reduced immobility during the forced swim and tail suspension tasks, suggesting reduced depressive-like behaviours compared to wild-type controls.

Evidence from other mouse models suggests that depressive-like behaviours are dependent on strain and may arise with age. Transgenic APP23 females at 2, 3, and 8 months of age and transgenic APP23 males at 3, 6, and 12 months of age exhibited no decrease in sucrose preference (a measure of anhedonia; Pfeffer et al, 2018). However, APP23 transgenic males exhibit reduced immobility and a longer latency to immobility in the tail suspension task at 6 months of age, and in the forced swim task at 3, 6, and 12 months of age, suggesting reduced depressive-like behaviours compared to wild-type controls (Vloeberghs, Van Dam, Coen, Staufenbiel, & De Deyn, 2006). In contrast to these findings, transgenic 3xTg-AD males at 18 months of age exhibited increased immobility on the tail suspension and forced swim tasks, as well as reduced sucrose preference compared to wild-type controls, suggesting increased depressive-like behaviours (Romano et al, 2014). In the hAPP strain, transgenic mice at 5 – 7 months of age exhibit no differences compared to wild-type controls in immobility on the tail suspension task, but increased immobility at 13 – 15 months of age, suggesting an age-related increase in depressive-like behaviours (Iascone et al, 2013). Overall, this suggests

that transgenic mouse models of AD may exhibit altered depressive-like behaviours, although whether they exhibit increased or decreased depressive-like behaviours is dependent on strain. It is also important to note that increased agitation present in some strains of transgenic AD mouse models could potentially present a confound for the forced swim test and tail suspension test by increasing struggling in these tasks (Vloeberghs et al, 2006).

1.4.6 Aggression

Aggression in mice is commonly seen between two unfamiliar conspecifics, and typically serves two purposes (Brain & Parmigiani, 1990; Miczek, Maxson, Fish, & Faccidomo, 2001; Williamson, Lee, & Curley, 2016): *1)* to establish a dominance ranking between two conspecifics; and *2)* to drive an intruder out of an owned territory. Attacks associated with this form of aggression are termed *offensive* and are characterized by bites to the back and flanks (Brain & Parmigiani, 1990; Miczek et al, 2001); these attacks are considered a social behaviour, and are not intended to break the skin (Grant & Mackintosh, 1963). By comparison, *defensive attacks* are seen in lactating females and are intended to defend the dam and her pups, and involve bites to vulnerable regions like the face, abdomen, and genitals that often occur without warning (Brain & Parmigiani, 1990; Miczek et al, 2001). Although aggression in mice can be studied in many situations, many researchers choose to use the resident-intruder paradigm. This paradigm involves a period of social isolation housing, previously found to increase aggression (Goldsmith, Brain, & Benton, 1976), followed by an encounter with a novel conspecific (the intruder) in the home-cage of the subject (Brain & Parmigiani, 1990), which can precipitate attack. However, while attack is the most overt behaviour in aggression, there

are a number of other acts associated with aggression, including chasing, mounting, and tail rattle, among others (Grant & Mackintosh, 1963; Miczek et al, 2001; Williamson et al, 2016).

An overall consensus among researchers is that transgenic 5xFAD males are highly aggressive. Early research with the 5xFAD background strain (C57 B6SJL) indicated that this strain exhibits high levels of home-cage aggression starting at approximately 8 weeks of age, with serious injuries and/or death occurring in group-housed males between 4 – 6 months of age (Kuby, 1997, p. 27; Lyon, Rastan, & Brown, 1996, p. 1562). Braun and Feinstein (2017) and MacGowan *et al* (2016) also indicate that their results could be influenced by intermale (offensive) aggression. Additionally, observations in our lab and others have indicated high levels of home-cage aggression exhibited by transgenic 5xFAD males (M.P. McDonald, personal communication, June 27, 2017). However, this data is largely anecdotal, and there are no thorough studies available yet regarding aggression in this strain.

Despite the lack of concrete information in the 5xFAD strain, there is ample evidence to indicate increased aggression in APP-overexpressing mouse strains. This increase in aggression is typically characterized by decreased latency to attack, increased number of attacks, and increased total attack duration in transgenic 7-month-old Tg2576 males (Alexander et al, 2011), 6- and 12-month old APP23 males (Vloeberghs et al, 2006), and 2.5- to 3-month-old males from three APP-overexpressing lines (containing wild-type, London, and Swedish mutations; Moechars et al, 1998) during the resident-intruder task. Additionally, a higher proportion of transgenic APP23 and APP-overexpressing males exhibit aggression during this task compared to wild-type controls (Moechars et al, 1998;

Vloeberghs et al, 2006). The APP-overexpressing lines examined by Moechars *et al* also exhibited higher levels of male—male and male—female aggression. While they do not specify whether these mice were housed in single-genotype (containing either transgenic or wild-type mice only) or mixed-genotype (containing both transgenic and wild-type mice) cages, they indicate that this aggression was specific to transgenic animals, suggesting that the genotype of both the aggressor and target may influence this behaviour. Conversely, transgenic 3xTg-AD males and females did not exhibit increased aggression compared to wild-type controls at 12 or 18 months of age (Bories et al, 2012). However, a combination of methodological differences could account for this low level of aggression relative to other strains: *1)* the use of a novel environment rather than the home-cage may eliminate a territorial component of aggression; *2)* the use of group-housing reduces the social isolation stress; and *3)* the use of mixed-genotype combinations in the home-cage and test environment may result in altered social interactions being exhibited by only one animal in an interacting pair, thereby de-escalating situations that could otherwise lead to aggression.

Overall, mouse models of AD exhibit increased intermale aggression, typically with decreased latency to attack, increased number of attacks, and increased total attack duration. However, this appears to be modulated by factors including the environment, stressors, and genotypes of both the aggressor and target, suggesting that this is not solely due to an innate increase in aggression.

1.5 The Present Study

1.5.1 Rationale and Aims

The negative impact of impaired social interactions on the lives of AD patients, their families, and caregivers, supports the need for continued research with animal models to elucidate the neurological underpinnings of altered social behaviours arising from the disease. However, four of the five mouse models most commonly used in AD—the Tg2576, APP23, APP/PS1, and 3xTg-AD strains—exhibit limitations, most notably a long time-scale for disease progression and a lack of widespread neurodegeneration congruous to human AD, making them more suitable for modeling early stages of the disease (Bilkei-Gorzo, 2014). Though the 5xFAD strain does not exhibit the tauopathy characteristic of human AD, it does exhibit rapid disease progression and widespread neurodegeneration, making it suitable for tracking the effects of neurodegeneration across early-, mid-, and late-stages of the disease. However, little information is available about the social effects that this may have in these mice. As a result, this thesis aims to further characterize the social changes exhibited by this strain over the course of disease progression, examining the effects of age, sex, and genotype on responses to social odour cues, sociability and social approach behaviour, social novelty preference, social recognition memory, and aggression.

While this work was originally conceived as a longitudinal study examining changes over time in both males and females, high levels of home-cage aggression observed in transgenic single-genotype cages necessitated the separation of all transgenic cage-mates between 3 – 6 months of age. As a result, a second study was performed to examine the factors leading to aggression in transgenic 5xFAD males. The original study is covered in

Chapter 2 (Experiment 1: Social Deficits in the 5xFAD Mouse Strain), and the second study is covered in Chapter 3 (Experiment 2: Phenotypic Contagion of Aggression in Male 5xFAD Mice). However, the specific aims and general overviews of the behavioural tasks used in each experiment are covered in the following sections.

1.5.2 Specific Aims of Experiment 1

Experiment 1 aims to characterize the changes in social behaviours exhibited by the 5xFAD strain. Specifically, it examines the effects of genotype, age, and sex on responses to social odour cues, sociability, social novelty preference, social recognition memory, and aggression; however, due to the home-cage aggression discussed previously, no group-housed transgenic males were available for testing at 6 months of age or later. As a result, sex effects were only examined at 3 months of age, and effects of age were only examined in females at 3, 6, and 9 months of age. The behavioural paradigms used were olfactory habituation/dishabituation, urine marking, the sociability/social novelty preference/social recognition tasks, and free social interaction. A brief overview of each task is given in Section 1.5.4.

1.5.3 Background and Specific Aims of Experiment 2

Prior to beginning Experiment 1, observations in the Brown lab (Dalhousie University) had indicated that transgenic males were aggressive when housed in mixed-genotype cages, although group-housing was a viable option. However, in order to prevent social behaviours in transgenic mice from affecting wild-type controls, and vice versa, mice for Experiment 1 were group-housed in single-sex, single-genotype cages (housing only transgenic mice or wild-type controls). This resulted in unexpectedly high levels of home-cage aggression in transgenic males, requiring all transgenic males

intended for Experiment 1 to be separated from cage-mates between 3 – 6 months of age to prevent injury. By comparison, wild-type males exhibited few issues with home-cage aggression even at 12 months of age, and reports from other labs indicated that mixed-genotype housing reduced—but did not eliminate—aggressive home-cage behaviours. Similarly, preliminary results from Experiment 1 suggested that transgenic males were not more aggressive in the free social interaction task compared to wild-type controls at three months of age; however, this task was performed using a novel wild-type conspecific as a stimulus, and behaviours in response to a transgenic stimulus had not been examined. Overall, this suggested that the environment and genotype of a conspecific—whether novel or familiar—could affect aggressive behaviours in 5xFAD males.

As a result, Experiment 2 was developed in order to examine how the genotypes—and by extension, the phenotypes—of two interacting males could mediate their behaviours, and how this was affected by familiarity with the environment and conspecific. Specifically, the aims were to examine the onset and progression of aggressive home-cage behaviours in transgenic males, whether levels of home-cage aggression differed from single-genotype to mixed-genotype cages, whether social approach behaviours differed in response to a novel transgenic vs wild-type conspecific, whether aggressive behaviours in the free social task were mediated in response to a novel transgenic or wild-type stimulus, and whether genotype affected dominance in 5xFAD males. As the highest levels of aggressive behaviour observed in transgenic males in Experiment 1 occurred before 6 months of age, Experiment 2 examined the development of aggressive home-cage behaviours between 2 – 6 months of age (starting

prior to observed aggression), as well as examining aggression in response to novel stimuli at 6 months of age (the previously observed peak of aggression). As a result, the sociability task, free social interaction, home-cage observations, and the tube task of dominance were examined; each of these is described in the following sections.

1.5.4 Behavioural Paradigms

1.5.4.1 Olfactory Habituation/Dishabituation

The olfactory habituation/dishabituation task is used to assess whether an animal can detect and differentiate between odours cues by examining investigation durations of novel and familiar odours (Yang & Crawley, 2009). While the present study utilized a protocol outlined in 2009, the basic task and its variants have been used to measure olfaction since the paradigm was described in 1981 (Yang & Crawley, 2009). Briefly, this task involves successive presentations (typically 1 – 2 minutes each) of one odour cue, during which the time spent investigating is recorded; this successive presentation of the same odour leads to a familiarization and decrease in investigation of the odour (*habituation*) over successive trials. After 3 – 6 successive presentations of the initial odour, a novel odour is presented for one or more trials; the presentation of a new odour should elicit an increase in investigation time (*dishabituation*), indicating that the animal can differentiate between odours. This pattern of habituation and dishabituation is characteristic of normal olfaction, indicating that the animal can perceive and differentiate between these odours, although sniffing duration in response to each odour can also be used as a measure of attractiveness of the odour (Yang & Crawley, 2009).

This paradigm has been used successfully in the past to examine olfactory function in rats and mice. Results have indicated that mouse urine from the same strain, or different

strains raised in a germ-free environment, was undistinguishable by rats despite normal olfaction (Schellinck, Rooney, & Brown, 1995), and altered responses to social and non-social odour cues have been associated with decreased sociability in mouse models of autism spectrum disorder (Rabaneda, Robles-Lanuza, Nieto-Gonzalez, & Scholl, 2014; Yang et al, 2012).

In the present study, the olfactory habituation/dishabituation task was used in Experiment 1 in order to assess whether transgenic 5xFAD mice were able to detect and differentiate between social and non-social odours, and to examine interest in social odours. Although previous studies indicate no deficits in olfactory discrimination or sensitivity in 5xFAD transgenic mice, this task was also performed to confirm that any behavioural changes in the present cohort were not due to impaired olfaction.

1.5.4.2 Urine Marking

Urine marking has previously been used to examine dominance hierarchies (Desjardins, Maruniak, & Bronson, 1973) and responses to social odour cues (Brown, 1977), and involves exposing the subject to a stimulus (such as an odour cue or a conspecific separated by a barrier) and then assessing the number and distribution of urine marks. Scent marking allows for long-term communication with conspecifics, even in the absence of the original marker, and mice use scent marking as a method of recognition of individuals, territory demarcation, determination of dominance, and advertisement of reproductive fitness (Arakawa, Blanchard, Arakawa, Dunlap, & Blanchard, 2008). As a result, a number of factors can influence the rate of urine deposition, including age, sex, dominance, and reproductive fitness of the marker, as well as presence of urine from a conspecific, and the age, sex, reproductive status, and

dominance of the conspecific (Arakawa, Arakawa, Blanchard, & Blanchard, 2007a; Drickamer, 1995; Drickamer, 2001; Ninomiya & Kimura, 1989). Therefore, the urine marking task was used in Experiment 1 to explore social communication by examining whether transgenic 5xFAD mice exhibit responses to social odour cues that are comparable to wild-type controls relative to the type of cue.

1.5.4.3 Sociability, Social Novelty Preference, and Social Recognition Memory

This paradigm involves successive trials in the same apparatus and allows for examination of three behaviours: sociability (preference for social vs non-social interactions); social novelty preference (preference for interaction with a novel vs familiar conspecific); and social recognition memory (ability to identify and differentiate between previously encountered and novel conspecifics; Filali et al, 2011a; Gunn, Huentelman, & Brown, 2011). These behaviours are examined using a three-chamber apparatus as previously described in Section 1.4.3. Placing a stimulus in each of the outer chambers creates a three-alternative forced choice task; subjects need to cross a threshold in order to interact with one stimulus or another, or neither. The choice of stimuli, their locations, and the delay between successive trials therefore determine the specific behaviour examined. The use of a novel conspecific vs a non-social stimulus (like a plastic toy) assesses sociability, whereas the use of a novel vs familiar conspecific is used to examine social novelty preference; varying the time between initial exposure to the familiar conspecific and the subsequent test is used to examine social recognition memory. Although variations on this task exist, it has been used successfully to assess sociability, social novelty preference, and social recognition in a number of mouse strains

(Deutsch, Burket, Jacome, Cannon, & Herndon, 2011; Fairless, Shah, Guthrie, Li, & Brodkin, 2011; Filali et al, 2011a; Gunn et al, 2011; Pobbe et al, 2011; Yang et al, 2012). While sociability, social novelty preference, and social recognition were performed during Experiment 1, a slightly modified protocol of sociability alone was used for Experiment 2.

1.5.4.4 Free Social Interaction

In order to study social interactions involving direct contact between animals, such as aggression, a free social interaction task was used. Variations on this task—also referred to as reciprocal social interaction—have been described by a number of researchers (Bories et al, 2012; Fairless et al, 2011; Koike et al, 2009; Yang et al, 2012), although the common features remain the same: the subject is placed in a novel environment (such as a clean cage, open field, or three-chamber apparatus), a novel conspecific is introduced, and the two are allowed to interact directly for a set amount of time. These interactions are scored for the number or duration of behaviours, including investigative behaviours (such as orofacial or anogenital sniffing), aggressive behaviours (including chasing and attacking), and self-grooming behaviours, among others. In the present study, this task was used specifically to assess aggression, although behaviours were also scored for durations of investigative and self-grooming behaviours, as well as affiliative behaviours (Experiment 1) and defensive behaviours (Experiment 2).

1.5.4.5 Home-cage Observations

Home-cage observations were performed in Experiment 2 to assess behaviours in a familiar environment with familiar conspecifics, thereby allowing for examination of behaviours over an extended period of time without inducing stress from repeated testing.

Briefly, subjects were pair-housed in modified rat cages (containing two enrichment tubes as well as food and water bowls to minimize obstruction of view) in either single-genotype (transgenic—transgenic or wild-type—wild-type) or mixed-genotype (transgenic—wild-type) cages, and recorded between 8 – 22 weeks of age to examine the progression of aggressive, defensive, investigative, affiliative, and solitary behaviours. Previous evidence (discussed in Section 1.4.6) suggests that aggression in mouse models of AD may vary based on the familiarity of the environment. While home-cage observations require no special apparatus or methodology and are simply used to monitor behaviours in consistent environment over long periods of time, behaviours tracked in the present study were based on those of Arakawa, Blanchard, and Blanchard (2007b), Grant and Mackintosh (1963), and Williamson *et al* (2016).

1.5.4.6 Tube Test of Dominance

The tube test of dominance was used in Experiment 2 to assess whether transgenic males differed from wild-type controls on overall dominance. Briefly, this test involves two "goal boxes" connected by a clear acrylic tube, approximately 3 cm in diameter and 30 cm long (Lindzey, Winston, & Manosevitz, 1961). A mouse is placed in each of the goal boxes and encouraged to enter the tube by gently pulling on the tail; once both have entered, their tails are released and they are allowed to meet in the middle of the apparatus. Due to the diameter of the tube, the two cannot pass around each other, and one must back out into the starting box to allow the other one through; the one that backs out is considered submissive, while the one that passes through to the opposite goal box is considered dominant. Since its inception, this paradigm has been used successfully to assess social dominance in a number of mouse strains (Flanigan et al, 2014; Wang,

Kessels, & Hu, 2014; Yang et al, 2015). Although aggression and dominance are two distinct phenomena, aggression can be required for initial determination of hierarchy (Wang et al, 2014), and consistently high levels of dominance in transgenic males relative to wild-type controls could point to a source of conflict in transgenic single-genotype cages, as "submissive" mice may test the dominant one for some shared resource (such as food, water, or preferred sleeping locations), ultimately leading to aggression aimed at restoring the balance of dominance.

Chapter 2: Experiment 1: Social Deficits in the 5xFAD Mouse Strain

2.1 Introduction

The use of animal models has provided a wealth of information regarding the effects of AD-related pathology on social behaviours. Such changes include impaired social recognition memory in 21-month old transgenic Tg2576 females and increased aggression in a resident-intruder task in 7 month old transgenic Tg2576 males (Filali et al, 2011a; Alexander et al., 2011; Deacon et al, 2009). Evidence for increased sociability in the 3xTg-AD mouse model has also been presented (Bories et al, 2012), whereas the Tg2576 strain exhibits no effect of AD pathology on sociability (Deacon et al, 2009). Overall, these results indicate that some mouse models of AD exhibit changes in social behaviour that include altered aggression and sociability. However, many transgenic mouse models of AD (e.g., 3xTg-AD, APP/PS1, and Tg2576) exhibit a slow rate of Aβ accumulation and can only model the initial phase of the disease which does not include widespread neurodegeneration (Bilkei-Gorzo, 2014).

By comparison, the 5xFAD strain exhibits rapid development and progression of amyloid plaques and neurodegeneration, allowing effects to be tracked across early-, mid-, and late-stage disease progression. There is increasing evidence that 5xFAD mice exhibit a range of social deficits that, in some cases, mirror the social symptoms presented by AD patients. Transgenic 5xFAD 9-month-old male mice and 12-month-old male and female mice present deficits in nest building, a behaviour that has been associated with affiliative behaviours (Schneider et al, 2014; Devi & Ohno, 2015), however they also engage in more home-cage social behaviours than wild-type controls

(Flanigan et al, 2014). They also exhibit impaired social recognition memory at 9-months of age (Flanigan et al, 2014), consistent with memory deficits exhibited in this strain and human AD patients. In our own lab, preliminary studies have indicated that transgenic 5xFAD males tended to spend less time than wild-type controls investigating a novel social odour, but more time investigating a novel stimulus mouse (MacGowan et al, 2016). In this case, this combination of behaviours was taken to indicate social withdrawal concomitant with increased aggression, as the increased investigation of the novel stimulus animal could represent increased attempts to attack the animal. This interpretation is consistent with the social withdrawal and increased A/A seen in human AD patients, as well as observations of high levels of aggression observed in transgenic 5xFAD males in our lab and others.

While these results suggest that the 5xFAD strain exhibits social deficits, a comprehensive investigation of social behaviours in this strain has—to the best of my knowledge—not yet been performed. The present study aimed to characterize a range of social behaviours in the 5xFAD strain in a genotype-, age-, and sex-dependent manner. Specifically, this study examined responses to odour cues, sociability, social novelty preference, social recognition memory, and aggression. Overall, it was hypothesized that transgenic 5xFAD mice would exhibit *1)* reduced response to social odour cues, *2)* increased sociability, *3)* increased social novelty preference, *4)* impaired social recognition memory, and *5)* increased aggression compared to wild-type controls in an age- and sex-dependent manner. However, during the study transgenic males were found to exhibit high levels of home-cage aggression between 3 – 6 months of age, necessitating separation of all transgenic males by 6 months of age. Due to the potential

for isolation to affect social interactions, such as increased aggression (Goldsmith et al, 1976), only group-housed animals were tested; as a result, there is no data available for transgenic males in the present study at 6 or 9 months of age, and this high level of home-cage aggression is further examined in Chapter 3 (Experiment 2: Phenotypic Contagion of Aggression in Male 5xFAD Mice).

2.2 Methods

2.2.1 Subjects

2.2.1.1 Groups

Subjects were separated into four groups: female transgenic, female wild-type, male transgenic, and male wild-type. Female subjects were tested at 13 weeks (3 months), 26 weeks (6 months), and 39 weeks (9 months) of age and male subjects were tested at 13 weeks of age. Mice exhibiting stereotypy (continuously running in circles, doing backflips, jumping in one corner of the cage) were excluded from testing once these behaviours were discovered to ensure that these behaviours did not affect results. Mice that were homozygous for a genetic mutation in the *Pde6b* gene, which is common in this mouse strain and leads to retinal degeneration, were not used to ensure that visual impairments with age did not affect results. Singly-housed subjects were also excluded from testing. See Table 2.1 for sample sizes at each age. All subjects were sexually naive.

Table 2.1

Sample Sizes by Sex, Genotype, and Age

	Female		Male	
Age	Transgenic	Wild-Type	Transgenic	Wild-Type
3 Months	24	17	12	6
6 Months	22	17	0	6
9 Months	21	17	0	6

2.2.1.2 Breeding

Animals were bred in-house using polygamous breeding. Two adult wild-type females (C57 B6SJL; obtained from The Jackson Laboratory, Bar Harbor, ME) were paired with a transgenic 5xFAD adult male (from the colony) for one week; following this period, all mice were housed individually. Breeder males were heterozygous for PSEN and homozygous for the wild-type Pde6b gene to reduce the likelihood of offspring having retinal degeneration. Offspring were ear-punched for genotyping and identification between P14 and P18. Animals were weaned between P23 and P26.

2.2.1.3 Genotyping

Animals were genotyped for PSEN and Pde6b genes prior to weaning. A 400 ml stock of digest buffer was made using the following: 1.6 ml of 20 mM NaCl; 20 ml of 50 mM Tris-HCl; 0.8 ml of 1 mM EDTA; 40 ml of 1% SDS; 337.6 ml PCR-grade water. The digest buffer was divided into 5 ml aliquots and stored at -20°C until use. Proteinase K (20 mg/ml) was also divided into 250 µl aliquots and stored at -20°C until use. A digest solution was prepared immediately prior to use at a ratio of 1:19 parts Proteinase K to digest buffer. DNA was extracted from ear punches by shaking tissue in 200 µl digest solution in 1.5 ml centrifuge tubes overnight (approximately 18 hours) at 56°C. Following digestion, samples were centrifuged at 14,000 rpm for 10 minutes to remove hair and debris, and the supernatant (approximately 180 µl) was transferred to a new centrifuge tube, to which 200 µl of isopropanol was added. Tubes were then vortexed and were centrifuged again at 14,000 rpm for 10 minutes to precipitate DNA. The supernatant was then removed, and 200 µl of 70% ethanol was added to the remaining precipitate. The tubes were again centrifuged at 14,000 rpm for 5 minutes, at which point the

supernatant was removed again and tubes were allowed to air dry; tubes were left open and placed upside-down on a rack on top of a heat plate set to 56°C to accelerate drying. Following drying, the DNA was re-suspended in 40 µl of PCR-grade water. DNA samples were then stored at -20°C until use.

Genotyping was performed to check for the presence of mutant PSEN and Pde6b genes. Genotyping for PSEN was performed using oIMR1644 (5'-AAT AGA GAA CGG CAG GAG CA-3'), oIMR1645 (5'-GCC ATG AGG GCA CTA ATC AT-3'), oIMR7338 (5'-CTA GGC CAC AGA ATT GAA AGA TCT-3'), and oIMR7339 (5'-GTA GGT GGA AAT TCT AGC ATC ATC C-3') primers, obtained from Sigma-Aldrich Canada Co. (Oakville, ON, Canada). Reaction components for each sample were combined as follows: 3.78 µl PCR-grade H_2O; 1.2 µl 10x PCR Buffer without $MgCl_2$ (Sigma-Aldrich); 1.2 µl 25 mM $MgCl_2$ solution (Sigma-Aldrich); 0.8 µl 20 µM oIMR1644 primer; 0.8 µl 20 µM oIMR1645 primer; 0.6 µl 20 µM oIMR7338 primer; 0.6 µl 20 µM oIMR7339 primer; 0.96 µl 2.5 mM dNTP; 0.06 µl JumpStart Taq DNA Polymerase (Sigma-Aldrich); 2 µl DNA. A master mix of reaction components (not including DNA) was made and gently mixed, then pipetted into PCR tubes; 10 µl of master mix was added to each tube, followed by 2 µl of DNA. A positive control (using a DNA sample from an animal previously determined to be heterozygous for PSEN) and a negative control (PCR-grade water from the same aliquot used to create the master mix) was also used. Samples were then capped and briefly spun on a mini centrifuge to remove bubbles before being placed into a thermocycler for amplification using the following pattern (min:sec): 94°C for 3:00; (94°C for 0:30; 60°C for 1:00; 72°C for 1:00) x35; 72°C for 2:00; 4°C until removal.

Genotyping for Pde6b was performed using oIMR2093 (5'-AAG CTA GCT GCA GTA ACG CCA TTT-3'), oIMR2094 (5'-ACC TGC ATG TGA ACC CAG TAT TCT ATC-3'), and oIMR2095 (5'-CTA CAG CCC CTC TCC AAG GTT TAT AG-3') primers (Sigma-Aldrich). Reaction components for each sample were combined as follows: 5.56 µl PCR-grade H_2O; 1.2 µl 10x PCR Buffer without $MgCl_2$ (Sigma-Aldrich); 0.72 µl 25 mM $MgCl_2$ solution (Sigma-Aldrich); 0.48 µl 20 µM oIMR2093 primer; 0.48 µl 20 µM oIMR2094 primer; 0.48 µl 20 µM oIMR2095 primer; 0.96 µl 2.5 mM dNTP; 0.12 µl JumpStart Taq DNA Polymerase (Sigma-Aldrich); 2 µl DNA. A master mix of reaction components (not including DNA) was made and gently mixed, then pipetted into PCR tubes; 10 µl of master mix was added to each tube, followed by 2 µl of DNA. A positive control (using a DNA sample from an animal previously determined to be heterozygous for Pde6b) and a negative control (PCR-grade water from the same aliquot used to create the master mix) was also used. Samples were then capped and briefly spun on a mini centrifuge to remove bubbles before being placed into a thermocycler for amplification using the following pattern (min:sec): 94°C for 5:00; (94°C for 0:30; 63°C for 0:30; 72°C for 1:30) x35; 72°C for 2:00; 4°C until removal.

During DNA amplification, a 1.5% gel was prepared using agarose and 1x TAE and allowed to set for 30 – 60 minutes prior to loading. Following PCR amplification, samples were removed from the thermocycler, gently mixed, and then spun on a mini centrifuge to transfer any liquid from the cap into the tube. After the spin, 2.4 µl of UView 6x Loading Dye (Bio-Rad Laboratories Canada Ltd., Mississauga, ON, Canada) was added to each sample. A molecular ruler (100 bp Molecular Ruler, Bio-Rad Laboratories Canada) was also prepared containing 8.33 µl of molecular ruler and 1.67 µl

of loading dye for each row of lanes. Two rows of wells were used: one for PSEN, one for Pde6b. Samples were loaded into each row of wells sequentially, starting with the molecular ruler, then the samples, and finally the positive and negative controls. Most gels were run with either 52 wells (26 lanes per row) or 104 wells (52 lanes per row) at 180 VDC for 60 minutes. Following electrophoresis, gels were imaged under UV light. Any samples that exhibited smearing or weak or non-existent fluorescence were amplified again by doubling the amount of DNA in each PCR sample (from 2 µl to 4 µl) and removing 2 µl of water from the master mix used for each sample (to maintain an overall volume of 12 µl per sample). Any samples that exhibited no bands following the second run were ear-punched a second time, and the DNA extraction and genotyping process was repeated. A mutant PSEN band was expected at 608 bp, with a wild-type band expected at 324 bp; heterozygous (transgenic) mice were expected to exhibit both bands. A mutant Pde6b bands was expected at 560 bp, while a wild-type band was expected at 240 bp; heterozygous animals exhibited a band at 560 and 240 bp.

2.2.1.4 Housing

Animals were group-housed in same-sex, same-genotype, same-litter cages following weaning; cages contained between 2 – 4 animals. Subjects were housed in polysulfone cages (30 x 19 x 13 cm; model PC7115HT; Allentown Caging Inc., Allentown, NJ, USA) containing wood-chip bedding (FreshBed; Shaw Resources, Shubenacadie, NS, Canada), a metal cage top containing food (Laboratory Rodent Diet #5001; Purina LabDiet, St. Louis, MO, USA) and a water bottle, and two black, opaque, polymer enrichment tubes (4 cm diameter, approx. 8 cm long); cages were topped with a micro-isolator filter to reduce the spread of airborne contaminants and diseases.

Animals were housed in a colony room on an inverted 12:12 light:dark cycle; the dark phase lasted from 09:30 to 21:30, and the light phase lasted from 21:30 to 09:30. Cages were changed weekly, prior to the end of the light cycle (approximately 08:30 – 09:30). To reduce instability of dominance hierarchies, a small amount of bedding was transferred from the old cage to the new cage.

2.2.1.5 Separation of Animals Due to Aggressive Behaviour

Animals were group-housed when possible; however, cages exhibiting abnormally high levels of home-cage aggression were separated to prevent injury to animals. In order to prevent unnecessary separation of animals, they were only separated when any of the animals in the cage exhibited signs injury or distress, or broken skin from bites.

When possible, the aggressor was removed from the cage and housed individually, leaving the submissive animals group-housed. When the aggressor could not be identified, the injured animal was separated. Injured animals were treated with antibiotic cream and monitored for healing. Any animals exhibiting distress were immediately euthanized by first inducing anaesthesia and then performing cervical dislocation or decapitation.

2.2.2 Behavioural Procedures

2.2.2.1 Testing Schedule

Subjects performed four tests over eight days, with one-day breaks between tasks to reduce stress due to daily testing. All testing was performed within an 11-hour window, starting a minimum of 30 minutes after onset of the dark phase, and ending at least 30 minutes prior to onset of the light phase. Tasks were performed in order of increasing stress, as follows: Day 1, olfactory habituation/dishabituation; Day 3, urine marking; Day

5, sociability, social novelty preference, and social recognition memory (30 minute delay); Day 6, social recognition memory (24 hour delay); Day 8, free social interaction.

2.2.2.2 Testing Conditions

Testing was performed in a quiet 2.4 x 2.4 m room containing a desk with a computer and a table for the testing apparatus. Two monochromatic network cameras were used to video record the tasks: one mounted approximately 1 m over the table on an overhead stand, and the other located on the table 15-30 cm away from the apparatus. Cameras were connected to the computer via a network hub. Tasks were recorded using the Biobserve Viewer 3.0 program (Biobserve GmbH, Bonn, Germany).

Testing was performed under minimal lighting conditions; the room was lit with a ceiling-mounted red light, and a desk lamp containing a red bulb. Although the computer display was not turned off during testing, brightness was reduced and the screen was angled away from the testing apparatus to reduce direct illumination. The door was kept closed to reduce light and noise from surrounding areas. Due to the possibility of aggression, the free social interaction protocol required the experimenter to remain in the room for the duration of the task; otherwise, the experimenter only entered the room at the beginning and end of every trial.

2.2.2.3 Olfactory Habituation/Dishabituation

2.2.2.3.1 Apparatus

The apparatus consisted of a standard polysulfone cage (30 x 19 x 13 cm; identical to the home cage) containing wood chip bedding and topped with a metal cage top. Wood-handled cotton swabs (15 cm long; Puritan Medical Products Company, Guildford, MN, USA) were inserted between the bars of the cage top and held in place with a binder clip,

with the swab and approximately 3 cm of the handle protruding into the cage approximately 5 cm above the base of the cage (see Figure 2.1 for representative layout). Cotton swabs were inserted from the handle end to avoid transferring scent from the swab to the metal cage top.

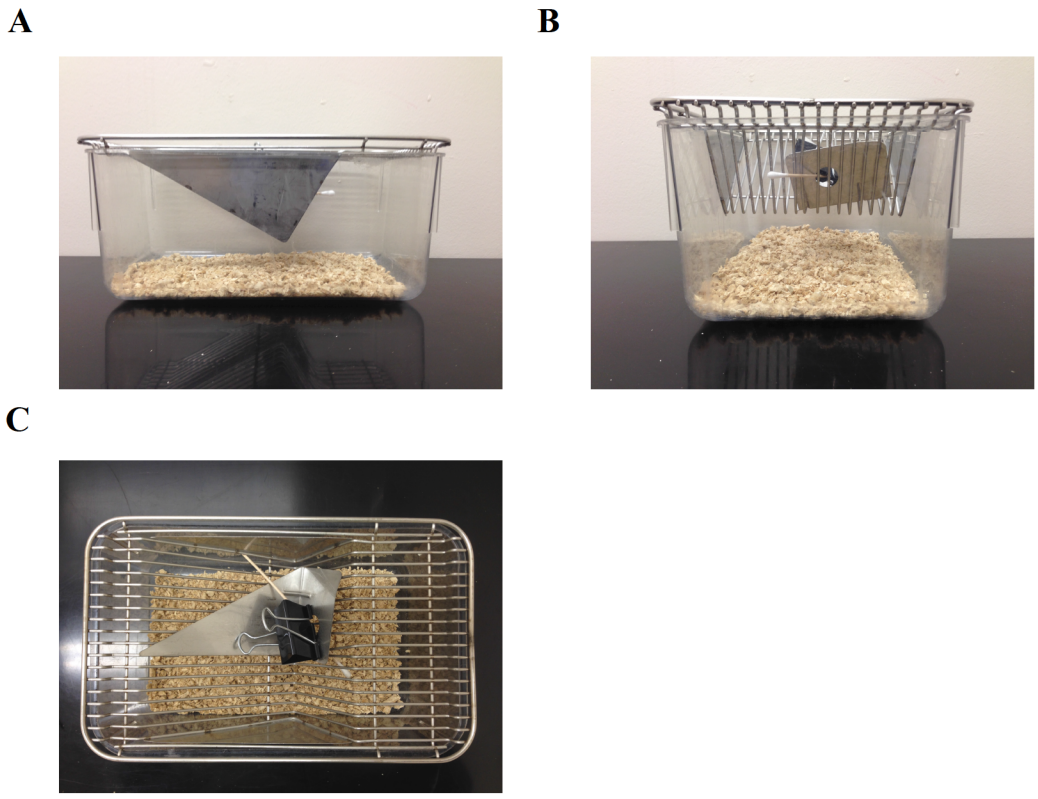

Figure 2.1. Representative layout of the apparatus used in the olfactory habituation/dishabituation task from the **A)** front, **B)** side, and **C)** top views.

2.2.2.3.2 Stimuli

Stimuli consisted of the following scents, in order: *1)* distilled water (neutral control); *2)* imitation almond extract (1:100 dilution; La Cie McCormick Canada, London, ON, Canada); *3)* imitation banana extract (1:100 dilution; La Cie McCormick Canada); *4)*

unfamiliar same-sex cage 1; *5)* unfamiliar same-sex cage 2. All stimuli were prepared on the day of testing and were only used for that day. Dilutions were performed using the same water as the neutral control, and stimuli were prepared on the day of testing. Non-social odour cues were made by preparing the dilution in a 15 mL conical centrifuge tube, and briefly immersing the swab in the dilution prior to use. Each of the non-social odour cues used throughout the experiment came from the same original bottles, which were stored at 4°C and vortexed prior to use. Social odour cues were prepared by swabbing the bottom of an unfamiliar, same-sex cage that had not been changed for at least three days prior; each stimulus cage contained at least two animals. Any bedding material remaining on the swabs was brushed off, and swabs were placed in a zipper storage bag until use.

2.2.2.3.3 Procedure

The procedure was performed as described by Yang and Crawley (2009). Subjects were placed into a clean cage, the metal cage top containing a clean, dry cotton swab was placed on top, and the cage was covered with a microisolator top; subjects were allowed to habituate to this apparatus for 30 minutes prior to testing to reduce the novelty of the cotton swab. During this time, subjects remained in the colony room. All subjects were removed from home-cages and placed into testing cages at the same time, and were returned to the home-cage once all cage-mates had completed testing.

Following the 30-minute habituation period, the dry swab was removed, and subjects were moved to the testing room. A swab was prepared with water alone and was placed into a metal cage top; this top was placed onto the cage, and the recording was started. Upon starting the recording, the experimenter left the room and prepared a second cage top with the next stimulus. When the recording finished, the cage top containing the

previous stimulus was replaced with the next stimulus, and the next recording was started. This process was repeated until all 15 stimuli had been presented.

Following testing, subjects were returned to the colony room. Subjects remained in the testing cage until all cage-mates had been tested before being returned to their home-cage. Subjects not undergoing habituation or testing were given a metal cage top containing a bottle of water to prevent dehydration.

2.2.2.3.4 Scoring and Data Analysis

Videos were scored manually for time in seconds spent sniffing during each presentation of each odour, as described by Yang and Crawley (2009). Sniffing was defined as orientation of the snout towards the cotton swab, with the swab 2 cm or less from the snout. Raters were blind to the genotypes of the animals.

Data analysis was performed in R version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria) using bootstrapped 95% confidence intervals of mean sniffing durations and effects of genotype for each presentation of each odour. Outliers were not excluded.

2.2.2.4 Urine Marking

2.2.2.4.1 Apparatus

The apparatus consisted of a polycarbonate arena, approximately 40 x 40 x 60 cm, with four opaque white walls and a colourless and transparent floor. The bottom of the arena was lined with a sheet of clean, absorbent paper (Strathmore 400 Series Recycled Drawing Paper; Strathmore Artist Papers, Neenah, WI, USA), similar to the apparatus described by Roullet, Wohr, and Crawley (2011). Sheets were prepared by writing the date, subject number, and stimulus on the underside of the sheet in pencil, which was

used to reduce odour and potential bleeding of ink through the sheet. Odour stimuli were prepared by placing 40 µL spots of of the odour cue on the paper in each corner of the arena for a total of 4 spots (160 µL), similar to that described by Ninomiya and Kimura (1989). Each sheet contained only a single odour cue placed in each corner, with no odours present during habituation.

2.2.2.4.2 Stimuli

Stimuli consisted of three odour cues: *1)* imitation vanilla extract (1:100 dilution; La Cie McCormick Canada); *2)* same-sex conspecific urine; *3)* opposite-sex conspecific urine. Vanilla scent (non-social cue) was prepared on the day of testing using distilled water. The vanilla extract used throughout the experiment came from the same original bottle and was stored at 4°C and vortexed prior to use. Urine was collected from unfamiliar, sexually-naive, adult, wild-type C57B6SJL mice. Urine collection was performed similarly to that described by Yang and Crawley (2009): mice were scruffed, a clean 1.5 mL microcentrifuge tube was held near the genitals, and the abdomen was palpated gently to promote urination. This method of urine collection was chosen over metabolic cages or similar apparatus as it restricts stress on the animal to the period immediately during urine collection and prevents feces from being mixed with the urine. Urine was collected from at least four mice per sex over the course of seven days. Following collection, urine was refrigerated at 4° C, then pooled and divided into 1 mL aliquots. Pooling was done to ensure odour cues are consistent across testing, and do not vary across stage of estrous or dominance of sample provider. Aliquots were then frozen at -80° C until use. Odour stimuli were kept on ice during testing.

2.2.2.4.3 Procedure

Subjects were transferred to a clean holding cage containing bedding and a food pellet, and a micro-isolator cover was placed on top. All subjects were removed from home-cages and placed into holding cages at the same time. They were not returned to the home-cage until all cage-mates had completed testing.

An arena was prepared with a clean sheet of paper with no odour, and the subject was placed into the arena and allowed to freely explore for 5 minutes to habituate. During this time, a second arena was prepared with the next paper sheet and stimulus; order of presentation of each stimulus was counterbalanced across subjects and across ages, and arenas were prepared in a separate room from testing to minimize odour in the testing room. At the end of the 5-minute habituation period, the subject was returned to its holding cage, the arena with the habituation sheet was removed, and the arena containing the next stimulus was put in its place. The mouse was then placed in the next arena and allowed to freely explore for 15 minutes. The first arena—containing the habituation sheet—was then moved to a separate room away from the testing area; this was done to reduce odours from reaching the testing room. A third arena was then prepared with the next stimulus, and the process was repeated until the mouse had performed one 5-minute habituation trial and three 15-minute testing trials. Sheets were allowed to dry for 15 minutes immediately following testing, and then were carefully removed from the apparatus and placed on a flat surface to further dry for approximately 24 hours. In between trials, arenas were cleaned with Fisherbrand Sparkleen 1 (Fisher Scientific, Pittsburgh, PA, USA) and room-temperature water, then thoroughly dried with paper towels.

Following testing, subjects were returned to the colony room. Subjects remained in the holding cage until all cage-mates had been tested before being returned to their home-cage. Subjects not undergoing testing were given a metal cage top containing a bottle of water to prevent dehydration.

2.2.2.4.4 Scoring and Data Analysis

Scoring was done by examining the surface area of subject urine covering the sheets. After drying, sheets were sliced into quarters (approximately 20 x 20 cm), then were imaged using a Bio-Rad ChemiDoc MP Imaging System and Image Lab Version 5.1 (Bio-Rad Laboratories, Hercules, CA, USA) using the following settings: blue light illumination; 530/28 filter; 50 msec exposure time. These settings were previously determined to provide the best fluorescence of urine marking while minimizing background fluorescence. All images were taken at a uniform zoom level to allow comparison of images at varying time points. Images were then exported as TIFF files for analysis in ImageJ Version 1.51 (U.S. National Institutes of Health, Bethesda, MD, USA). Image thresholding was used to identify fluorescence, using the following settings: 14500 lower limit; 65535 upper limit; MaxEntropy protocol; B&W output; dark background. As with imaging, these settings were previously found to provide the best differentiation between fluorescence of urine spots and background fluorescence. Following thresholding, a 1 x 1 cm grid was overlaid on the image, and the number of grid squares containing urine spots was counted (Arakawa et al, 2007a; Arakawa et al, 2008; Drickamer, 2001; Roullet et al, 2011). Previous studies (Arakawa et al, 2007a; Arakawa et al., 2008) defined urine pools as any spots encompassing more than 4 square grids (2 x 2 cm); however, placement of the grid may affect whether a spot is marked as a

urine pool, and urine pools may shift during handling of the apparatus, thereby affecting their shape. As a result, the present study deemed urine pools to be any spots greater than 4 cm^2, regardless of shape. Urine pools and spots corresponding to the original locations of the odour stimuli were removed from analysis, and the number of grid squares covered by remaining marks was summed for each sheet.

Data analysis was performed in R version 3.4.1 (R Foundation for Statistical Computing) using multilevel modelling. Subjects that were separated or exhibiting stereotypy were not testing, resulting in missing data at certain ages; in order to make use of the remaining data, multilevel modelling was chosen for data analysis over repeated-measures ANOVA (which requires that all subjects have data for each time-point). Sex and genotype were used as between-subjects factors, while age and stimulus were used as within-subjects factors. The effect of subject was used as the error term. A preliminary examination indicated that the data exhibited a negative binomial distribution; as a result, a logarithm transformation was used to normalize the residuals in order to perform accurate data analysis. As data for transgenic males was only available at 3 months of age, two analyses were performed: one examining the effects of sex, genotype, and stimulus at 3 months of age in males and females, and one examining the effects of age, genotype, and stimulus from 3 – 9 months of age in females only. Bootstrapped 95% confidence intervals were used to examine patterns of significant effects.

Due to the presence of liquid on the sheet (including odour stimuli and urine pools from the subjects), subjects could artificially increase the surface area of the fluorescence by dragging their tails through or stepping in liquid. To avoid bias by the rater and prevent removal of actual urine marking, these marks were not excluded from analysis;

instead, outliers (sheets where the number of grid squares covered was over two standard deviations from the mean) were excluded from analysis. Outliers were determined by calculating the mean and standard deviation for each sex, genotype, age, and stimulus separately, then comparing the results of each sheet to those values. Outliers were flagged and removed from data analysis, and the remaining data was used in the final analysis.

2.2.2.5 Sociability, Social Novelty Preference, and Social Recognition Memory

2.2.2.5.1 Apparatus

The apparatus was a three-chamber box similar to that described by Moy *et al* (2004). The apparatus consisted of a clear, acrylic box, 69 x 20 x 20 cm, divided into three compartments of equal size (23 x 20 x 20 cm) by two clear, acrylic walls. Access to each compartment was through a 6 x 6 cm, floor-level opening in the middle of the dividing walls. Opaque sections of acrylic were used as barriers between the three chambers. The floor of the apparatus was lined with the same wood-chip bedding as used in the home-cages, and a round wire cage (Galaxy Cup; Spectrum Diversified Designs Inc., Streetsboro, OH, USA) was used in each of the two outer chambers to contain stimuli. White, opaque, 500 mL HDPE bottles (Nalge Nunc International Corporation, Rochester, NY, USA) filled with water were placed on top of the Galaxy Cups to prevent subjects from climbing on top. See Figure 2.2 for a representative layout.

2.2.2.5.2 Procedure

Prior to testing, subjects were transferred to a clean holding cage containing wood-chip bedding and a food pellet, and a micro-isolator cover was placed on top. All subjects were removed from home-cages and placed into holding cages at the same time. They

were not returned to the home-cage until all cage-mates had completed testing. Stimuli mice were also transferred to holding cages for testing.

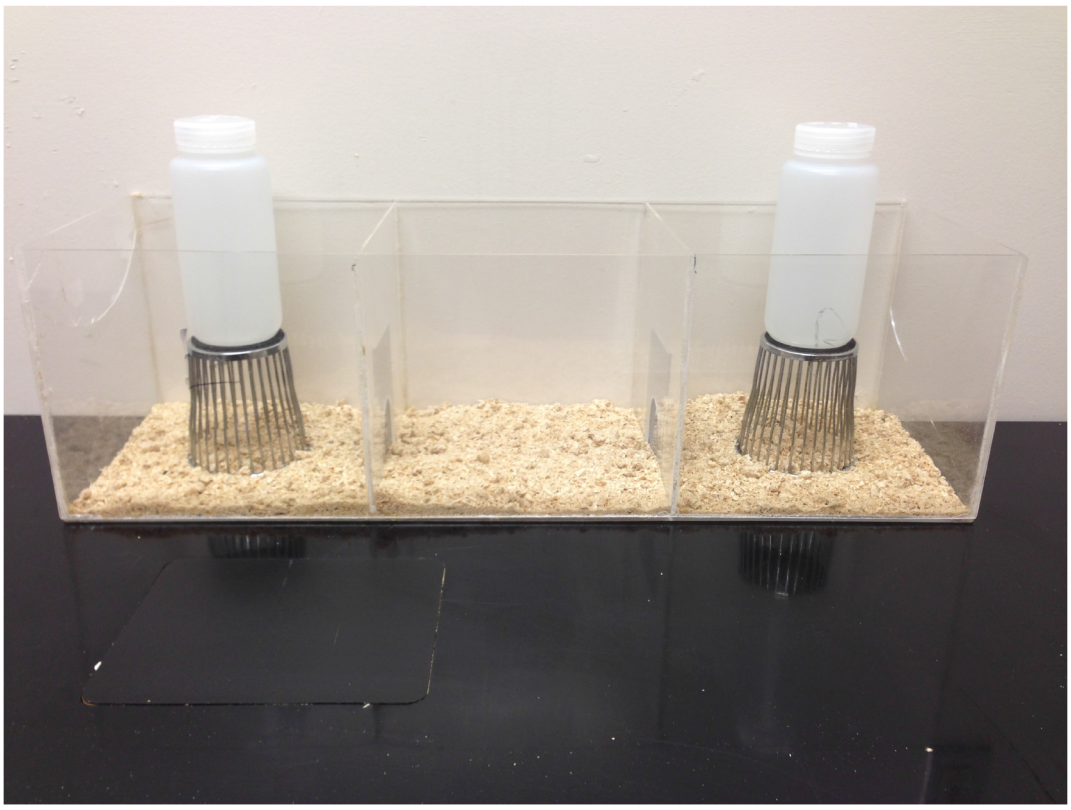

Figure 2.2. Representative layout of the apparatus used in the sociability, social novelty preference, and social recognition memory tasks.

The sociability and social recognition task was performed similarly to that described by Gunn *et al* (2011). This task involved one habituation trial and four testing trials; however, with the exception of stimuli, inter-trial intervals (ITI), and trial duration, the procedure was identical; see Figure 2.3 for an overview of stimuli, ITIs, and trial durations. Each trial began with the opaque barriers in place, blocking access between the three chambers. Stimuli were placed under the Galaxy Cups, with water bottles on top, and the subject was placed in the centre chamber. Once the subject and stimuli were in

place, both barriers were removed simultaneously, and the subject was allowed to freely explore the apparatus for the duration of the trial; habituation trials were 5 min in length, while testing trials were 10 min in length. At the end of the trial, the subject was coaxed into the centre chamber and both barriers were replaced. To reduce stress due to repeated handling, subjects were allowed to remain in the centre chamber of the apparatus while the stimuli were replaced for 1-minute ITIs; subjects were returned to their holding cages for 30-minute ITIs, and to the home-cage for 24-hour ITIs.

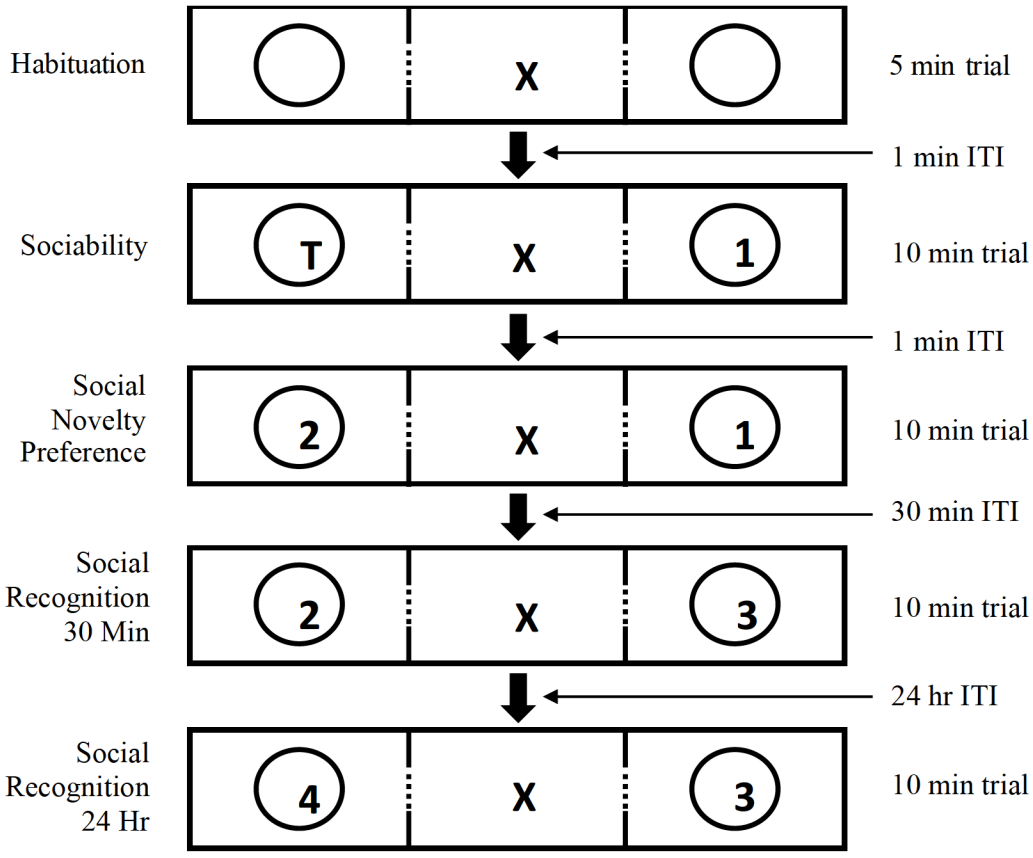

Figure 2.3. Overview of layout, stimuli, trial durations, and intertrial intervals (ITI) of the sociability, social novelty preference, and social recognition memory tasks. X = location of subject at the start of the trial; T = novel toy (non-social stimulus); 1 – 4 = wild-type stimulus conspecifics.

Habituation trials included no stimulus under either Galaxy Cup. The first testing trial began one minute after the habituation trial, and the stimuli consisted of a small, unfamiliar plastic animal toy (non-social stimulus) placed under one Galaxy Cup, and the first unfamiliar, same-sex, wild-type stimulus mouse placed under the Galaxy Cup in the opposite chamber; this trial was used to examine sociability, and the side of presentation of the toy and mouse were counterbalanced across subjects and across ages. All stimulus mice were habituated to the Galaxy Cups for 15 minutes prior to testing. The second testing trial began one minute after the first testing trial. For the second testing trial, the toy was removed, and a second unfamiliar, same-sex, wild-type stimulus mouse was placed in its location; the first stimulus mouse remained, and due to being exposed to the subject during the previous trial, was now considered the familiar mouse. This trial examined social novelty preference, and subsequent ones examined social recognition memory. The pattern of using the novel stimulus mouse from one trial as the familiar mouse for the subsequent trial was continued throughout the experiment; this maintained the level of familiarity across stimulus mice and across ages, as the subject had only interacted with the familiar mouse for 10 minutes in the previous trial. Following the second trial, subjects (and stimuli mice) were returned to their holding cage for a 30-minute ITI. After 30 minutes, the second stimulus mouse (now considered the familiar stimulus) was placed under the Galaxy Cup in the same location as previously, and a third, unfamiliar, same-sex, wild-type stimulus mouse was placed under the opposite Galaxy Cup. The subject was then returned to the centre chamber and the trial was started. The final trial took place approximately 24 hours following the end of the third trial; the third stimulus mouse from the previous day (now considered the familiar

mouse) was placed in the same location as the previous trial, a fourth, unfamiliar, same-sex, wild-type stimulus mouse was placed under the opposite Galaxy Cup, the subject was placed in the middle chamber, and the trial was started.

Following testing, subjects were returned to the colony room. Subjects remained in the holding cage until all cage-mates had been tested before being returned to their home-cage. Subjects not undergoing testing were given a metal cage top containing a bottle of water to prevent dehydration.

The apparatus was cleaned in between subjects; bedding was dumped out and any remaining particles were vacuumed out, and the apparatus was cleaned using Fisherbrand Sparkleen 1 (Fisher Scientific) and room-temperature water, then thoroughly dried with paper towels. Galaxy Cups were cleaned with Fisherbrand Sparkleen 1 and room-temperature water, then dried with paper towels, between testing different sexes and/or different cohorts of subjects, to reduce any possible odours from urine marking.

2.2.2.5.3 Scoring and Data Analysis

Videos were manually scored for duration (in seconds) spent in each chamber during each trial, as well as duration (in seconds) of interaction with each stimulus. Subjects were considered to have entered a chamber as soon as their head and forelimbs were across the threshold into the chamber. Interaction with a stimulus was defined as orientation of the snout towards the Galaxy Cup, with the snout 1 cm or less away from the Galaxy Cup. Interaction duration was used to calculate preference ratios for the novel stimulus animal over the novel toy (sociability), or the novel stimulus animal over the familiar stimulus animal (social novelty preference and social recognition memory). Preference ratios were calculated by dividing the time spent interacting with the novel

stimulus animal over the total time spent interacting with either stimulus. Additionally, to ensure that preference ratio differences were not due to overall differences in exploration, the total time (in seconds) spent interacting with both stimuli was also calculated. Previous work by Fairless *et al* (2011) indicated that cylinder time (time spent investigating the cylinder/cage containing a stimulus) was a more consistent measure of sociability than time spent in chamber; as a result, all analyses were performed using investigation time.

Data analysis was performed in R version 3.4.1 (R Foundation for Statistical Computing) using multilevel modelling; see Section 2.2.2.4.4 for rationale. Sex and genotype were used as between-subjects factors, and age was used as the within-subjects factor. The effect of subject was used as the error term. As each trial measured a different behaviour, trials were analyzed independently: Trial 1 examined sociability (social vs non-social stimuli), Trial 2 examined social novelty preference (novel vs familiar social stimuli), and Trials 3 and 4 examined social recognition memory (recognition of a familiar social stimulus either 30 minutes or 24 hours after initial encounter).

As data for transgenic males was only available at 3 months of age, two analyses were performed for both preference ratio and total interaction time for each trial: one examining the effects of sex and genotype at 3 months of age in males and females, and one examining the effects of age and genotype from 3 – 9 months of age in females only. Bootstrapped 95% confidence intervals were used to examine patterns of significant effects. Outliers were not removed from analysis.

2.2.2.6 Free Social Interaction

2.2.2.6.1 Apparatus

The apparatus consisted of a 38 x 38 x 40 cm box, with the floor raised 5 cm off of the base. Three of the walls were made of plywood and painted beige, and the fourth wall was made of clear acrylic to allow for recording from the front. The floor was made of clear acrylic to facilitate cleaning between animals; a sheet of black paper was placed immediately below the acrylic to make it appear solid.

2.2.2.6.2 Procedure

Prior to testing, subjects were transferred to a clean holding cage containing wood-chip bedding and a food pellet, and a micro-isolator cover was placed on top. All subjects were removed from home-cages and placed into holding cages at the same time, and were not returned to the home-cage until all cage-mates had completed testing. Stimulus mice were also transferred to holding cages for testing.

The protocol was largely based on existing free social interaction protocols (Bories et al, 2012; Fairless et al, 2011; Koike et al, 2009; Yang et al, 2012). The subject was placed into the apparatus and allowed to habituate by freely exploring for five minutes. Following habituation, subjects remained in the apparatus while an unfamiliar, same-sex, wild-type stimulus mouse was placed into the apparatus; the mice were then allowed to freely interact for 10 minutes. To ensure safety of both mice, the experimenter remained in the room, observed quietly for the duration of the trial, and intervened if excessive aggression was observed. Mice were physically separated within the apparatus if continuous fighting was observed for more than 30 seconds. If fighting continued after

separation, or if more than five attacks were performed by any mouse, the test was stopped immediately and both mice were returned to their holding cages.

Following testing, subjects were returned to the colony room. Subjects remained in the holding cage until all cage-mates had been tested before being returned to their home-cage. Subjects not undergoing testing were given a metal cage top containing a bottle of water to prevent dehydration. The apparatus was cleaned between subjects using Fisherbrand Sparkleen 1 (Fisher Scientific) and room-temperature water, then thoroughly dried with paper towels.

2.2.2.6.3 Scoring and Data Analysis

Videos were manually scored for duration (in seconds) of investigative (follow; orofacial sniffing; anogenital sniffing), affiliative (allogrooming; sitting in contact), aggressive (chase; mounting; attack), and self-grooming behaviours. Definitions of these behaviours were adapted from Arakawa, Blanchard, & Blanchard (2007b) and Grant and Mackintosh (1963), and were as follows: follow (slow speed locomotion towards the other animal that is also moving); chase (rapid locomotion towards the other animal that is rapidly moving away); orofacial sniffing (orientation of the snout towards and nearly touching the snout of the other animal); anogenital sniffing (orientation of the snout towards and nearly touching the anogenital region of the other animal); mounting (mounting the other animal from behind and thrusting the pelvis); attack (initiating biting and/or rolling); sitting in contact (self-grooming, allogrooming, or sitting quietly while in contact with the other animal); allogrooming (licking the other animal). Although Grant and Mackintosh (1963) put anogenital sniffing under mating behaviour, it is also used to

determine dominance in mice; as a result, it was considered an investigative behaviour in the present study.

Data analysis was performed in R version 3.4.1 (R Foundation for Statistical Computing) using multilevel modelling; see Section 2.2.2.4.4 for rationale. Sex and genotype were used as between-subjects factors, while age was used as the within-subjects factor. The effect of subject was used as the error term. Due to behaviours being relatively independent of each other, each behaviour was analyzed separately.

As data for transgenic males was only available at 3 months of age, two analyses were performed: one examining the effects of sex and genotype at 3 months of age in males and females, and one examining the effects of age and genotype from 3 – 9 months of age in females only. Bootstrapped 95% confidence intervals were used to examine patterns of significant effects. Outliers were not removed from analysis.

To identify whether changes in behaviour with age were due to locomotor activity, the automatic tracking function in Viewer 3.0 was used to assess distance travelled per minute during the 10-minute interaction period. Due to video quality and sensitivity of the tracking software, only white-coloured females could be accurately tracked; as a result, only a subset of transgenic females (n = 10) were scored for distance travelled at 3, 6, and 9 months of age. Due to the possibility of the automatic tracking failing to accurately track the subject, outliers (distances greater than two standard deviations from the mean) were removed on a minute-by-minute basis, and the remaining values were used to calculate a mean distance travelled per minute for each subject at each age. Bootstrapped 95% confidence intervals were used to analyze age effects on mean distance travelled per minute in these females only.

2.2.2.7 Ethics

The present study was performed under approval of the Dalhousie University Committee on Laboratory Animals, protocol number 16-021.

2.3 Results

2.3.1 Olfactory Habituation/Dishabituation

Both transgenic 5xFAD males and females and wild-type controls exhibit olfactory habituation (decrease in sniffing duration) over successive presentations of a given odour cue, as well as olfactory dishabituation (increase in sniffing duration) in response to the first presentation of a different odour cue (Figures 2.4 – 2.7).

Compared to wild-type control females, transgenic 5xFAD females typically exhibit decreased sniffing durations in response to social odours across all ages, particularly for dishabituation to the first social odour (95% CIs [-25.20, 1.92], [-17.86, 0.69], and [-18.02, 0.64] for genotype differences in sniffing durations at 3, 6, and 9 months, respectively). The exception to this is an increased sniffing duration for the third presentation of the second social odour at 3 months of age (95% CI [-0.88, 10.43]). Confidence intervals for mean sniffing durations for all odour cues are listed in Table 2.2, and genotype effects are for each odour are listed in Table 2.3.

2.3.2 Urine Marking

Analysis of females from 3 to 9 months of age indicated a main effect of age (χ^2 (1) = 62.92, $p < .001$) and odour cue (χ^2 (2) = 62.92, $p < .001$), with increased urine marking in response to social cues over non-social cues (with no difference in responses to same- vs opposite-sex urine), and an overall decrease in urine marking with age, particularly from

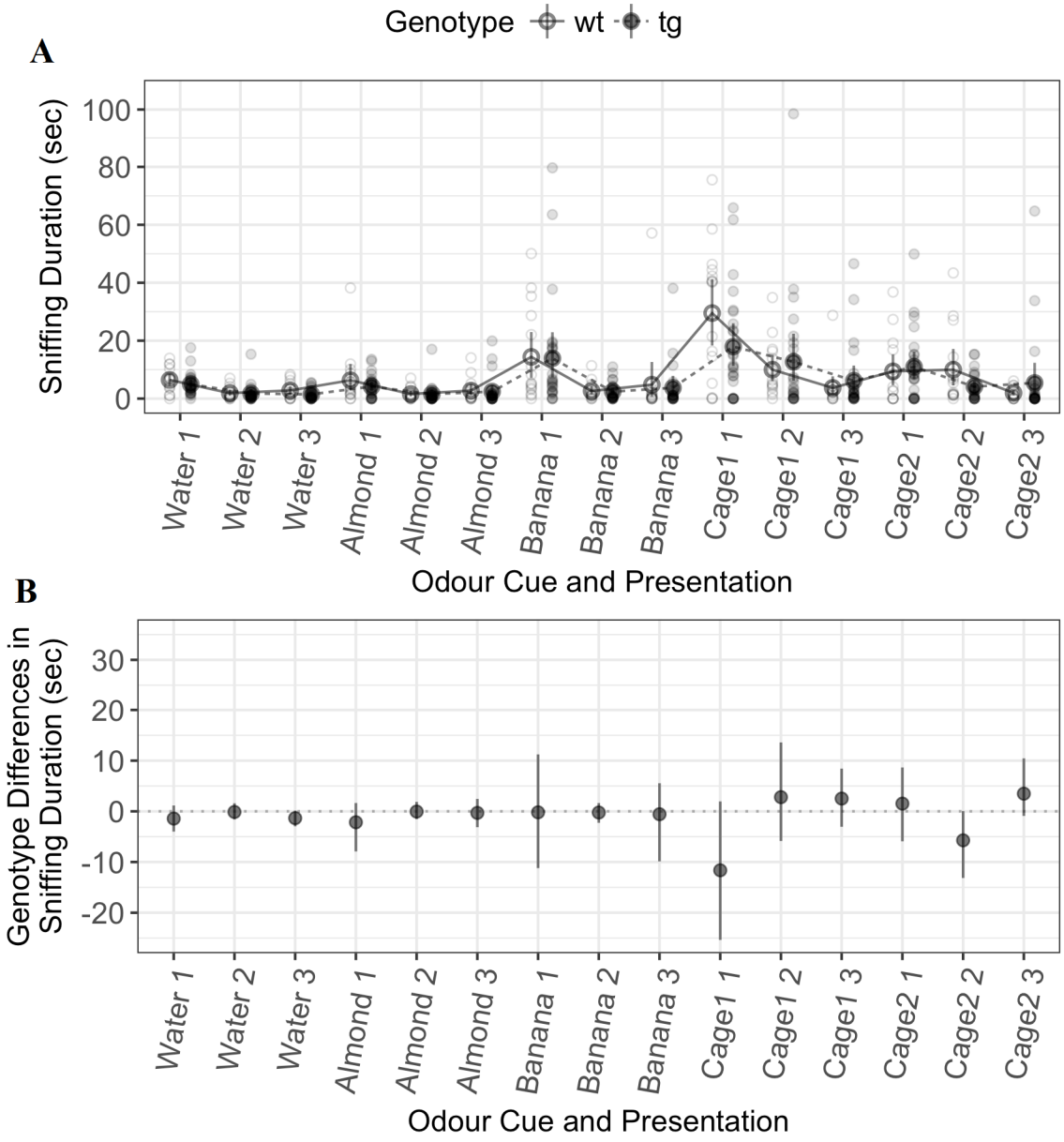

Figure 2.4. 95% CIs for **A)** mean sniffing duration by genotype (in sec) and **B)** genotype differences in sniffing duration (transgenic relative to wild-type, in sec) in females at 3 months of age.

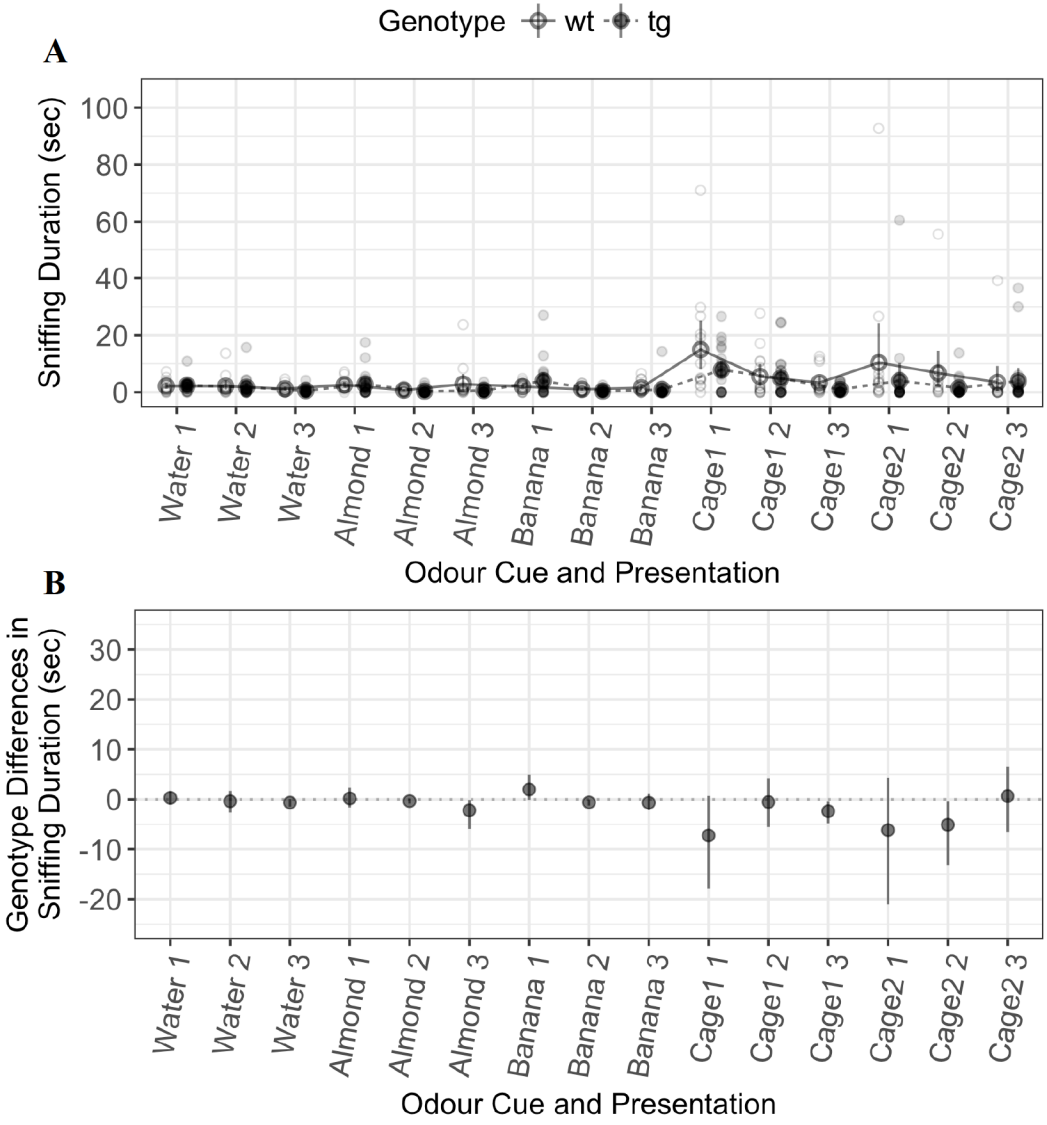

Figure 2.5. 95% CIs for **A)** mean sniffing duration by genotype (in sec) and **B)** genotype differences in sniffing duration (transgenic relative to wild-type, in sec) in females at 6 months of age.

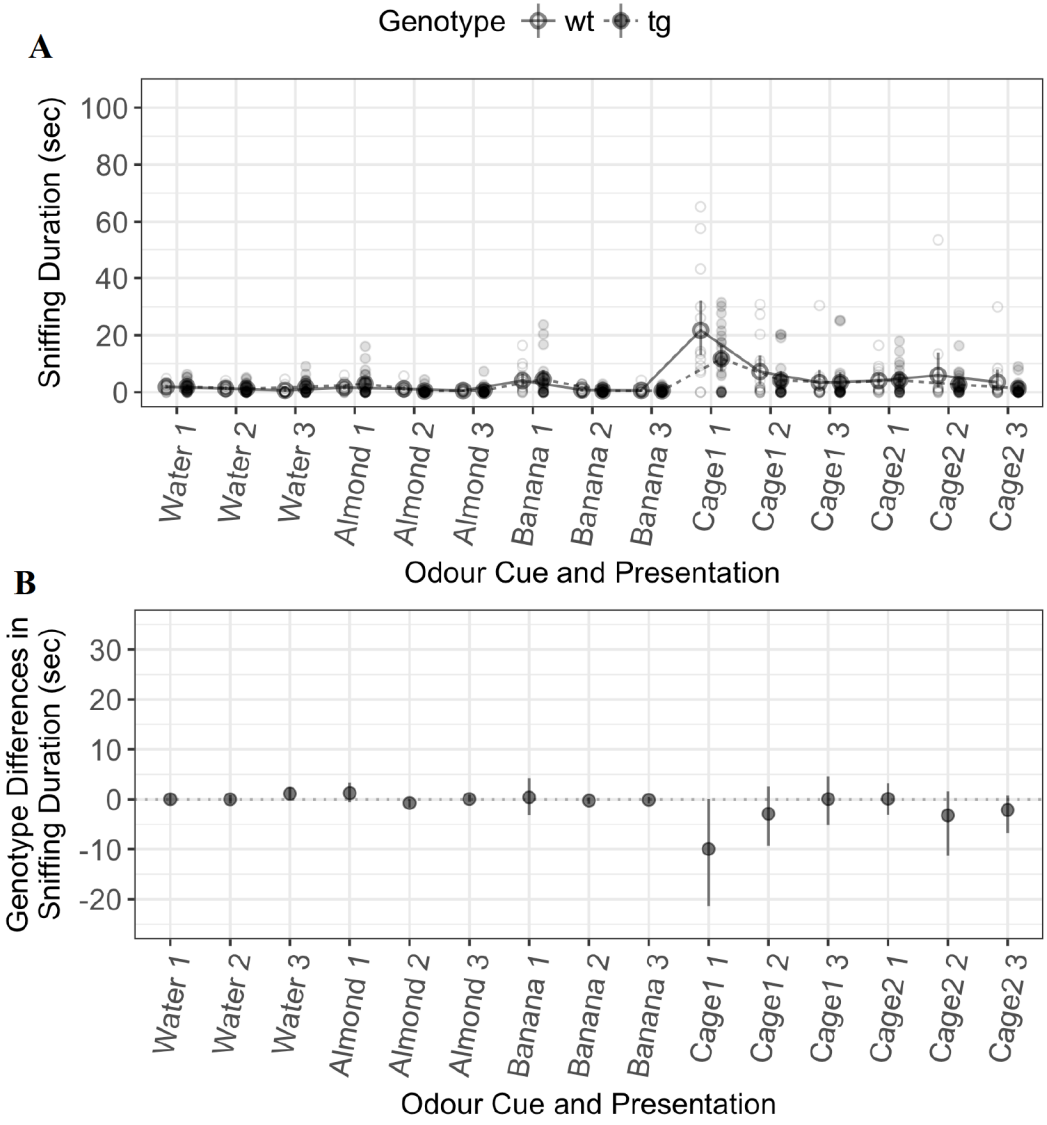

Figure 2.6. 95% CIs for **A)** mean sniffing duration by genotype (in sec) and **B)** genotype differences in sniffing duration (transgenic relative to wild-type, in sec) in females at 9 months of age.

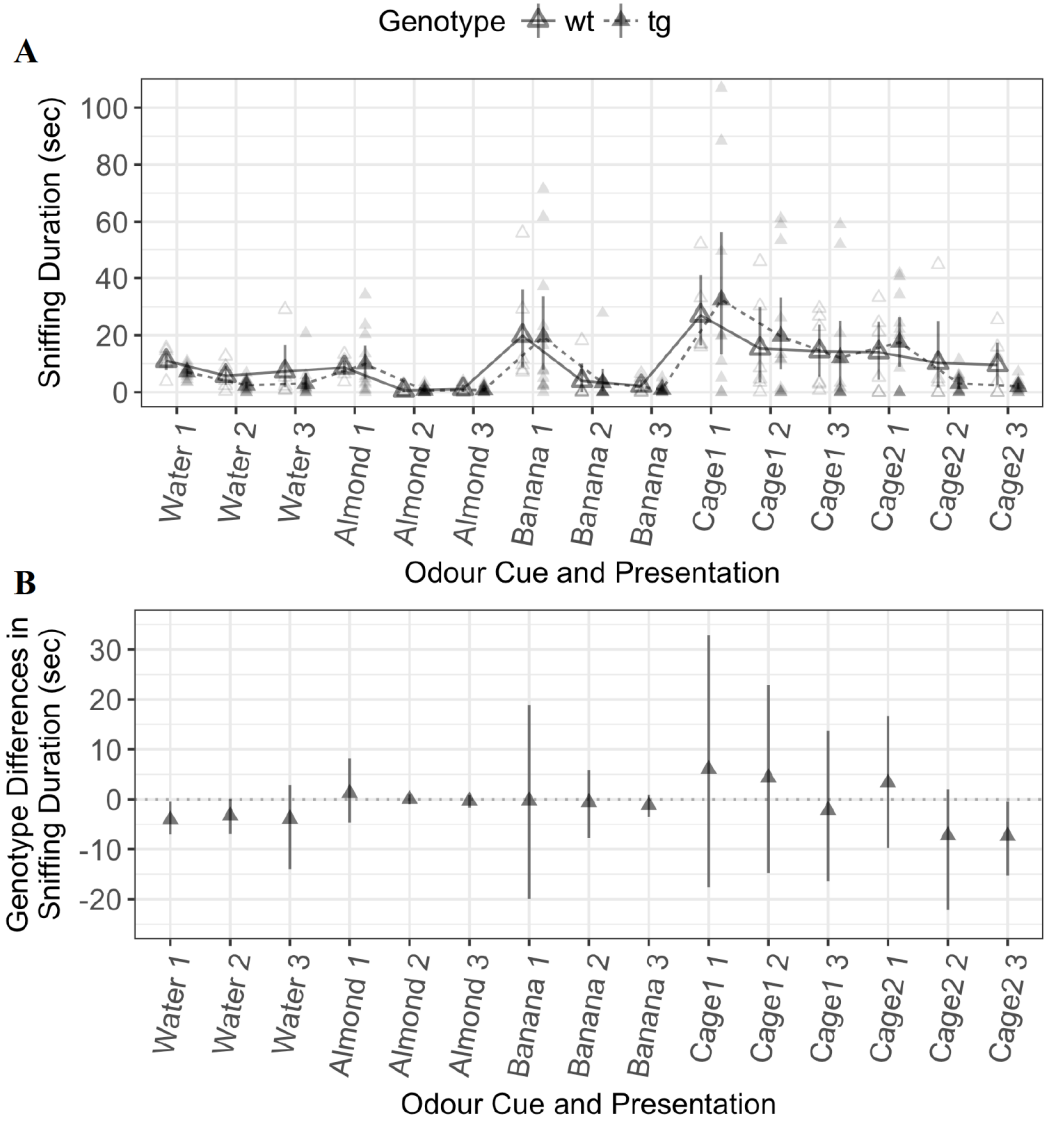

Figure 2.7. 95% CIs for **A)** mean sniffing duration by genotype (in sec) and **B)** genotype differences in sniffing duration (transgenic relative to wild-type, in sec) in males at 3 months of age.

Table 2.2

95% CIs for Sniffing Duration (in sec) during Olfactory Habituation/Dishabituation Task

	Female						Male	
	3 Months of Age		6 Months of Age		9 Months of Age		3 Months of Age	
Presentation	tg	wt	tg	wt	tg	wt	tg	wt
				Water				
1	[3.62, 6.66]	[4.40, 8.52]	[1.69, 3.44]	[1.22, 3.25]	[1.10, 2.73]	[1.22, 2.56]	[5.68, 8.34]	[7.74, 13.73]
2	[0.85, 3.25]	[0.96, 3.03]	[0.77, 3.48]	[0.92, 4.15]	[0.68, 2.01]	[0.78, 1.93]	[1.31, 3.71]	[2.61, 9.25]
3	[0.84, 2.19]	[1.56, 4.25]	[0.26, 1.04]	[0.67, 1.97]	[0.88, 2.95]	[0.16, 1.46]	[0.94, 6.75]	[1.75, 16.65]
				Almond				
1	[2.72, 5.81]	[3.00, 11.92]	[1.31, 4.82]	[1.74, 3.65]	[1.29, 4.97]	[0.95, 2.51]	[4.68, 16.48]	[6.22, 11.10]
2	[0.65, 3.14]	[0.74, 2.69]	[0.06, 0.78]	[0.40, 1.10]	[0.10, 1.01]	[0.66, 2.02]	[0.19, 1.36]	[0.06, 1.62]
3	[0.80, 4.72]	[1.15, 4.99]	[0.29, 1.04]	[0.82, 6.39]	[0.19, 1.54]	[0.38, 0.90]	[0.35, 1.44]	[0.21, 2.36]
				Banana				
1	[7.33, 22.91]	[7.14, 22.98]	[2.04, 6.90]	[1.29, 2.72]	[1.99, 7.74]	[2.13, 6.56]	[7.78, 33.65]	[8.64, 36.07]
2	[1.29, 3.69]	[1.34, 4.34]	[0.17, 0.72]	[0.54, 1.63]	[0.23, 0.94]	[0.31, 1.38]	[0.41, 8.29]	[0.12, 10.05]
3	[1.30, 7.84]	[0.67, 12.64]	[0.15, 2.39]	[0.73, 2.68]	[0.26, 0.93]	[0.26, 1.32]	[0.34, 1.98]	[0.32, 4.44]
				Cage 1				
1	[11.27, 26.00]	[18.33, 41.26]	[4.97, 11.04]	[8.09, 25.15]	[7.36, 16.50]	[12.96, 32.06]	[13.21, 56.29]	[16.50, 41.15]
2	[6.16, 22.41]	[6.01, 15.15]	[2.42, 8.11]	[2.20, 9.94]	[1.73, 7.39]	[2.78, 12.83]	[8.08, 33.25]	[3.51, 29.98]
3	[2.39, 11.42]	[1.46, 7.67]	[0.41, 1.59]	[1.45, 5.67]	[0.85, 6.97]	[0.91, 7.77]	[2.35, 25.05]	[5.59, 23.68]
				Cage 2				
1	[6.71, 16.12]	[4.63, 15.23]	[0.78, 10.21]	[2.82, 24.19]	[2.24, 6.51]	[2.16, 6.61]	[8.96, 26.25]	[4.40, 24.78]
2	[2.43, 6.08]	[4.52, 17.14]	[0.49, 3.00]	[2.37, 14.52]	[1.34, 4.54]	[1.61, 13.95]	[1.10, 5.32]	[1.60, 24.96]
3	[1.18, 12.36]	[1.07, 2.94]	[0.89, 8.54]	[0.43, 9.23]	[0.46, 2.59]	[0.91, 7.93]	[1.02, 3.38]	[2.69, 17.41]

Note. tg = transgenic; wt = wild-type

Table 2.3

95% CIs of Genotype Differences in Sniffing Durations on the Olfactory Habituation/Dishabituation Task

	Female			Male
Presentation	3 Months of Age	6 Months of Age	9 Months of Age	3 Months of Age
			Water	
1	[-3.98, 1.21]	[-1.08, 1.61]	[-1.06, 1.67]	[-7.10, -0.49]
2	[-1.61, 1.60]	[-2.67, 1.74]	[-2.64, 1.73]	[-7.02, 0.06]
3	[-2.94, 0.08]	[-1.42, 0.07]	[-1.48, 0.09]	[-14.02, 2.90]
			Almond	
1	[-7.86, 1.75]	[-1.62, 2.44]	[-1.61, 2.34]	[-4.69, 8.05]
2	[-1.53, 1.78]	[-0.84, 0.16]	[-0.83, 0.16]	[-1.02, 0.95]
3	[-3.05, 2.44]	[-5.86, -0.14]	[-5.79, -0.12]	[-1.61, 0.81]
			Banana	
1	[-11.20, 10.97]	[-0.13, 5.04]	[-0.14, 4.95]	[-20.24, 18.53]
2	[-2.25, 1.58]	[-1.25, -0.03]	[-1.24, -0.02]	[-7.51, 6.11]
3	[-10.07, 5.54]	[-2.07, 1.09]	[-2.06, 1.07]	[-3.62, 0.85]
			Cage 1	
1	[-25.20, 1.92]	[-17.86, 0.69]	[-18.02, 0.64]	[-17.83, 32.91]
2	[-5.75, 13.08]	[-5.66, 4.18]	[-5.74, 4.08]	[-14.67, 22.84]
3	[-3.19, 8.46]	[-4.78, -0.40]	[-4.88, -0.38]	[-16.26, 14.06]
			Cage 2	
1	[-5.86, 8.60]	[-20.84, 3.99]	[-21.53, 4.14]	[-10.28, 16.66]
2	[-13.13, -0.02]	[-13.26, -0.48]	[-13.17, -0.45]	[-22.28, 1.98]
3	[-0.88, 10.43]	[-6.47, 6.52]	[-6.39, 6.62]	[-15.13, -0.34]

Note. All values are given for transgenic 5xFAD mice relative to wild-type controls, in seconds.

6 to 9 months of age (Figure 2.8). There was also an age by odour cue interaction (χ^2 (2) = 11.61, p = .003), with less urine marking at 6 months of age in response to opposite-sex urine compared to same-sex urine, and less urine marking in response to opposite-sex urine at 6 months of age compared to 3 months of age. There were no other effects.

Analysis of males and females at 3 months of age indicated a main effect of odour cue (χ^2 (2) = 71.27, p < .001), with increased urine marking present in response to social odour cues over non-social odour cues; there was no difference in responses to same- vs opposite-sex urine. There were no other effects. See Table 2.4 for results of data analysis, Table 2.5 for 95% CIs of means squares covered by age and odour cue, and Figure 2.9 for 95% CIs of genotype effects.

2.3.3 Sociability and Social Novelty Preference

Analysis of sociability in females from 3 – 9 months of age indicated that both transgenic females and wild-type controls preferred social over non-social interactions, but there were no effects of genotype, age, or a genotype by age interaction for preference ratio. For total interaction time, there was a genotype by age interaction for the sociability task (χ^2 (1) = 5.80, p = .016), with transgenic females exhibiting reduced investigation time (social and non-social investigation) compared to wild-type controls at 9 months of age (95% CIs [-23.91, 58.69], [-56.07, 31.39], and [-70.60, -3.71] for genotype differences at 3, 6, and 9 months of age, respectively). There were no main effects of genotype or age for time investigating both stimuli. See Tables 2.6 – 2.10 and Figures 2.10 – 2.13.

Analysis of social novelty preference in females from 3 – 9 months of age indicated that both transgenic females and wild-type controls preferred the novel over familiar

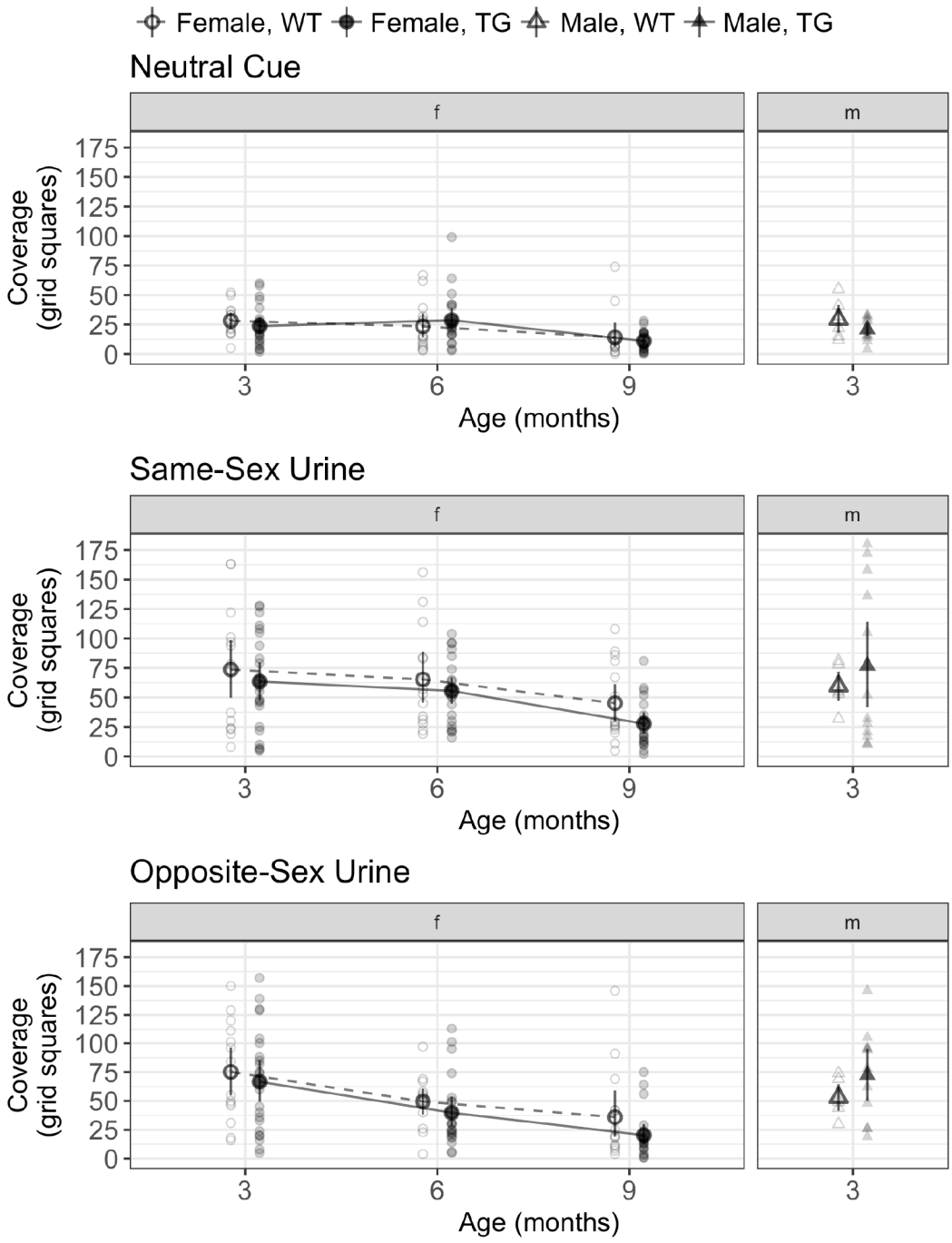

Figure 2.8. Raw scores and 95% CIs for mean urine marking (in grid squares) in response to olfactory cues in females and males.

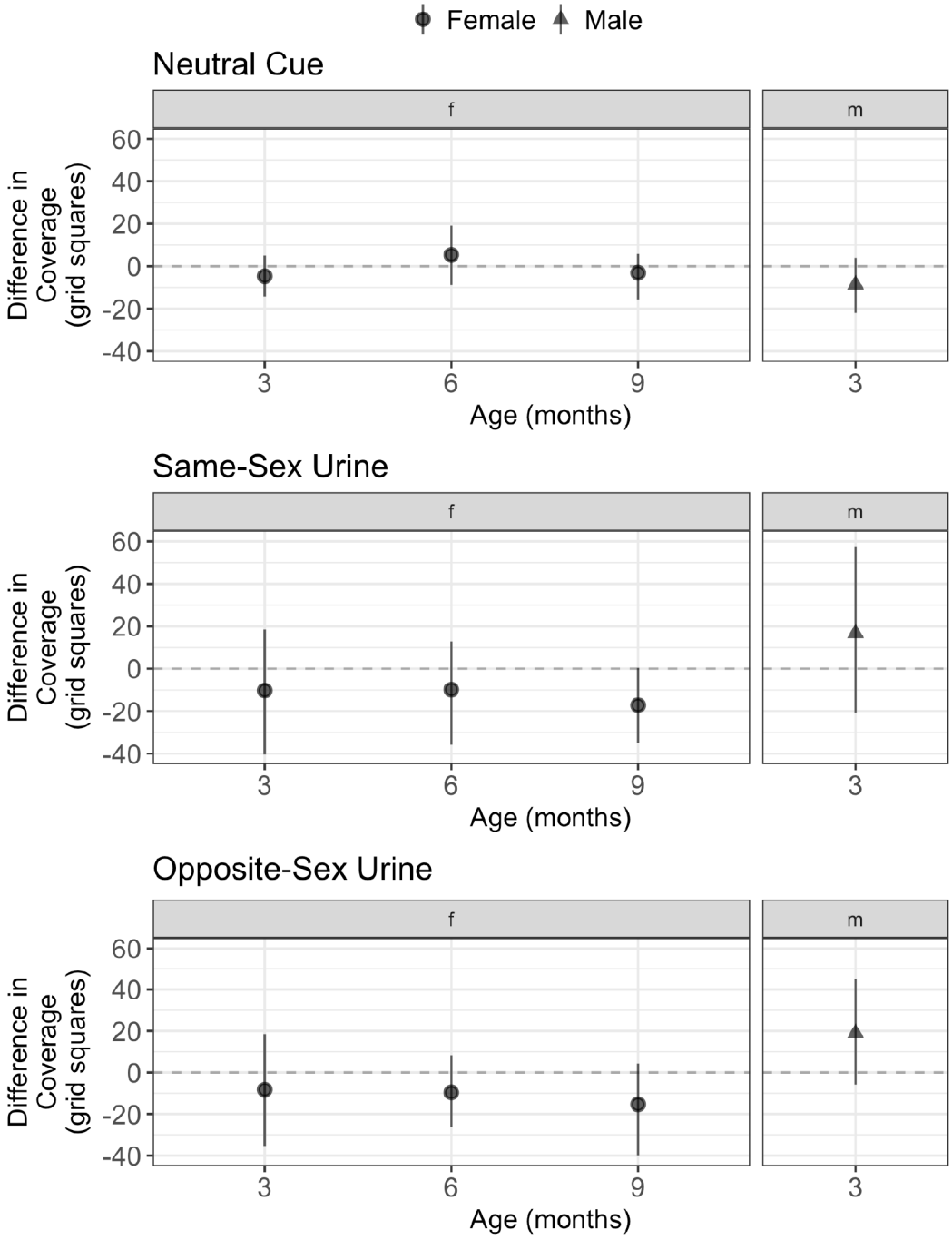

Figure 2.9. 95% CIs for genotype differences (transgenic relative to wild-type) in urine marking (in grid squares) in response to olfactory cues in females and males.

Table 2.4

Analysis of Deviance for the Urine Marking Task

Effect	χ^2	df	*p*
	Females, 3 - 9 Months of Age		
Genotype	1.42	1	.234
Age	62.92	1	**.000**
Odour Cue	62.92	2	**.000**
Genotype:Age	0.36	1	.551
Genotype:Odour Cue	2.07	2	.354
Age:Odour Cue	11.61	2	**.003**
Genotype:Age:Odour Cue	0.31	2	.857
	Females and Males, 3 Months of Age		
Genotype	0.09	1	.762
Sex	0.02	1	.887
Odour Cue	71.27	2	**.000**
Genotype:Sex	1.05	1	.305
Genotype:Odour Cue	0.34	2	.842
Sex:Odour Cue	0.43	2	.806
Genotype:Sex:Odour Cue	2.18	2	.335

Note. p-values less than .05 shown in **bold**.

Table 2.5

95% CIs for Squares Covered in the Urine Marking Task, by Sex, Age, Genotype, and Odour Cue

Females						Males	
3 Months of Age		6 Months of Age		9 Months of Age		3 Months of Age	
tg	wt	tg	wt	tg	wt	tg	wt
Neutral Cue							
[17.14, 30.77]	[21.86, 35.07]	[20.57, 39.00]	[14.14, 34.57]	[7.60, 14.75]	[6.14, 26.50]	[15.55, 26.00]	[18.00, 41.67]
Same-Sex Urine							
[47.35, 79.65]	[49.73, 98.60]	[44.68, 66.86]	[45.57, 88.71]	[19.55, 37.55]	[29.73, 61.33]	[41.92, 114.33]	[47.00, 72.00]
Opposite-Sex Urine							
[49.22, 84.91]	[54.80, 96.20]	[28.29, 53.52]	[38.36, 60.64]	[12.80, 29.95]	[19.07, 59.14]	[50.18, 95.09]	[41.50, 64.50]

Note. tg = transgenic; wt = wild-type

stimulus mouse, but there were no effects of genotype, age, or a genotype by age interaction for preference ratio. For total interaction time, there was a main effect of age (χ^2 (1) = 12.91, p < .001), with both transgenic and wild-type females exhibiting increased investigation of both stimuli with age (95% CIs [93.14, 122.43], [114.43, 148.23], and [122.60, 162.25] for mean total investigation time at 3, 6, and 9 months of age, respectively). There were no main effects of genotype, and no genotype by age interaction for total investigation time.

Analysis of social recognition memory after a 30 minute delay in females from 3 – 9 months of age indicated no preference for novel or familiar social stimuli for either transgenic females or wild-type controls at any age. There were no effects of genotype, age, or a genotype by age interaction for preference ratio or total investigation time.

Analysis of social recognition memory after 24 hours for females from 3 – 9 months of age indicated a main effect of age for preference ratio (χ^2 (1) = 4.60, p = .032), with females exhibiting a preference for novel over familiar stimuli at 9 months of age only (see Table 2.7). There were no effects of genotype or a genotype by age interaction for preference ratio. For total interaction time, there were main effects of genotype (χ^2 (1) = 4.36, p = .037), age (χ^2 (1) = 15.81, p < .001), and a genotype by age interaction (χ^2 (1) = 10.15, p = .001), with transgenic females exhibiting less total interaction time with age compared to wild-type controls (95% CIs [-15.96, 42.96], [-44.74, 5.19], and [-63.31, -11.24] for genotype differences at 3, 6, and 9 months of age, respectively). See Table 2.10.

Analysis of sociability in males and females at 3 months of age indicated that all groups had a preference for social over non-social interactions, with no effects of

Table 2.6

Analysis of Deviance for the Sociability, Social Novelty Preference, and Social Recognition Tasks for Females, 3 – 9 Months of Age

	Preference Ratio			Total Investigation Time		
Effect	χ^2	df	*p*	χ^2	df	*p*
			Sociability			
Genotype	0.18	1	.676	2.65	1	.104
Age	0.30	1	.584	2.34	1	.126
Genotype:Age	0.07	1	.785	5.80	1	**.016**
			Social Novelty Preference			
Genotype	2.72	1	.099	0.95	1	.330
Age	0.25	1	.619	12.91	1	**.000**
Genotype:Age	0.25	1	.620	0.25	1	.619
			Social Recognition, 30 Minute Delay			
Genotype	0.22	1	.639	2.59	1	.107
Age	0.30	1	.585	4.60	1	**.032**
Genotype:Age	1.51	1	.219	1.26	1	.261
			Social Recognition, 24 Hour Delay			
Genotype	2.59	1	.107	4.36	1	**.037**
Age	4.60	1	**.032**	15.81	1	**.000**
Genotype:Age	1.26	1	.261	10.15	1	**.001**

Note. p-values less than .05 shown in **bold**.

Table 2.7

95% CIs for Preference Ratio on the Sociability, Social Novelty Preference, and Social Recognition Tasks

Female						Male	
3 Months of Age		6 Months of Age		9 Months of Age		3 Months of Age	
tg	wt	tg	wt	tg	wt	tg	wt
Sociability							
[0.69, 0.81]	[0.71, 0.83]	[0.69, 0.76]	[0.65, 0.82]	[0.72, 0.78]	[0.66, 0.83]	[0.63, 0.93]	[0.80, 0.91]
Social Novelty Preference							
[0.59, 0.72]	[0.59, 0.69]	[0.62, 0.71]	[0.48, 0.69]	[0.64, 0.74]	[0.57, 0.71]	[0.61, 0.78]	[0.55, 0.85]
Social Recognition, 30 Minute Delay							
[0.45, 0.56]	[0.49, 0.64]	[0.49, 0.59]	[0.51, 0.64]	[0.49, 0.61]	[0.53, 0.63]	[0.38, 0.54]	[0.34, 0.65]
Social Recognition, 24 Hour Delay							
[0.47, 0.57]	[0.45, 0.56]	[0.48, 0.55]	[0.47, 0.56]	[0.55, 0.68]	[0.46, 0.59]	[0.51, 0.65]	[0.41, 0.62]

Note. tg = transgenic; wt = wild-type

Table 2.8

95% CIs for Total Investigation Time on the Sociability, Social Novelty Preference, and Social Recognition Tasks

Female						Male	
3		6		9		3	
tg	wt	tg	wt	tg	wt	tg	wt
Sociability							
[191.92, 244.39]	[168.06, 233.28]	[190.26, 248.55]	[199.60, 264.95]	[191.91, 240.41]	[230.19, 276.41]	[151.69, 250.96]	[162.30, 281.80]
Social Novelty Preference							
[146.10, 197.60]	[127.43, 170.98]	[180.23, 226.41]	[153.08, 233.69]	[184.16, 247.29]	[174.37, 237.26]	[167.15, 248.84]	[180.18, 358.10]
Social Recognition, 30 Minute Delay							
[164.68, 219.76]	[150.71, 222.57]	[165.38, 218.39]	[173.76, 216.78]	[146.71, 206.09]	[174.65, 218.95]	[108.78, 213.56]	[73.68, 270.54]
Social Recognition, 24 Hour Delay							
[148.31, 179.65]	[125.17, 175.39]	[149.18, 184.60]	[168.18, 204.43]	[159.62, 191.26]	[192.53, 232.73]	[123.51, 197.38]	[139.60, 222.65]

Note. tg = transgenic; wt = wild-type

Table 2.9

95% CIs for Genotype Differences in Preference Ratio on the Sociability, Social Novelty Preference, and Social Recognition Tasks

Female			Male
3	6	9	3
Sociability			
[-0.10, 0.07]	[-0.11, 0.09]	[-0.08, 0.09]	[-0.23, 0.10]
Social Novelty Preference			
[-0.07, 0.09]	[-0.04, 0.19]	[-0.04, 0.14]	[-0.18, 0.15]
Social Recognition, 30 Minute Delay			
[-0.15, 0.04]	[-0.12, 0.05]	[-0.10, 0.05]	[-0.21, 0.14]
Social Recognition, 24 Hour Delay			
[-0.05, 0.09]	[-0.06, 0.06]	[0.00, 0.18]	[-0.06, 0.21]

Note. All values given for transgenic 5xFAD mice relative to wild-type controls.

Table 2.10

95% CIs for Genotype Differences in Total Interaction Time (in sec) on the Sociability, Social Novelty Preference, and Social Recognition Tasks

Female			Male
3	6	9	3
Sociability			
[-23.91, 58.69]	[-56.07, 31.39]	[-70.60, -3.71]	[-99.42, 53.05]
Social Novelty Preference			
[-11.06, 57.12]	[-36.91, 56.54]	[-35.31, 54.50]	[-157.64, 36.33]
Social Recognition, 30 Minute Delay			
[-39.00, 50.36]	[-37.16, 32.51]	[-57.22, 16.28]	[-115.77, 113.58]
Social Recognition, 24 Hour Delay			
[-15.96, 42.96]	[-44.74, 5.19]	[-63.31, -11.24]	[-78.18, 33.49]

Note. All values given for transgenic 5xFAD mice relative to wild-type controls.

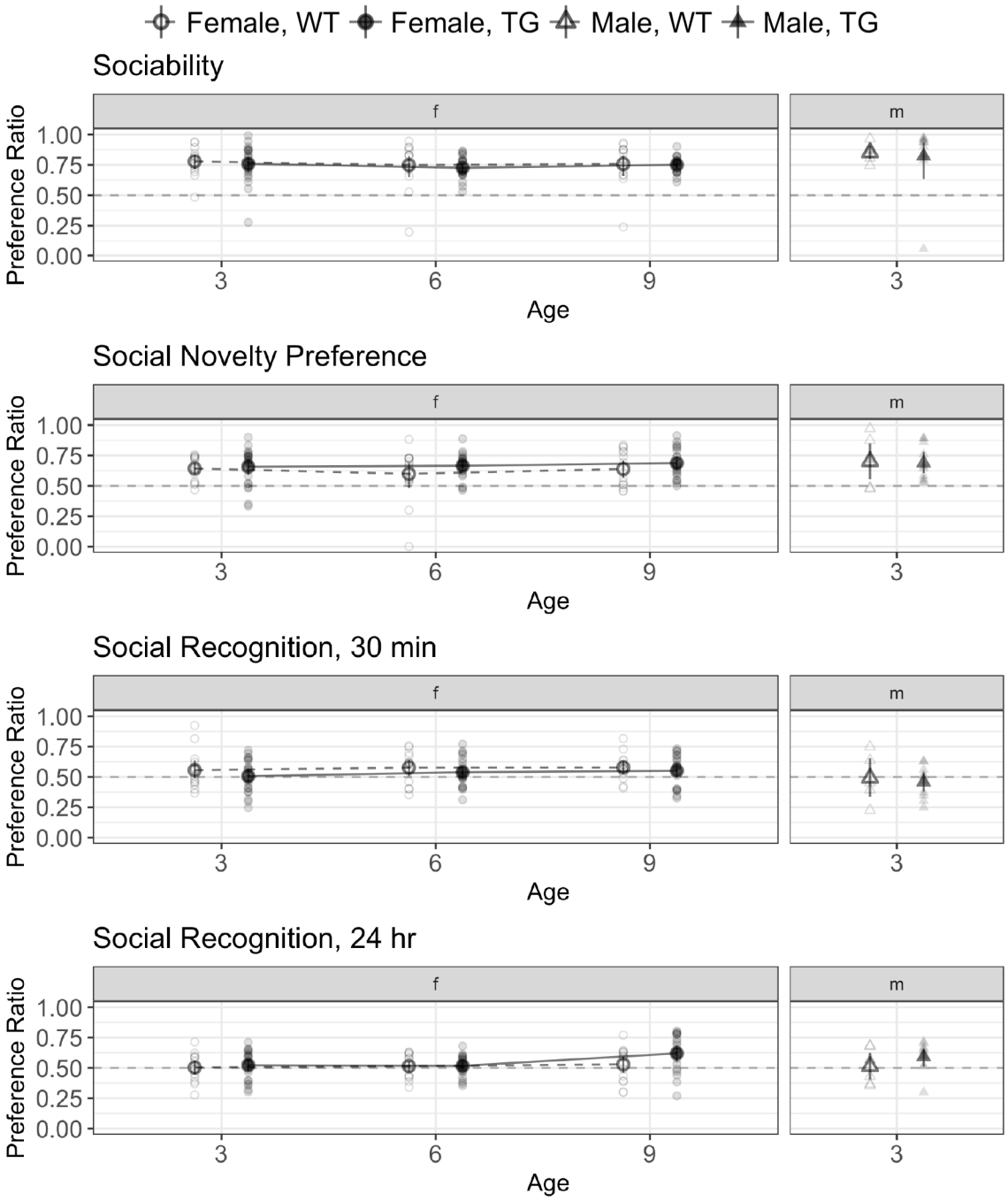

Figure 2.10. Raw data and 95% CIs for mean preference ratios in the Sociability, Social Novelty Preference, and Social Recognition tasks in females and males.

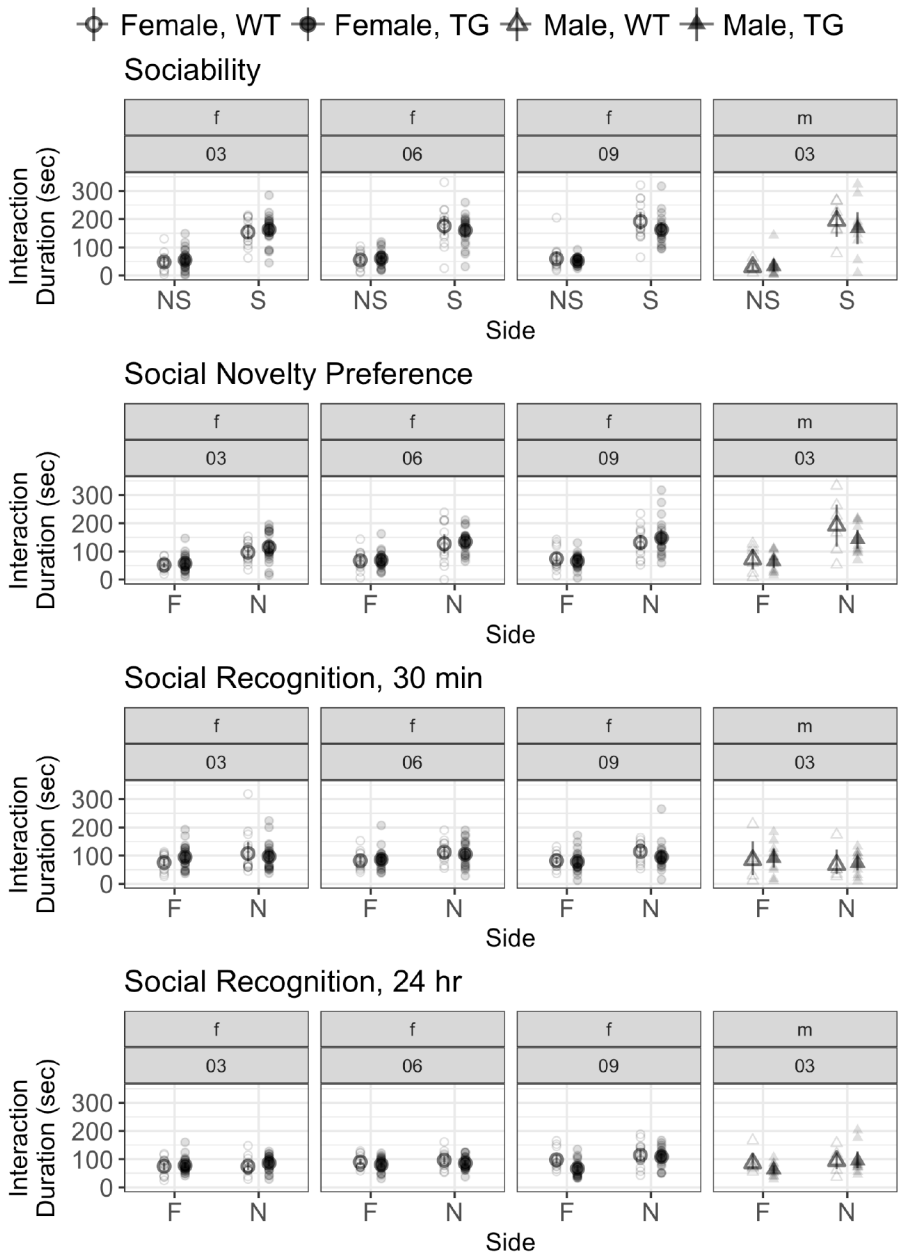

Figure 2.11. Raw data and 95% CIs for mean interaction time (in seconds) in the Sociability, Social Novelty Preference, and Social Recognition tasks in females (f) and males (m). S = social stimulus; NS = non-social stimulus; F = familiar conspecific; N = novel conspecific.

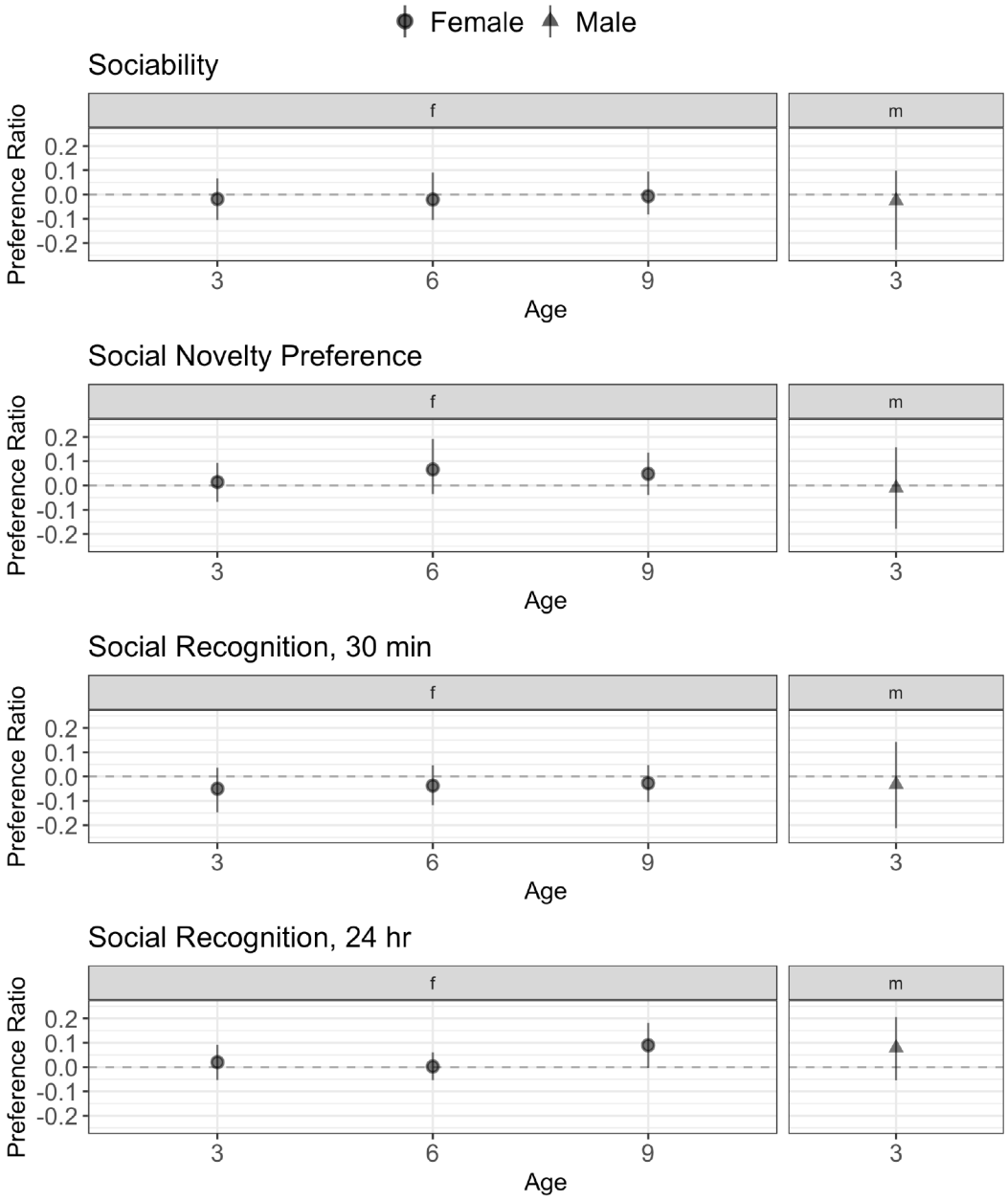

Figure 2.12. 95% CIs for genotype differences (transgenic relative to wild-type) in the Sociability, Social Novelty Preference, and Social Recognition tasks in females and males.

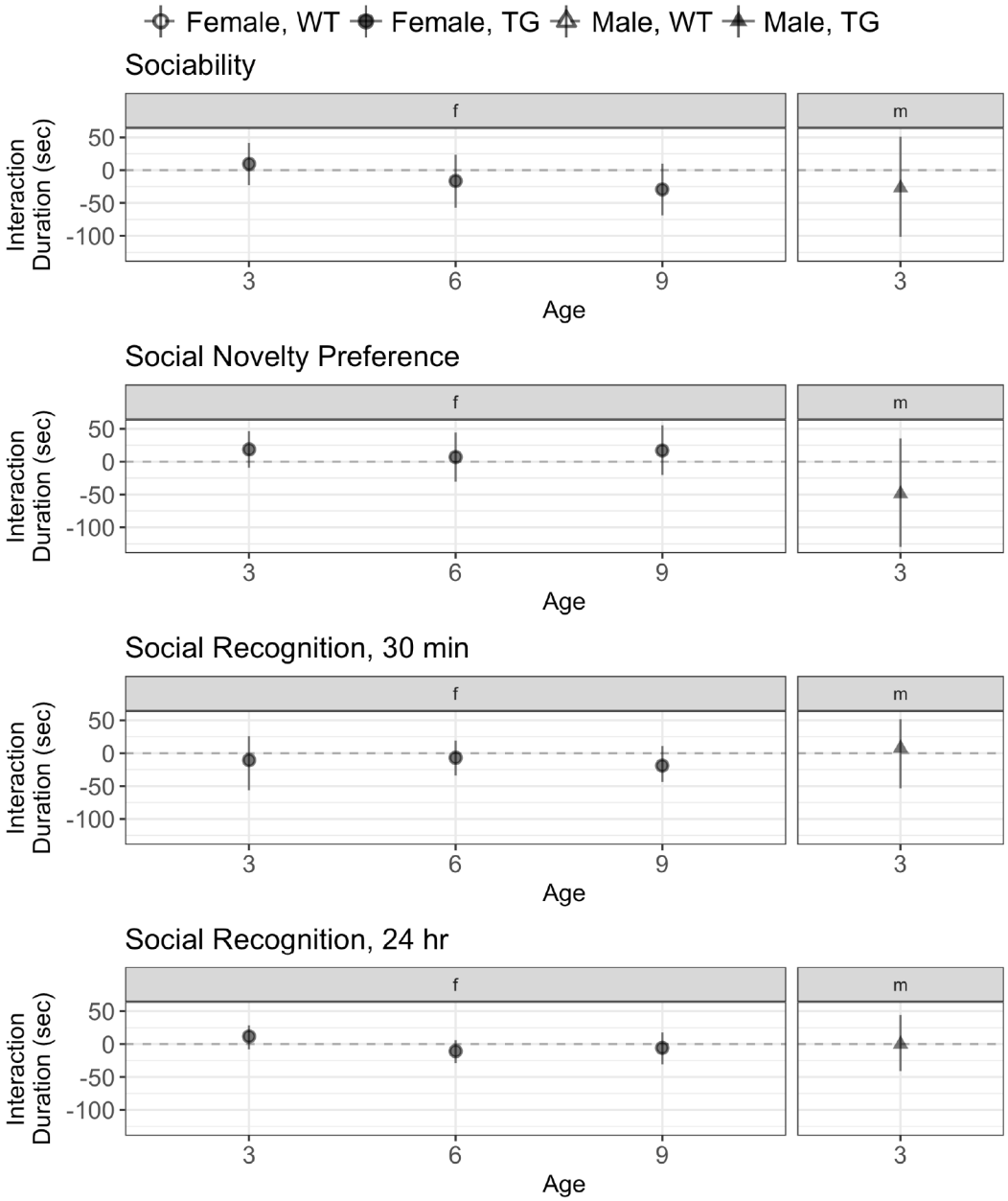

Figure 2.13. 95% CIs for genotype differences (transgenic relative to wild-type) for total interaction durations (in seconds) in the Sociability, Social Novelty Preference, and Social Recognition tasks in females (f) and males (m).

Table 2.11

Analysis of Deviance for the Sociability, Social Novelty Preference, and Social Recognition Tasks for Females and Males, 3 Months of Age

	Preference Ratio			Total Investigation Time		
Effect	χ^2	df	*p*	χ^2	df	*p*
			Sociability			
Genotype	0.26	1	.612	0.05	1	.825
Sex	1.69	1	.194	0.02	1	.877
Genotype:Sex	0.02	1	.877	0.90	1	.342
			Social Novelty Preference			
Genotype	0.02	1	.878	0.00	1	.963
Sex	1.03	1	.311	10.31	1	**.001**
Genotype:Sex	0.08	1	.773	3.68	1	.055
			Social Recognition, 30 Minute Delay			
Genotype	1.22	1	.269	0.13	1	.715
Sex	1.46	1	.227	1.51	1	.219
Genotype:Sex	0.04	1	.846	0.01	1	.926
			Social Recognition, 24 Hour Delay			
Genotype	1.13	1	.288	0.05	1	.819
Sex	1.57	1	.210	0.27	1	.601
Genotype:Sex	0.60	1	.440	1.53	1	.216

Note. p-values less than .05 shown in **bold**.

genotype, sex, or genotype by sex interactions for either preference ratio or total investigation time. See Table 2.11.

Analysis of social novelty preference in males and females at 3 months of age indicated that all groups had a preference for novel over familiar stimulus mice, but there were no effects of genotype, sex, or genotype by sex interactions for preference ratio. For total interaction time, there was an effect of sex (χ^2 (1) = 10.31, p = .001), with females spending less time interacting with both stimuli than males (95% CI [-94.77, -14.78] for interaction duration for females relative to males). There was no effect of genotype or a genotype by sex interaction for total interaction time. See Tables 2.7 – 2.11.

Analysis of social recognition memory in males and females at 3 months of age indicates no preference for novel over familiar stimulus mice after 30 minute or 24 hour delays. There were also no effects of genotype, sex, or genotype by sex interactions for either preference ratio or total investigation time. See Table 2.11.

2.3.4 Free Social Interaction

Analysis of behaviours in females from 3 – 9 months of age indicates a genotype by age interaction for orofacial sniffing (χ^2 (1) = 8.71, p = .003), with transgenic females exhibiting decreased orofacial sniffing durations with age compared to wild-type controls (95% CI [-17.58, 16.02], [-24.65, 2.26], and [-41.63, -11.78] for genotype differences at 3, 6, and 9 months of age, respectively). There was also a main effect of genotype on anogenital sniffing (χ^2 (1) = 6.39, p = .011) ; 95% CI [-16.24, -0.83]) and following (χ^2 (1) = 4.57, p = .033; 95% CI [-7.62, 1.46]), with transgenic females exhibiting decreased durations compared to wild-type controls. There were no other effects in females; see Tables 2.12 – 2.14 and Figures 2.14 – 2.19.

Table 2.12

Analysis of Deviance for the Free Social Interaction Task for Females, 3 – 9 Months of Age

Effect	χ^2	df	*p*
		Orofacial Sniffing	
Genotype	7.74	1	**.005**
Age	4.77	1	**.029**
Genotype:Age	8.71	1	**.003**
		Anogenital Sniffing	
Genotype	6.39	1	**.011**
Age	13.39	1	**.000**
Genotype:Age	3.29	1	.070
		Follow	
Genotype	4.57	1	**.033**
Age	28.31	1	**.000**
Genotype:Age	2.62	1	.105
		Chase	
Genotype	1.03	1	.310
Age	0.24	1	.624
Genotype:Age	0.15	1	.699
		Mount	
Genotype	0.80	1	.370
Age	0.93	1	.336
Genotype:Age	1.26	1	.261
		Attack	
Genotype	0.67	1	.414
Age	0.00	1	.956
Genotype:Age	0.00	1	.965
		Sitting in Contact	
Genotype	0.00	1	.955
Age	2.00	1	.157
Genotype:Age	0.16	1	.687
		Allogrooming	
Genotype	0.23	1	.632
Age	0.04	1	.837
Genotype:Age	1.11	1	.292
		Self-Grooming	
Genotype	0.52	1	.469
Age	0.47	1	.492
Genotype:Age	0.38	1	.537

Note. p-values less than .05 shown in **bold**.

Table 2.13

95% CIs for Duration (in seconds) of Behaviours on the Free Social Interaction Task

Female						Male	
3		6		9		3	
tg	wt	tg	wt	tg	wt	tg	wt
Orofacial Sniffing							
[42.38, 63.30]	[40.69, 66.78]	[32.84, 48.84]	[41.70, 62.96]	[29.33, 38.01]	[45.93, 74.56]	[23.28, 45.92]	[15.67, 30.07]
Anogenital Sniffing							
[47.24, 67.01]	[44.91, 68.01]	[30.75, 48.72]	[46.77, 58.63]	[29.07, 41.31]	[39.73, 59.04]	[42.99, 70.82]	[39.38, 58.07]
Follow							
[22.13, 33.20]	[18.58, 31.83]	[10.86, 17.52]	[15.77, 26.42]	[8.04, 13.64]	[12.42, 21.16]	[16.08, 56.59]	[13.76, 36.51]
Chase							
[0.00, 0.00]	[0.00, 0.00]	[0.00, 1.19]	[0.00, 0.22]	[0.00, 0.39]	[0.00, 0.00]	[0.00, 1.30]	[0.59, 10.12]
Mount							
[0.00, 0.27]	[0.00, 10.14]	[0.00, 0.00]	[0.00, 0.75]	[0.00, 0.00]	[0.00, 1.05]	[0.00, 2.65]	[0.00, 3.73]
Attack							
[0.00, 0.00]	[0.00, 0.00]	[0.00, 0.07]	[0.00, 0.00]	[0.00, 0.00]	[0.00, 0.00]	[1.38, 18.71]	[0.56, 37.62]
Sitting in Contact							
[0.30, 8.81]	[0.06, 5.36]	[0.16, 2.60]	[0.00, 3.16]	[0.00, 1.20]	[0.00, 1.32]	[0.00, 0.00]	[0.00, 1.41]
Allogrooming							
[0.00, 1.51]	[0.00, 0.11]	[0.00, 2.15]	[0.00, 0.28]	[0.00, 0.00]	[0.00, 1.65]	[0.00, 0.00]	[0.00, 0.00]
Self-Grooming							
[15.15, 28.32]	[14.49, 25.88]	[15.53, 27.82]	[7.28, 19.63]	[15.05, 30.46]	[13.45, 62.50]	[4.37, 12.60]	[4.84, 20.52]

Note. tg = transgenic; wt = wild-type

Table 2.14

95% CIs for Genotype Differences in Duration of Behaviours on the Free Social Interaction Task

Female			Male
3	6	9	3
Orofacial Sniffing			
[-17.58, 16.02]	[-24.65, 2.26]	[-41.63, -11.78]	[-2.38, 24.87]
Anogenital Sniffing			
[-14.32, 15.74]	[-23.68, -2.73]	[-25.69, -2.77]	[-9.05, 24.43]
Follow			
[-6.24, 11.50]	[-13.12, -0.51]	[-11.38, -0.84]	[-13.03, 33.73]
Chase			
[0.00, 0.00]	[-0.15, 1.12]	[0.00, 0.39]	[-9.82, -0.08]
Mount			
[-10.01, 0.18]	[-0.75, 0.00]	[-1.05, 0.00]	[-3.21, 2.28]
Attack			
[0.00, 0.00]	[0.00, 0.07]	[0.00, 0.00]	[-29.26, 11.92]
Sitting in Contact			
[-3.49, 7.57]	[-2.14, 1.92]	[-1.08, 0.96]	[-1.41, 0.00]
Allogrooming			
[-0.11, 1.51]	[-0.28, 2.15]	[-1.65, 0.00]	[0.00, 0.00]
Self-Grooming			
[-7.45, 10.20]	[-0.45, 17.35]	[-42.53, 12.46]	[-13.20, 5.07]

Note. All values given for transgenic 5xFAD mice relative to wild-type controls, in seconds.

Table 2.15

Analysis of Deviance for the Free Social Interaction Task for Females and Males, 3 Months of Age

Effect	χ^2	df	*p*
		Orofacial Sniffing	
Genotype	0.17	1	.682
Sex	10.30	1	**.001**
Genotype:Sex	0.63	1	.427
		Anogenital Sniffing	
Genotype	0.21	1	.643
Sex	0.13	1	.716
Genotype:Sex	0.22	1	.636
		Follow	
Genotype	0.62	1	.432
Sex	0.44	1	.506
Genotype:Sex	0.27	1	.604
		Chase	
Genotype	5.41	1	**.020**
Sex	12.12	1	**.000**
Genotype:Sex	12.87	1	**.000**
		Mount	
Genotype	1.70	1	.192
Sex	0.02	1	.886
Genotype:Sex	0.52	1	.471
		Attack	
Genotype	0.48	1	.487
Sex	14.43	1	**.000**
Genotype:Sex	1.15	1	.283
		Sitting in Contact	
Genotype	0.11	1	.745
Sex	1.18	1	.277
Genotype:Sex	0.11	1	.736
		Allogrooming	
Genotype	0.52	1	.469
Sex	0.49	1	.483
Genotype:Sex	0.22	1	.639
		Self-Grooming	
Genotype	0.00	1	.971
Sex	8.31	1	**.004**
Genotype:Sex	0.26	1	.611

Note. p-values less than .05 shown in **bold**.

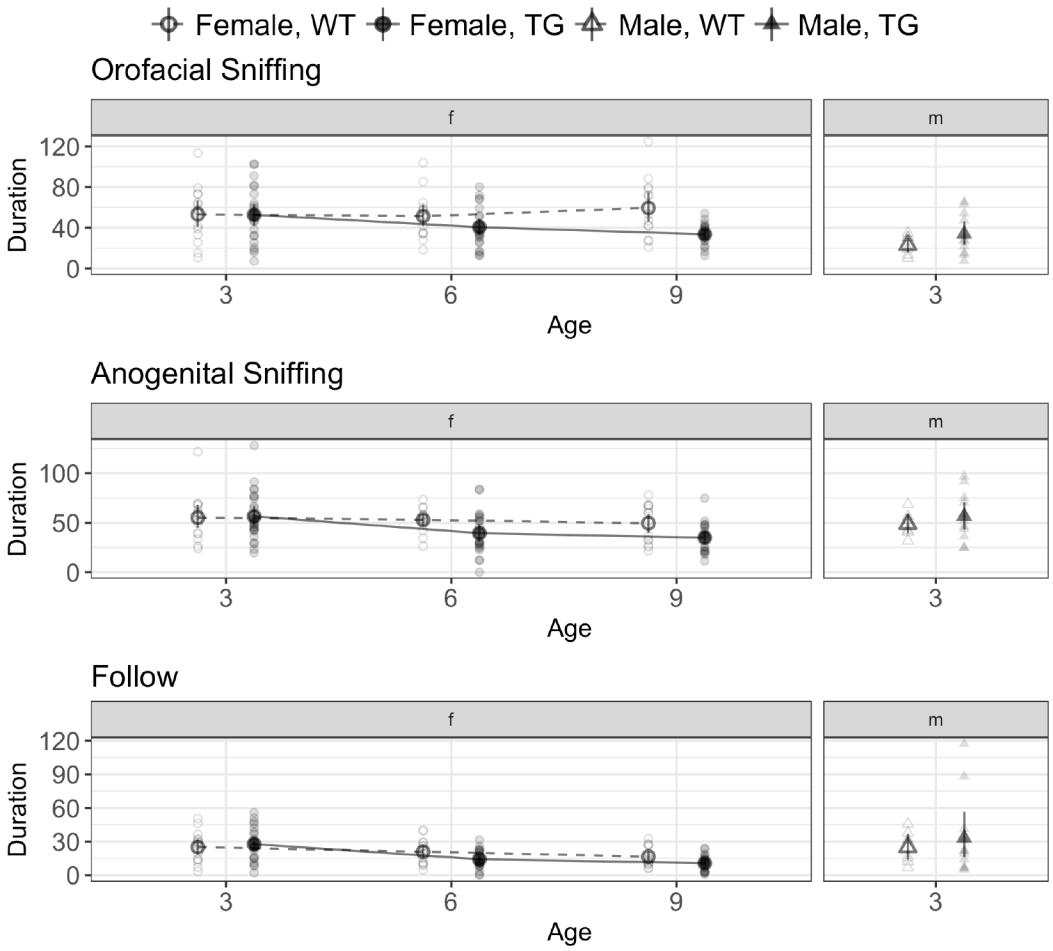

Figure 2.14. Raw scores and 95% CIs for mean behaviour duration (in seconds) for investigative behaviours in the Free Social Interaction task in females (f) and males (m).

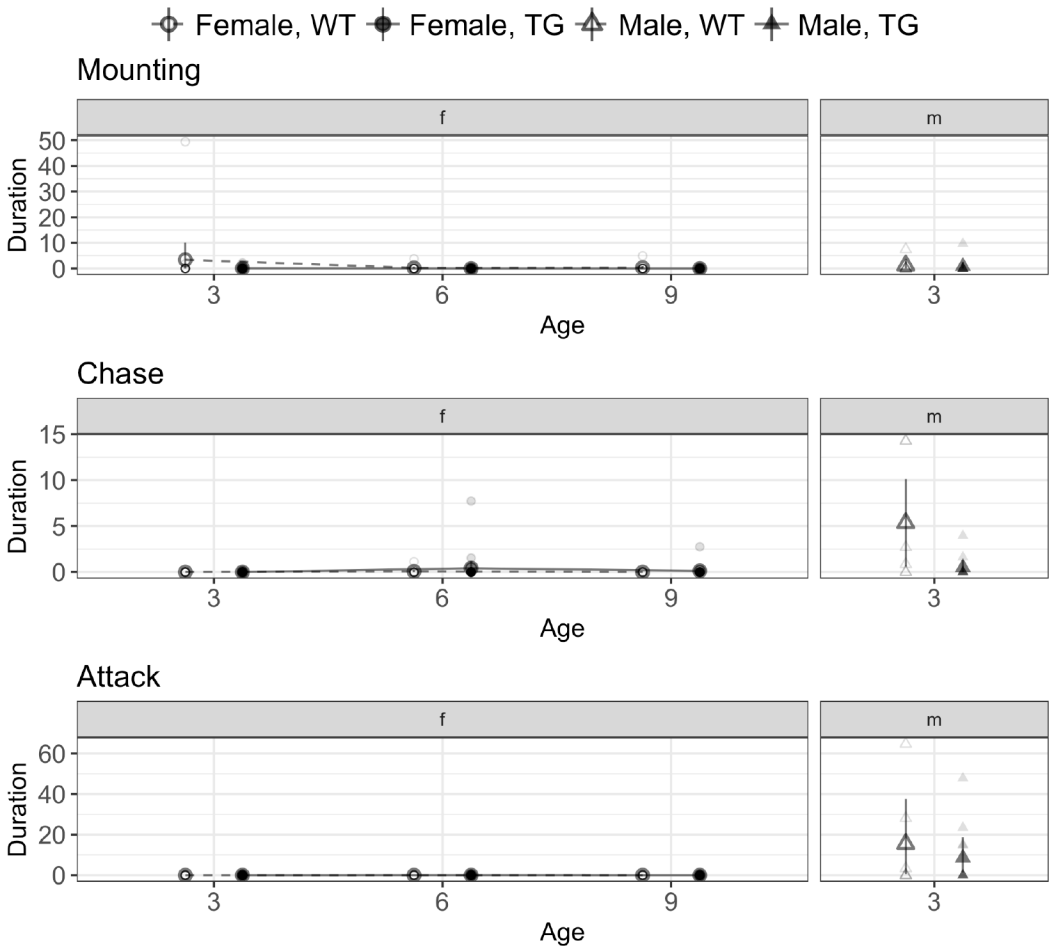

Figure 2.15. Raw scores and 95% CIs for mean behaviour duration (in seconds) of aggressive behaviours in the Free Social Interaction task in females (f) and males (m).

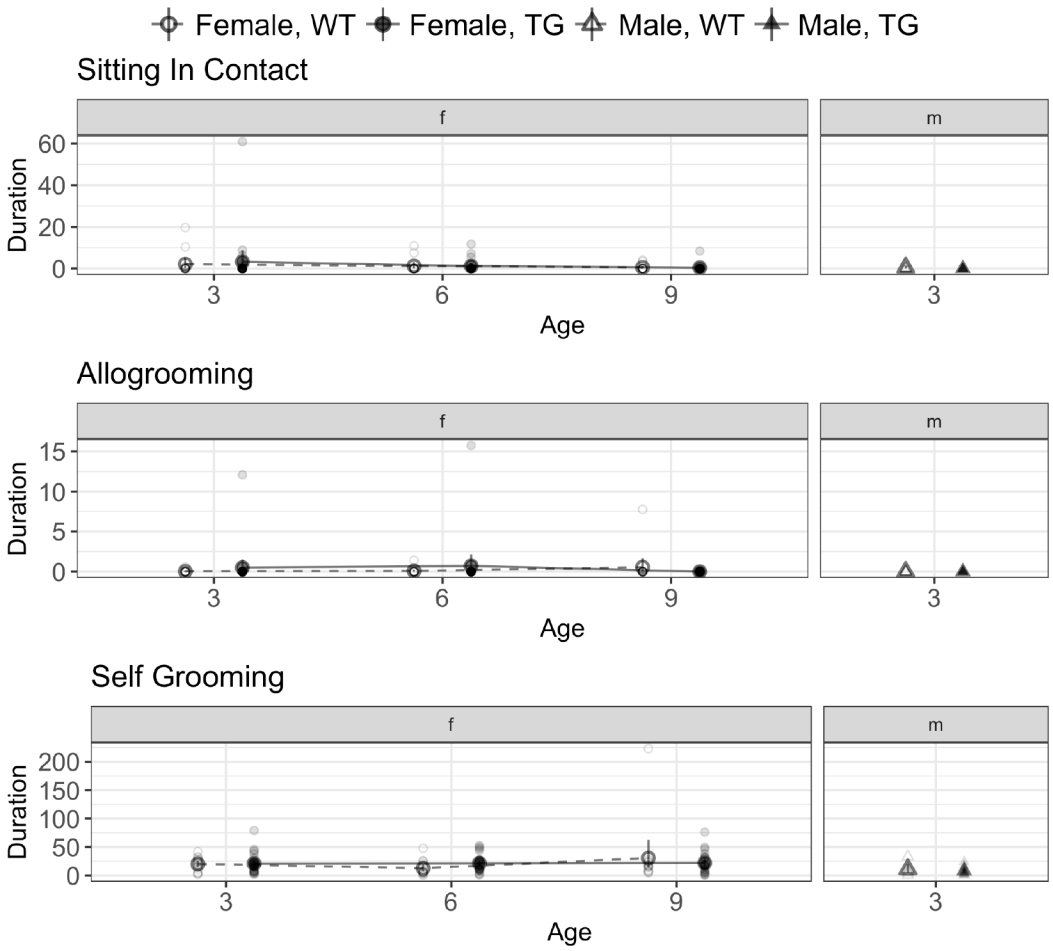

Figure 2.16. Raw scores and 95% CIs for mean behaviour duration (in seconds) of affiliative and self-grooming behaviours in the Free Social Interaction task in females (f) and males (m).

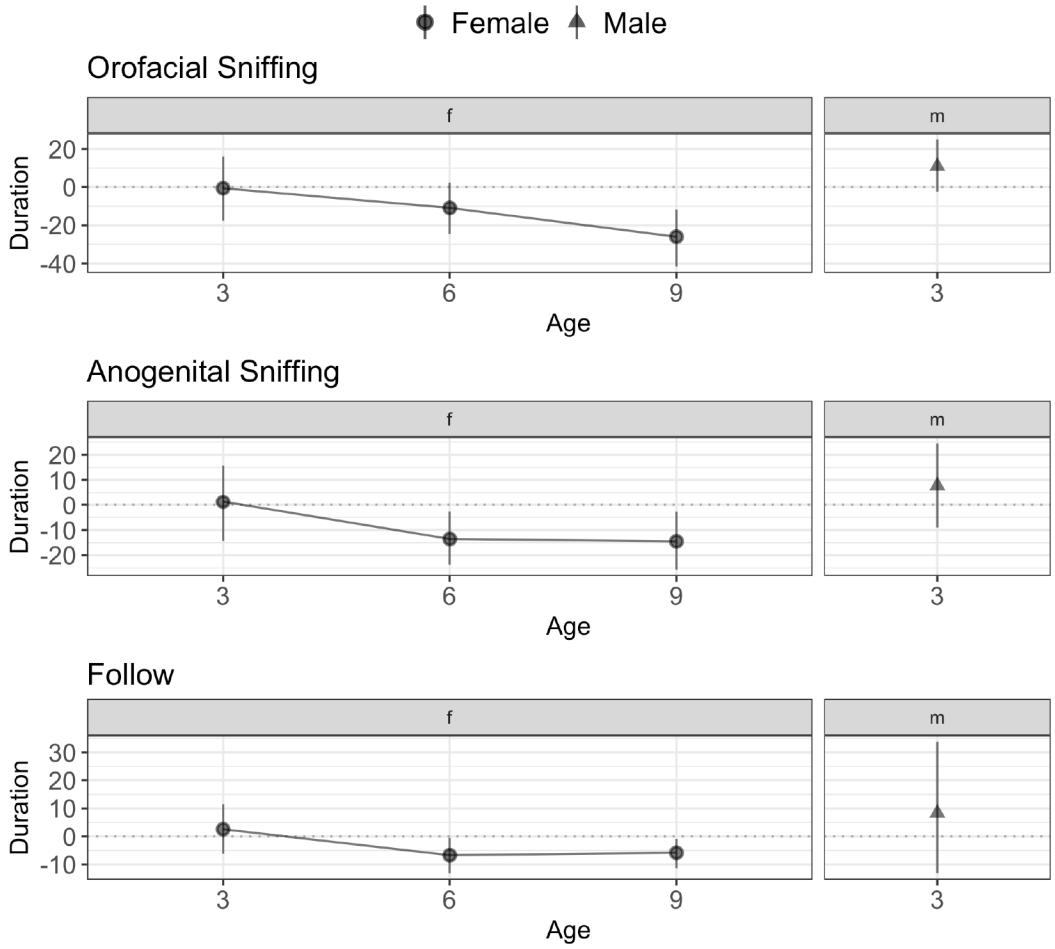

Figure 2.17. 95% CIs for genotype differences (transgenic relative to wild-type) for mean behaviour durations of investigative behaviours in the Free Social Interaction task in females (f) and males (m).

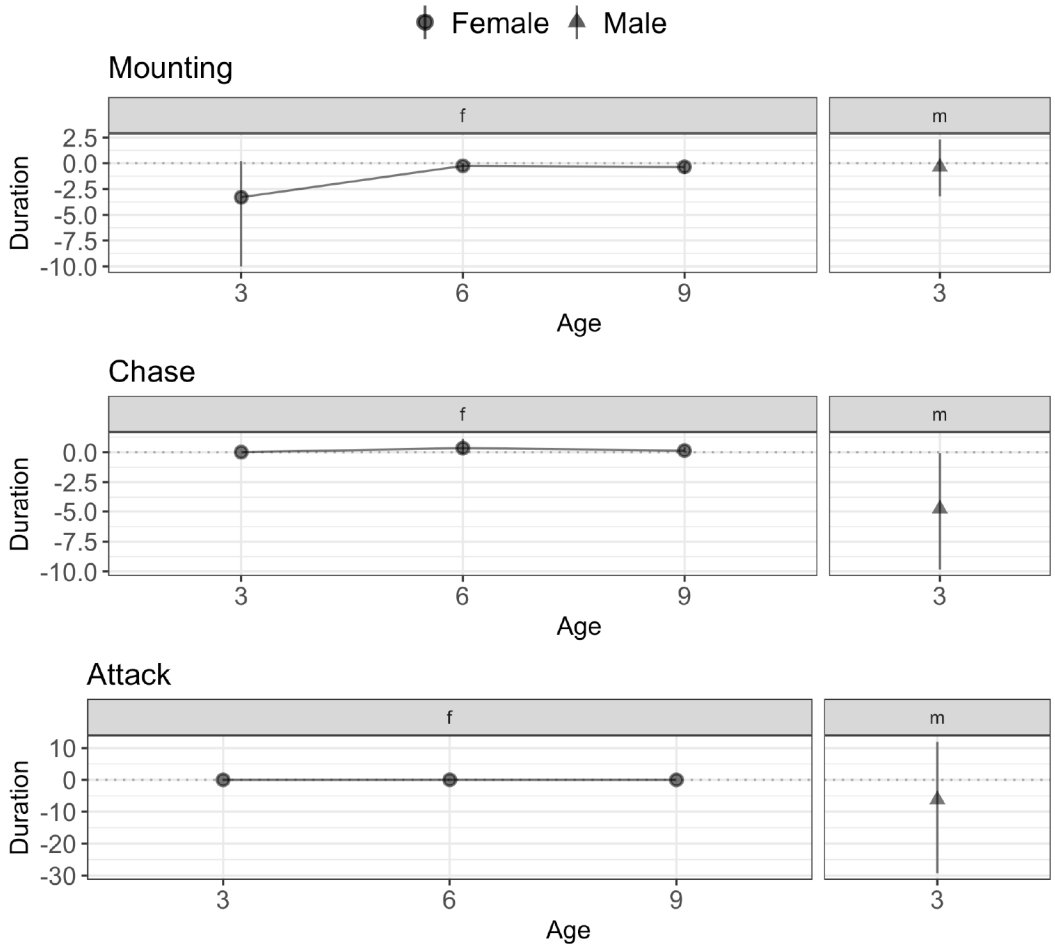

Figure 2.18. 95% CIs for genotype differences (transgenic relative to wild-type) for mean behaviour durations of aggressive behaviours in the Free Social Interaction task in females (f) and males (m).

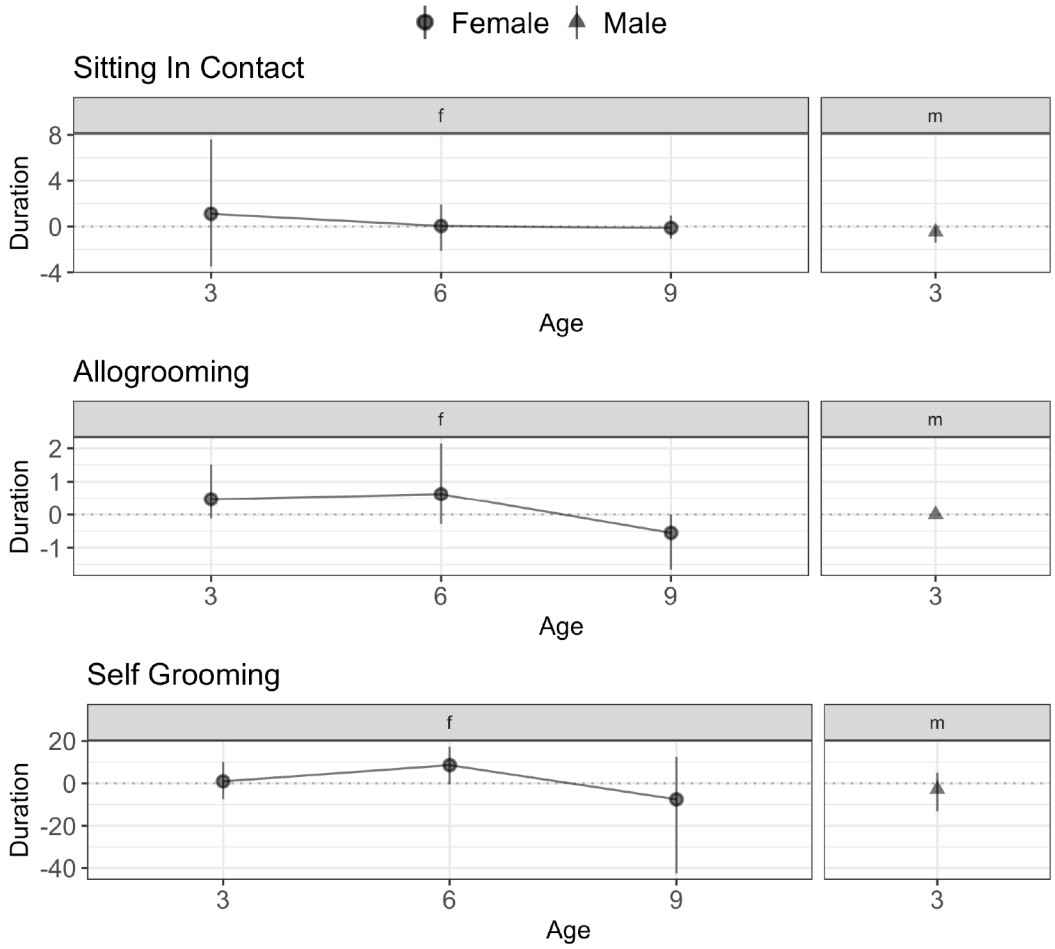

Figure 2.19. 95% CIs for genotype differences (transgenic relative to wild-type) for mean behaviour durations of affiliative and self-grooming behaviours in the Free Social Interaction task in females (f) and males (m).

Analysis of behaviours in males and females at 3 months of age indicates a genotype by sex interaction for chasing (χ^2 (1) = 12.87, $p < .001$), with wild-type males exhibiting more chasing than all other groups. There was also a main effect of sex on orofacial sniffing (χ^2 (1) = 10.30, $p = .001$), attack (χ^2 (1) = 14.43, $p < .001$), and self-grooming (χ^2 (1) = 8.31, $p = .004$), with females exhibiting more orofacial sniffing and self-grooming, but less attacking, than males. There were no other effects in males and females at 3 months of age; see Tables 2.13 – 2.15 and Figures 2.14 – 2.19.

Analysis of distance tracking suggests that there is no effect of age on the distance travelled by white transgenic females from 3-9 months of age (95% CIs [-99.90, 62.96], [-129.98, 57.10], and [-106.83, 72.43] cm/min difference from 3 to 6, 6 to 9, and 3 to 9 months of age, respectively).

2.4 Discussion

Transgenic 5xFAD females exhibited reduced interest in social odours during the olfactory habituation/dishabituation task, but no obvious olfactory deficits and no changes in scent marking behaviour compared to wild-type controls. Transgenic females also displayed an age-related decrease in social investigation in the free social interaction task. No differences in sociability in the three-chamber apparatus were observed at any age, and no overall differences were seen between transgenic 5xFAD males and wild-type controls at 3 months of age. This suggests that transgenic 5xFAD females exhibit impaired social interaction with age, but that this change is context-specific.

2.4.1 Transgenic 5xFAD Mice Exhibit Decreased Interest in Social Odours, but Normal Olfactory Perception and Scent-based Social Communication

As hypothesized, transgenic 5xFAD females from 3 – 9 months of age, and transgenic males at 3 months of age, exhibited no signs of non-social olfactory deficits relative to wild-type controls. The overall pattern of responses on the olfactory habituation/dishabituation task indicates that transgenic mice are able to detect non-social odours and discriminate between two different non-social odours (Figures 2.4 – 2.6). Findings on olfactory perception in 5xFAD mice has been mixed; prior reports support our findings that male and female 5xFAD mice have no overall deficits in olfactory perception (O'Leary, 2013; Roddick et al, 2014, 2016) although one study has reported that transgenic 5xFAD male mice display age-related olfactory deficits beginning at 3 months of age (Xiao et al., 2015).

Transgenic 5xFAD females exhibited decreased interest in social odour cues by 3 months of age, and this persisted through to 9 months of age; males exhibited no genotype differences for interest in social odour cues at three months of age (Figure 2.7). This extends previous work in our lab that indicated that transgenic 5xFAD males display reduced interest in social odours at 6 – 8 months of age (MacGowan et al, 2016). Interestingly, transgenic 3xTg-AD females have shown decreased preference for male social odours compared to wild-type controls, although these mice also exhibited a concomitant impairment in odour discrimination (Coronas-Samano et al, 2014). This suggests that the sexual olfactory deficits demonstrated by female transgenic 3xTG-AD mice is due to overall deficits in olfaction while the social olfactory deficits exhibited in

my study by transgenic 5xFAD females is due to reduced motivation to explore social odours specifically.

While there was minimal dishabituation to the second social odour during the olfactory habituation/dishabituation task, the same effect was seen for both transgenic and wild-type females, suggesting that this is due to similarities in major histocompatibility complex (MHC) and/or major urinary proteins (MUPs) between the two social odours (Arakawa et al, 2007a; Schellinck et al, 1995; Singh, Brown, & Roser, 1987). Both of these are proteins associated with determination of individuality in animals and are influenced by both genetics and environment, and given that the 5xFAD strain is highly inbred, and that subjects are kept under nearly identical conditions, it is likely that subjects have difficulty in identifying that these cues are from two different donors.

Although transgenic mice were hypothesized to exhibit impaired responses to social odour cues, the pattern of urine marking in response to social vs non-social odour cues was comparable between transgenic mice and wild-type controls at all ages (Figures 2.8 – 2.9). Additionally, while previous evidence suggests that males exhibit a more dispersed pattern of urine marking (Ninomiya & Kimura, 1989; Palanza, Parmigiani, & Vom Saal, 1995; Powell & Wolff, 1982) and an increased rate of excretion (Drickamer, 1995) than females, as well as increased urine marking in response to opposite sex urine (Arakawa et al, 2007a), there were no effects of subject sex at three months of age, nor was there more urine marking in response to opposite-sex compared to same-sex urine at any age. However, Brown (1991) previously indicated that sexual experience mediated urine marking, and the mice in the present study were sexually naive which most likely

accounts for this variation. However, the overall increase in urine marking in response to social compared to non-social odour cues indicates that transgenic mice are able to discriminate between these cues and mediate their response accordingly, thereby indicating no deficits in scent-based communication.

While these normal levels of urine marking may seem counter to the decreased interest in social odour cues, they are similar to effects seen in the transgenic BTBR model of autism spectrum disorder. Compared to wild-type C57BL6 controls, BTBR males and females at ~3 months of age exhibit reduced sociability (Pobbe et al, 2010) and an overall decrease in sniffing durations during the olfactory habituation/dishabituation task (Yang et al, 2012), while exhibiting similar levels of countermarking in response to urine from novel male conspecifics (Roullet et al, 2011). While these results are not directly comparable to mouse models of AD, they do indicate that interest in social odours can be dissociated from social communication via urine marking, and that this form of social communication is distinct from sociability. My findings indicate that transgenic 5xFAD females exhibit decreased motivation for investigating social odours but are able to maintain normal urine marking behaviours.

2.4.2 Transgenic 5xFAD Females Exhibit Context-based Alterations in Social Approach

Contrary to my hypothesis, 5xFAD males at three months of age, and transgenic females at any age, exhibit no difference in sociability or social novelty preference compared to wild-type controls (Figures 2.10 – 2.13). My lab has previously reported that transgenic males exhibit increased sociability compared to wild-type controls at 6 – 8 months of age (MacGowan et al, 2016), suggesting a possible sex difference in transgenic

5xFAD sociability and a possible age-dependent progression of sociability in transgenic 5xFAD males. While the present study did indicate an effect of genotype on total exploration time (Figure 2.13), this was due to an age-related increase in exploration time by wild-type females during the sociability task rather than a decrease in exploration by transgenic females. However, there was no genotype difference in total exploration time during the social novelty preference task at any age, suggesting that this effect was only present during the initial exposure to a novel conspecific. This difference during the first trial could be the result of repeated testing; wild-type females may have increased their exploration time as their habituation to the procedure progressed from their initial testing at three months of age, while transgenic mice may have re-habituated to the procedure during the sociability trial at each timepoint. This would also result in the comparable exploration times between transgenic females and wild-type controls during the social novelty preference trial immediately following the sociability trial. Further experiments could address this by testing a cross-section of subjects at each age, rather than repeated testing over time.

Although I aimed to measure short-term and long-term social recognition memory, wild-type controls exhibited no preference for novel or familiar conspecifics after 30 minute or 24 hour delays, so it was not possible to assess memory deficits in transgenic mice. While the reasons for this are unclear, it is possible that this lack of preference is due to similarities between stimulus mice (due to the strain being heavily inbred and mice being kept in identical conditions) making it difficult for subjects to differentiate between novel and familiar conspecifics. Further experiments could address this by testing a cross-section of mice at each delay, thereby reducing effects of repeated testing, as well

as using mice from different strains as stimulus animals. This latter proposal requires care to ensure that results are not biased by motivation to approach each strain (such as the use of one highly aggressive vs one highly subordinate stimulus animal).

Curiously, while transgenic females exhibited no difference in social approach during the sociability or social novelty preference tasks, they did display a clear age-related decrease specifically in investigative approach behaviours during the free social interaction task (Figure 2.14). Though transgenic 5xFAD mice exhibit an age-related impairment in locomotor activity, these effects typically are not seen until 12 months of age (Jawhar et al, 2012; O'Leary, 2013), and analysis of distance travelled in a subset of transgenic females during the free social interaction task in the present study indicated no decrease in locomotor activity from 3 – 9 months of age, suggesting that the decreased investigation time observed in females was not due to changes in locomotor activity. Thus, together these findings suggest that social approach is dependent on the context, with free-roaming conspecifics leading to decreased motivation for social interaction compared to a restricted conspecific. Although the mechanisms for this are unclear, there are two likely explanations: *1)* increased social anxiety that is mediated by restraint of the novel conspecific; or *2)* altered sensitivity or responses to physiological arousal. Increased social anxiety would manifest as an aversion to social interaction without an overall increase in anxiety, whereas altered physiological arousal would be characterized by apathy in low-stimulus situations and aversion in high-stimulus situations.

In the case of increased social anxiety, transgenic 5xFAD females may exhibit heightened anxiety in social situations compared to wild-type controls, thereby leading to altered approach behaviours. The free social interaction paradigm allows approach to be

initiated by either the subject or stimulus mouse, and the arena prevents escape from the novel mouse, potentially resulting in high levels of anxiety. By comparison, the use of a restrained conspecific during the sociability and social novelty preference tasks may provide an increased level of comfort, as the subject is able to initiate approach or escape from the conspecific as desired. This is also consistent with the decrease in total interaction time with age relative to wild-type controls on the sociability task; specifically, that decrease may arise due to transgenic females not remembering the task from the previous testing session, thereby exhibiting reduced motivation to approach the stimulus mouse. However, subjects may exhibit an increased level of comfort with the restrained stimulus mice by the second trial, thereby leading to no differences in social interaction relative to wild-type controls. Transgenic 5xFAD mice also exhibit reduced social interaction times relative to wild-type controls during the 24-hour delay trial, further suggesting an inability to remember prior experience in the testing procedure.

Support for heightened levels of social anxiety during reciprocal social interactions comes from similar effects of sociability and free social interaction seen in Fmr1-KO mice (a model of Fragile X Syndrome), which exhibit a preference for social over non-social interactions in a three-chamber apparatus, but a decrease in social interactions during the second half of a free social interaction task (Pietropaolo et al, 2014). Pietropaolo *et al* used a 6-minute trial, compared to the 10-minute trial used in the present study, and they indicate a significant effect of genotype during the last 3 minutes of the trial; as a result, it is possible that a longer trial would have resulted in a progressive decrease of social interaction behaviour, thereby leading to results similar to the present study. Although Pietropaolo *et al* (2014) do not discuss the reasons underlying this

pattern of effects, Spencer, Alekseyenko, Serysheva, Yuva-Paylor, and Paylor (2005) indicate that Fmr1-KO mice exhibit decreased anxiety in open field and light/dark tests, yet increased social anxiety on the mirrored chamber test, a test that uses a mirrored chamber to create the visual illusion that another mouse is present, and compares exploration of this chamber to chambers without mirrors. Spencer *et al* (2005) indicate that treatment with anxiolytics reduces the latency of mice to enter the mirrored chamber, suggesting its validity as a method for assessing social anxiety. As a result, these studies indicate that Fmr1-KO mice exhibit increased social anxiety to unrestricted social stimuli, but no difference in response to the restricted social stimulus in the three-chamber sociability task, similar to my findings in transgenic 5xFAD females.

In the case of abnormal sensitivity to physiological arousal, it is possible that transgenic 5xFAD females exhibit dysregulation in physiological arousal leading to very high or very low levels of arousal in response to stimuli that have moderate effects on wild-type controls. In this scenario, the olfactory social odour cue during the olfactory habituation/dishabituation task may represent a low level of arousal, thereby leading to apathy, whereas the presence of a free-roaming conspecific—which includes visual, auditory, olfactory, and tactile information—in the free social interaction task may represent a high level of arousal, thereby leading to avoidance. Although the sociability task also involves visual, auditory, olfactory, and tactile cues, the use of a cage to contain the conspecific and the ability to escape to another chamber may reduce arousal to a level that promotes interaction rather than inhibiting it. Previous evidence has indicated that adenosine A_1 and A_{2A} receptors mediate arousal in mice (Van Dort, Baghdoyan, & Lydic, 2009; Ribeiro, Pfaff, & Devidze, 2009) and that there is an upregulation of adenosine A_{2A}

in AD in both human patients (Rahman, 2009) and the Tg2576 model (Da Silva et al, 2016). The use of selective agonists/antagonists could allow for direct modulation of arousal without changing external stimuli (Yacoubi, Ledent, Parmentier, Costentin, & Vaugeois, 2000). Transgenic 5xFAD mice exhibit no difference in adenosine A_{2A} receptor expression in the hippocampus compared to wild-type controls (Gyoneva, Swanger, Zhang, Weinshenker, & Traynelis, 2016), however arousal in mice by adenosine A_{2A} is mediated through the prefrontal cortex and brainstem via cholinergic neurons (Van Dort et al, 2009), and degeneration of cholinergic neurons has been reported in the 5xFAD strain (Devi & Ohno, 2010). Thus, the possibility of differences in adenosine-mediated arousal in the 5xFAD mouse strain, and its potential role in social function, is an interesting avenue for future exploration.

Finally, while transgenic males were hypothesized to be more aggressive than wild-type controls, wild-type males exhibited more chase behaviour than all other groups, with no other genotype differences in behaviours for males (Figure 2.15). This suggests that the transgenic males are less likely to initiate an aggressive encounter and that increased social withdrawal may play a role in this finding.

Overall, this pattern of behaviour—consistent social approach to a restricted mouse but an age-related decrease in investigative behaviour during reciprocal interactions—has not been reported in other mouse models of AD. Transgenic APP/PS1 males at 6 months of age exhibit similar levels of social approach behaviour as wild-type controls in the three-chamber apparatus, but high levels of non-social exploration (Filali et al, 2011a). Compared to wild-type controls, transgenic 3xTg-AD females exhibited fewer social behaviours during a free social interaction task at 18 months of age, a deficit that was

preceded by higher levels of social interaction at 12 months of age (Bories et al, 2012). Transgenic Tg2576 females at 21 months of age exhibited no difference in social investigation compared to wild-type controls during free social interaction (Deacon et al, 2009). Thus, social behaviours in mouse models of AD appear to be strain dependent, and previous research has not performed both the three-chamber sociability test and the free social interaction test in the same animals making it difficult to assess whether the particular combination of effects observed in the current study is unique to the 5xFAD strain.

2.4.3 Possible Neurological Underpinnings of Social Deficits in the 5xFAD Strain

Transgenic 5xFAD mice exhibit rapid and widespread accumulation of Aβ between 3 – 6 months of age (Oakley et al, 2006), which may account for a number of the behavioural changes observed. As discussed in Section 2.4.2, increased adenosine A_{2A} receptor expression similar to that seen in the Tg2576 strain (Da Silva et al, 2016) coupled with degeneration of cholinergic neurons (Devi & Ohno, 2010), could lead to altered modulation of arousal in 5xFAD mice, thereby leading to apathy or aversion based on salience of a stimulus.

In addition to neurodegeneration of cholinergic neurons, progressive age-related deficits in axon myelination have been observed in transgenic 5xFAD mice as early as 1 month of age, specifically within the prelimbic area, entorhinal cortex, and CA1 region of the hippocampus, followed by the retrosplenial granular cortex (Gu et al, 2018). Previous research has indicated that selective lesions in the medial prefrontal cortex (mPFC; including prelimbic area and entorhinal cortex) in rats can increase fear responses and

anxiety (Heidbreder & Groenewegen, 2003), that silencing mPFC—dorsal periaqueductal grey projections can lead to social avoidance in mice (Franklin et al., 2017), and that prelimbic cortex—nucleus accumbens projections are activated in response to conditioned place preference for social interaction in rats (El Rawas et al, 2012). These findings suggest that impaired connections to and from the medial prefrontal cortex of 5xFAD mice may drive changes in anxiety and motivation, and may be critical for social function. Similarly, evidence in the 3xTg-AD strain indicates that social interaction is better correlated with background synaptic activity in the medial prefrontal cortex rather than Aβ or tau pathology (Bories et al, 2012).

Finally, although evidence suggests that transgenic 5xFAD mice exhibit no olfactory deficits (O'Leary, 2013; Roddick et al, 2014; 2016), glucose hypometabolism has been reported in the olfactory bulb as early as at three months of age (Xiao et al, 2015), and it is possible that altered olfactory system function may be involved in mediating interest in social odour cues. Similarly, neurodegeneration of cholinergic neurons may affect function of the accessory olfactory system (including the vomeronasal organ), thereby leading to impaired processing of social odour cues (Smith & Araneda, 2010; Smith et al, 2015), although the similar levels of scent marking between transgenic 5xFAD females and wild-type controls in response to social odour cues suggests that transgenic females do not exhibit impaired processing of social odour cues.

2.4.4 Conclusions

Overall, transgenic 5xFAD female mice show an age-related decrease in social interest in the olfactory habituation/dishabituation task and the free social interaction task. Although the underlying mechanisms driving this behaviour are unclear, it is

possible that transgenic females may exhibit increased social anxiety or altered sensitivity to physiological arousal. Transgenic 5xFAD mice exhibit limited deficits in social-related behaviours at 3 months of age, with no overall genotype differences evident in males or females, with the exception of reduced investigation of social odours by transgenic females. Transgenic 5xFAD females also exhibit no overall changes in scent marking behaviour relative to wild-type controls at any age, as well as no differences in sociability or social novelty preference in the three-chamber apparatus.

While these results indicate that transgenic 5xFAD mice exhibit changes in social interactions, they also raise additional questions, predominantly in regards to the pattern of social interaction observed in transgenic females. As discussed previously, further exploration could determine whether the altered social approach behaviour during the free social interaction task is due to social anxiety or altered arousal. One major difference between this task and the sociability/social novelty preference tasks is the use of a restricted stimulus animal (by the use of a cage) compared to a free-roaming animal. The presence of this cage may affect approach behaviours by providing a barrier between the subject and the stimulus animal. In order to test this, a modified social approach task could be performed using a three-chamber apparatus with two novel stimulus animals, with one restricted to one chamber by a tether (as previously described by Rissman, Early, Taylor, Korach, & Lubahn, 1997) and the other contained within a cage. A consistent preference by transgenic females towards the caged animal over the tethered one would indicate that the presence of the cage mediates approach behaviour, suggesting that the difference in investigation seen during the sociability/social novelty preference tasks compared to the free social interaction task may be due to differences in social

anxiety. Additionally, altering arousal through the use of adenosine agonists or antagonists during olfactory habituation/dishabituation, sociability, and free social interaction tasks could identify whether altered levels of arousal mediate responses to each stimulus.

Chapter 3: Experiment 2: Phenotypic Contagion of Aggression in Male 5xFAD Mice

3.1 Introduction

While apathy and associated social withdrawal are major neuropsychiatric symptoms of AD, agitation/aggression (A/A) is also common in AD patients (Chung & Cummings, 2000; Chow et al, 2002; Frisoni et al, 1999; Lyketsos et al, 2002; Steffens et al, 2005; Zhao et al, 2016). A recent study has placed the world-wide prevalence of A/A at around 40% (Zhao et al., 2016), with aggressive behaviours being more common in males than females (Lovheim et al, 2009). The presence of A/A has also been linked with increased functional disability (Chung & Cummings, 2000), increased caregiver distress (Craig et al, 2005), and is a leading factor in institutionalization (Chenoweth & Spencer, 1986; Steele et al, 1990). As a result, a large number of AD patients in nursing homes exhibit A/A (Wood et al, 2000).

Mouse models are an important tool for understanding the underlying causes of A/A. Transgenic mouse models of AD (including the Tg2576, APP23, and mice exhibiting wild-type, London, and Swedish APP mutations) exhibit increased aggression compared to wild-type controls against a novel intruder in the resident/intruder task as early as six months of age (Alexander et al, 2011; Jager et al, 2018; Moechars, Gilis, Kuiperi, Laenen, & Van Leuven, 1998; Vloeberghs et al, 2006). Conversely, Bories *et al* (2012) found that transgenic 3xTg-AD mice did not exhibit increased aggression compared to wild-type controls in response to novel mice in a novel environment, even at 18 months of age, suggesting that the environment of the encounter—novel arena vs familiar home-cage—plays a role.

Prior stressors may also influence or trigger A/A in AD mouse models. Prior to testing AD mouse models in the resident-intruder tasks described above, subjects were placed in social isolation housing, a chronic stress paradigm that has been previously shown to increase aggression and to result in a number of additional behavioural and physiological changes (Goldsmith et al, 1976; Koike et al, 2009; Matsumoto, Pinna, Puia, Guidotti, & Costa, 2005). Moechars and colleagues (1998) also indicated that transgenic APP mice exhibited high levels of male-male and male-female aggression, but only for mice housed in vivaria containing a large number of other transgenic mice. While home-cage aggression was not indicated in other studies, and these mice were group-housed, the number and phenotypes of other mice in the room may represent an environmental stressor; one possible scenario is that aggression in one cage may incite aggression in adjacent cages, thereby propagating throughout the room.

Though Moechars *et al* do not specify whether mice were housed in single-genotype (transgenic or wild-type only) or mixed-genotype (transgenic and wild-type) cages, they indicate that the home-cage aggression observed was only present in transgenic mice. Additionally, Bories *et al* (2012) used mixed-genotype dyads for assessing social behaviour and found no differences in aggression between transgenic mice and wild-type controls. These findings suggest that the genotype of both the aggressor and the receiver (target) mediate the level of aggression.

Overall, previous findings aimed at understanding A/A in AD mouse models indicate that aggression in transgenic mice is a complex trait mediated by a number of factors including environment, genotype of the aggressor, and genotype of the receiver (target).

While the rapid progression of neurodegeneration in the 5xFAD strain makes it

suitable for examining changes in behaviour over time (Bilkei-Gorzo, 2014), little research has examined aggression in this model. Early work with the background strain (C57 B6SJL) indicated that males exhibited high levels of home-cage aggression starting at 8 weeks of age, with serious injuries and/or death occurring in group-housed males between 4-6 months of age (Kuby, 1997, p. 27; Lyon et al, 1996, p. 1562). However, observations in our lab and others have suggested that high levels of home-cage aggression occur predominantly in cages housing transgenic 5xFAD males, especially when housed in single-genotype cages (containing only transgenic males). In our lab, transgenic males housed in single-genotype cages had to be separated due to aggression by six months of age, with many being separated at approximately 4-5 months of age (see Table 2.1 for numbers of group-housed subjects at 3, 6, and 9 months of age in Experiment 1). By comparison, wild-type single-genotype cages exhibited relatively low levels of home-cage aggression, with few wild-type cages requiring separation even at 12 months of age. Observations in the Brown lab (Dalhousie University) suggested that housing males in mixed-genotype cages (containing both wild-type and transgenic males) decreased—but did not abolish—the level of home-cage aggression compared to transgenic-only cages. Overall, this indicated that transgenic males were more aggressive than wild-type controls, although the results from Experiment 1 did not support this: at three months of age, transgenic males exhibited less chasing, and no difference in attacking or mounting, compared to wild-type controls in the free social interaction task. However, minimal neurodegeneration was expected at this age, and aggressive behaviours may not have begun to develop yet. Additionally, the protocol used only wild-type conspecifics as stimulus animals, similar to Bories *et al* (2012). As a result, this

created a mixed-genotype testing environment for transgenic males, and it is possible that this pairing served to reduce aggression in a similar manner to mixed-genotype home-cages. Overall, this led to my hypothesis that transgenic males are more aggressive in the presence of other transgenic males vs wild-type controls, and that this aggression may develop shortly after three months of age.

The phenomenon of *interacting phenotypes* is not a novel concept. Scott (1977) indicated that all social behaviours are, by definition, interactions between two or more conspecifics. This relationship itself is affected by the genotypes of the conspecifics and evolves over time: initial interactions between unfamiliar conspecifics are characterized by feedback and adaptation—wherein behaviour of each is modified by the behaviour of the other—while later stages of a relationship are characterized by a differentiation of behaviours—wherein each conspecific takes on a particular social role. Moore, Brodie, and Wolf (1997) expanded on this, indicating that the phenotype of an organism is the product of both genetics and the environment. As a result, the relationship itself is both the environment, which leads to the modification of behaviour, and an "evolving trait", which itself is modified by changes in behaviours. More recently, Baud *et al* (2017) reported that group-housing mice of different strains together can lead to similarities in gene expression in these mice, suggesting that phenotype can influence genotype. However, these studies indicated that relationships between unfamiliar conspecifics would either rapidly degenerate—as in the case of aggression against a novel intruder—or would stabilize over time, neither of which is the case in this scenario. Instead, group-housing transgenic 5xFAD male littermates should result in a familiarity from the earliest stages of life and a stable relationship that persists through adulthood, as is the case in

females and wild-type males. However, unlike females and wild-type males, transgenic males exhibit an imbalance in this relationship as time progresses, which leads to a rapid change in the relationship. This phenomenon was originally thought to be the result of aggressive (rather than subordinate) responses to aggressive behaviours, thereby perpetuating further aggressive acts and resulting in a positive-feedback loop; as a result, this phenomenon is referred to as *phenotypic contagion* (A. Baud, personal communication, June 27, 2017) in the present study.

Overall, the concepts put forth by Scott (1977) and Moore *et al* (1997) indicate that behaviours in a dyadic relationship are dependent on both the emitter (the individual initiating a behaviour) and the receiver (the individual responding to it). With respect to aggression in AD models, Alexander *et al* (2011) indicated that while transgenic Tg2576 males exhibited decreased latency to first attack and increased number of attacks, the overall duration of attacks did not differ between transgenic males and wild-type controls. This indicated that transgenic males in that strain were able to identify subordinate behaviour and cease attacks similar to wild-type controls. Further, these encounters are influenced by the level of both aggressive behaviour by aggressors and subordinate behaviour of targets, as well as the appropriate identification of both aggressive and subordinate in both individuals. With respect to the levels of aggression observed in our lab, this suggested that transgenic 5xFAD males exhibit deficits in any, all, or some combination therein of these behaviours.

The present study was developed to examine when aggression in the 5xFAD strain develops and how it progresses. Though transgenic single-genotype cages had to be separated more frequently than wild-type cages, it was unclear whether this was the result

of a stable increase in aggression or a rapid escalation. Moreover, though mixed-genotype cages appear to require less frequent separation than transgenic single-genotype cages, it is unclear whether these cages exhibit less frequency and/or severity of aggression than transgenic single-genotype cages. To this end, home-cage observations were used to examine behaviours in both single- and mixed-genotype cages.

The results of the aggression studies discussed above suggest that transgenic mice exhibited less aggression in novel environments (open field or clean cage) than in familiar (home-cage) environments. As a result, males were also tested in novel environments on both the social approach and free social interaction tasks. The social approach task was performed to assess investigative behaviours, and the free social interaction task was performed to examine aggressive behaviours, in response to a novel conspecific in a novel environment. Additionally, both tasks used transgenic and wild-type stimulus males to examine whether the genotype of a conspecific affects investigation and aggression. Finally, mice use aggression to establish dominance hierarchies (Brain & Parmigiani, 1990; Miczek et al, 2001; Williamson et al, 2016), and aggression has been associated with unstable dominance hierarchies (Howerton, Garner, & Mench, 2008), so the tube test of dominance was used to assess dominance between transgenic 5xFAD males and wild-type controls.

3.2 Methods

3.2.1 Subjects

3.2.1.1 Cohorts

Subjects were tested in two cohorts: Cohort 1 was tested on social approach and free social interaction; Cohort 2 was used for home-cage observations and dominance

hierarchy testing. Both cohorts included both transgenic males and littermate wild-type controls. Cohort 1 was tested at 26 weeks (6 months) of age. Home-cage recordings were taken of Cohort 2 between 8 weeks (2 months) and 23 (5.25 months) of age, with dominance hierarchy tested at 24-26 weeks (5.5 to 6 months) of age; however, only mixed-genotype pairings were used to examine dominance hierarchy. Animals were bred and genotyped in-house, as described in Chapter 2.2.1.2 and 2.2.1.3. All subjects were sexually naive.

Due to a limited number of mixed-genotype pairings available for home-cage observations, there were insufficient subjects to perform the dominance hierarchy task using only the above-described mice. As a result, additional subjects (n = 1 transgenic, 2 wild-type) were used. These subjects were 24-week old transgenic and wild-type males group-housed in a same-sex, same-litter, mixed-genotype cage, although they had been housed in a standard mouse cage rather than the modified rat cages used for home-cage recordings. Additional subjects (n = 3 transgenic, 5 wild-type) were also required to increase sample sizes for the social approach and free social interaction tasks. Three of these (n = 1 transgenic, 2 wild-type) were the same ones discussed above, although the social approach and free social interaction tasks were performed two weeks following the tube test of dominance. The remaining 4 subjects (n = 2 transgenic, 2 wild-type) had previously been used for home-cage observations, although had been transferred to standard mouse cages 3 weeks prior to testing, and were tested at 26 weeks of age. The larger cages used during home-cage observations and the use of food and water bowls could constitute environmental enrichment, and previous evidence suggests that environmental enrichment improves cognition in transgenic AD mouse models, although

there is mixed support for the effects on Aβ production (Arendash et al, 2004; Jankowsky et al, 2005; Jankowsky, Xu, Fromholt, Gonzales, & Borchelt, 2003). Evidence in rats has also suggested that environmental enrichment could affect social behaviours by increasing social investigation (Sparling, Baker, & Bielajew, 2018), although it is not possible to examine whether these mice differ significantly from experimentally naive mice on social approach or free social interaction due to the small sample sizes in the present study.

3.2.1.2 Housing

Cohort 1 was group-housed in groups of 2-3 in same-sex, same-litter, mixed-genotype (containing both transgenic males and wild-type controls; n = 5 transgenic, 6 wild-type) cages following weaning. Cages were standard mouse cages (30 x 19 x 13 cm, model PC7115HT; Allentown Caging Inc., Allentown, NJ, USA) containing wood-chip bedding (FreshBed; Shaw Resources, Shubenacadie, NS, Canada), a metal cage top containing food (Laboratory Rodent Diet #5001; Purina LabDiet, St. Louis, MO, USA) a water bottle, and two black, opaque, polymer enrichment tubes (4 cm diameter, approx. 8 cm long). All cages were topped with a micro-isolator filter to reduce the spread of airborne contaminants and diseases. Animals were housed in a colony room on an inverted 12:12 light:dark cycle; the dark phase lasted from 09:30 to 21:30, and the light phase lasted from 21:30 to 09:30. Cages were changed weekly, prior to the end of the light cycle (approximately 08:30-09:30). To reduce fighting between males following cage changes, a small amount of bedding was transferred from the old cage to the new cage to retain the scent of the animals.

Cohort 2 was group housed in pairs in same-sex, same-litter cages from weaning until

approximately 8 weeks of age; during this time, subjects were housed in conditions identical to the ones described for Cohort 1. Subjects were housed in transgenic single-genotype cages (n = 6 cages), wild-type single-genotype cages (n = 6 cages), and mixed-genotype cages (n = 3 cages), representing the three possible group- or pair-housing scenarios. At 8 weeks of age, subjects were moved to a separate recording colony room, and were transferred to the home-cage observation apparatus (standard rat cages; 47 x 26 x 21 cm; Allentown Caging Inc., Allentown, NJ, USA) in place of their normal cage change. These cages contained wood-chip bedding (FreshBed) and two black, opaque, polymer enrichment tubes. Nylon cable ties were used to hold enrichment tubes together and in parallel; this reduced the likelihood of tubes shifting and obscuring activity. Cages also contained two flat-bottomed clay bowls (approx. 10 cm diameter x 4 cm high); one contained food (Rodent Diet #5001) and the other contained tap water. To reduce obstructions, metal cage tops were not used, and cages were covered with clear acrylic tops containing ventilation holes (see Figure 3.1 for a representative image). The recording room used the same inverted 12:12 light:dark cycle, with the dark phase lasting from 09:30 to 21:30, and the light phase lasting from 21:30 to 09:30. Due to lighting requirements for video recordings, red lamps were also used; red lamps were turned on at 15:30 and off at 03:30. To maintain a consistent phase length throughout the experiment duration, the light cycle was shifted by 1 hour to adjust for Daylight Saving Time. To reduce effects from other animals, the recording room was only used to house subjects undergoing home-cage observations. Cages were changed weekly; however, food and water bowls were emptied and refilled every two days in-between cage changes to remove bedding and feces. Subjects remained in the same cages until home-cage

observations and dominance hierarchy tests were completed, at which point they were returned to standard mouse cages.

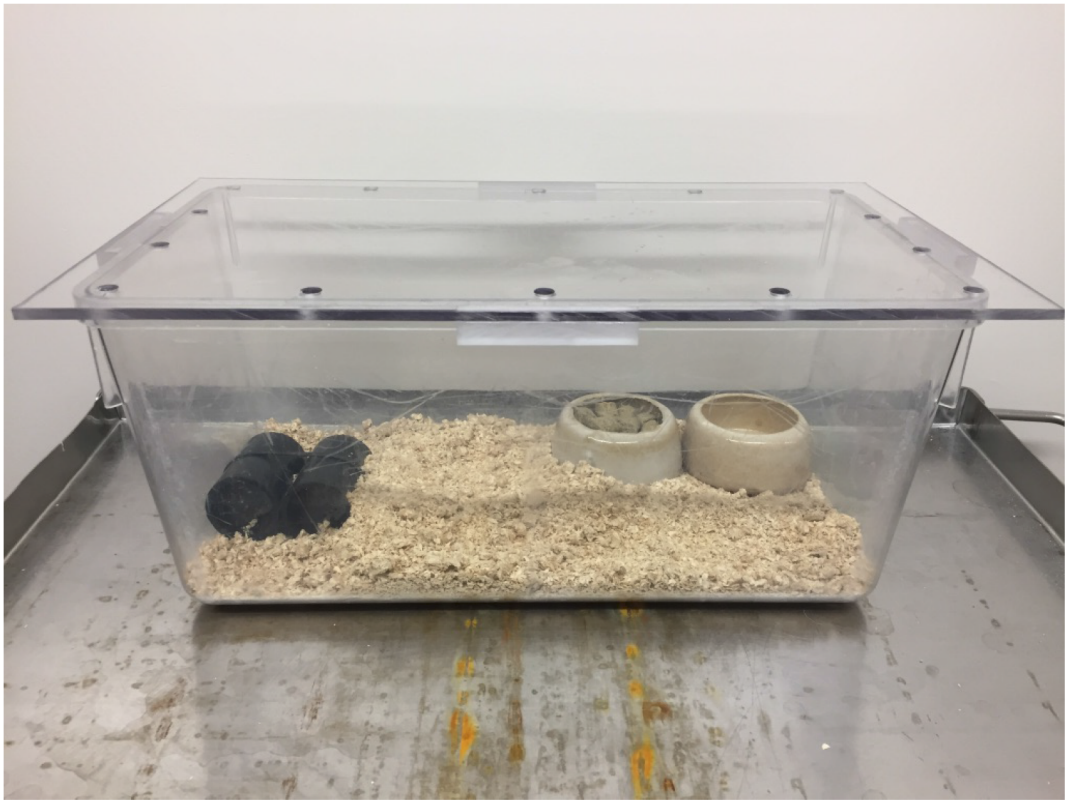

Figure 3.1. Representative layout of the home-cage apparatus used during home-cage recordings.

3.2.1.3 Separation of animals due to aggressive behaviour

Due to the possibility of home-cage aggression, animals were monitored regularly for general health and to check for injuries. Animals exhibiting signs of injury (such as broken skin from bites) were immediately separated from their cage-mate and placed in their own cage; any open wounds were treated with antibiotic ointment, and the animal was monitored closely over subsequent days to ensure proper healing. Any animals exhibiting distress were immediately euthanized by first inducing anaesthesia and then

performing cervical dislocation and decapitation. Once separated, animals were removed from further testing, although any data collected up to that point was used in the final analysis.

3.2.2 Behavioural Procedures

3.2.2.1 Testing Schedule

Subjects in Cohort 1 were tested over 11 days, with testing sessions taking place near the end of the dark phase (approximately 17:00 to 20:30). Recent research has indicated that male AD-model mice exhibit a circadian rhythm of aggression (Todd et al, 2018), so all testing was performed during the same period. Each subject performed a single testing session on a given day; these were as follows: Day 1, social approach session 1; Day 4, social approach session 2; Day 8, free social interaction session 1; Day 11, free social interaction session 2.

Subjects in Cohort 2 began home-cage observations at 8 weeks of age. Subjects were recorded for 3 days per week, every second week, from 8 weeks to 23 weeks of age; recordings were taken on the day of the cage change (Day 1), two days after a cage change (Day 3), and the day before a cage change (Day 7).

3.2.2.2 Testing Conditions

Social approach, free social interaction, and the tube test of dominance were performed in the same room described in Chapter 2.2.2.2; this was a quiet 2.4 x 2.4 m room containing a desk with a computer, and a table for the testing apparatus. Two monochromatic network cameras were used to video record the tasks: one mounted approximately 1 m over the table on an overhead stand, and the other located on the table 15-30 cm away from the apparatus. Cameras were connected to the computer via a

network hub. Tasks were recorded using the Biobserve Viewer 3.0 program (Biobserve GmbH, Bonn, Germany). Testing was performed under minimal lighting conditions; the room was lit with a ceiling-mounted red light, and a desk lamp containing a red bulb. Although the computer display was not turned off during testing, brightness was reduced and the screen was angled away from the testing apparatus to reduce direct illumination. The door was kept closed to reduce light and noise from surrounding areas. Due to the short duration of the tube test for dominance and the possibility of aggression during free social interaction, the experimenter remained in the room for both of these tasks.

Home-cage recordings were performed in the Cohort 2 colony room (3 m x 3.5 m). The room contained two metal cage racks (1.2 m x 0.5 m x 1.5 m tall) against the walls, each with four shelves plus the top panel; only one of these racks was used for recording. The room also contained a stainless steel counter and sink, and two video camcorders (Canon Vixia HF R800; Canon USA Inc, Melville, NY, USA) on tripods; the cameras were located 1.2 m from the rack, with the tripods adjusted to place the cameras in line vertically with each other as much as possible. One camera was adjusted to maximize viewing of the upper two shelves, while the other was adjusted to view either the middle or lower two shelves as needed. The room was lit by two ceiling-mounted white fluorescent light fixtures, as well as clamp-mounted desk lamps containing red bulbs on the end of each shelf; see Section 3.2.1.2 for light cycle details. The lamps faced the centre of the rack and were angled slightly towards the wall to reduce glare into the camera. Cages were arranged on racks with the broad side facing the front and slightly angled towards the camera (see Figure 3.2 for a representative image); this allowed up to three cages to be placed on a shelf without any obstructions. Shelves were initially filled

starting at the top; however, to maintain consistency in the videos, each cage was placed in the same spot within a shelf (either the left, middle, or right side) for all recordings. Cages were not placed on the top panel to prevent direct lighting by overhead lights.

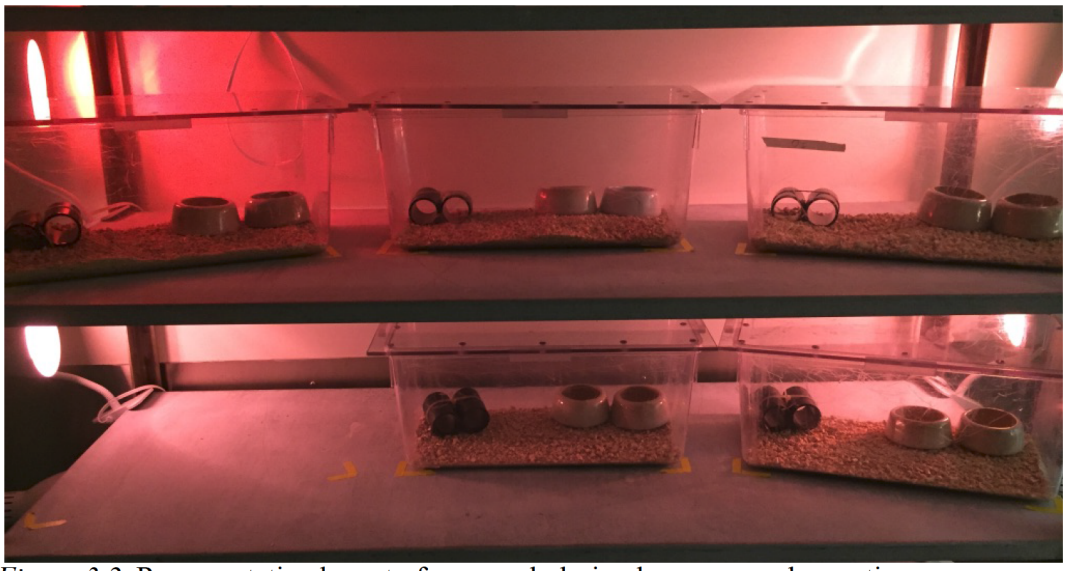

Figure 3.2. Representative layout of cage rack during home-cage observations.

3.2.2.3 Social Approach Behaviour

3.2.2.3.1 Apparatus

The apparatus was the same as that used for the Sociability and Social Novelty Preference task in Experiment 1 (Section 2.2.2.5). The apparatus consisted of a clear, acrylic box, 69 x 20 x 20 cm, separated by two clear, acrylic walls into three compartments of equal size (23 x 20 x 20 cm; Figure 2.2). Access to each compartment is through a 6 x 6 cm, floor-level opening in the middle of the dividing walls. Opaque sections of acrylic were used as barriers between the three chambers. The floor of the apparatus was lined with the same wood-chip bedding as used in the home-cages, and a

round wire cage (Galaxy Cup; Spectrum Diversified Designs Inc., Streetsboro, OH, USA) was used in each of the two outer chambers to contain stimuli. White, opaque, 500 mL HDPE bottles (Nalge Nunc International Corporation, Rochester, NY, USA) filled with water were placed on top of the Galaxy Cups to prevent subjects from climbing on top.

3.2.2.3.2 Procedure

The Social Approach task was performed similarly to the Sociability task described in Section 2.2.2.5.2; however, subjects performed one session on each of two separate days (see Section 3.2.2.1). The protocol was identical for each session, with the exception of the genotype of the stimulus animal used; one session used a transgenic male stimulus animal, while the other used a wild-type male stimulus animal. Siegfried, Frischknecht, and Waser (1982) indicated that an encounter with an aggressive novel conspecific could induce subordinate behaviours in response to a subsequent encounter with a non-aggressive novel conspecific. As a result, the order of presentation of each genotype was counterbalanced across subjects to ensure that interaction with one stimulus genotype did not consistently affect interaction with the other.

Prior to testing, subjects were transferred to a clean holding cage containing wood-chip bedding and a food pellet, and a micro-isolator cover was placed on top; holding cages were used to reduce scent from home-cages in the testing room. All subjects were removed from home-cages and placed into holding cages at the same time, and were returned to the home-cage after all cage-mates had completed testing. Stimuli mice were also transferred to holding cages for testing.

Each session consisted of one habituation trial and one testing trial. For the

habituation trial, the Galaxy Cups were inverted and placed in the two outer chambers without any stimuli, and the water bottles were placed on top. With the barriers in place, the subject was placed in the centre chamber, both barriers were removed simultaneously, and the subject was allowed to freely explore for 5 minutes. At the end of the 5 minutes, the subject was trapped in the centre chamber with the barriers, and an unfamiliar stimulus male was placed under one of the Galaxy Cups, while the opposite Galaxy Cup remained empty; the side of the stimulus animal was counterbalanced across subjects and stimulus animal genotypes. Once the stimulus animal was in place, both barriers were lifted simultaneously and the subject was allowed to freely explore for 10 minutes. Following the 10-minute testing trial, subjects and stimulus mice were returned their holding cages. Both subjects and stimulus animals were returned to their home-cages once all cage-mates had completed testing.

The apparatus was cleaned in between subjects; bedding was dumped out and any remaining particles were vacuumed out, and the apparatus was cleaned using Fisherbrand Sparkleen 1 (Fisher Scientific, Pittsburgh, PA, USA) and room-temperature water, then thoroughly dried with paper towels. Galaxy Cups were cleaned with Fisherbrand Sparkleen 1 and room-temperature water, then dried with paper towels, before and after testing.

3.2.2.3.3 Scoring and Data Analysis

Scoring was performed as described in Section 2.2.2.5.3. Videos were manually scored for duration spent in each chamber during the testing trial, as well as duration of interaction with the social stimulus and investigation of the empty Galaxy Cup. Subjects were considered to have entered a chamber as soon as their head and forelimbs were

across the threshold into the chamber. Interaction with a stimulus was defined as orientation of the snout towards the Galaxy Cup, with the snout 1 cm or less away from the Galaxy Cup. Interaction duration was used to calculate preference ratios for the social vs non-social stimulus; preference ratios were calculated by dividing the time spent interacting with the stimulus animal over the total time spent interacting with both stimuli.

In order to maintain a consistent method of analysis, data analysis was performed in R version 3.4.1 (R Foundation for Statistical Computing) using multilevel modelling; see Section 2.2.2.4.4 for rationale. Subject genotype was used as the between-subjects factor, while stimulus animal genotype was used as the within-subjects factor; the effect of subject was used as the error term. Analyses were performed for preference ratio and total interaction time. Bootstrapped 95% confidence intervals were used to examine means and effects. Outliers were not removed from analysis.

3.2.2.4 Free Social Interaction

3.2.2.4.1 Apparatus

The apparatus was the same as that used for the Free Social Interaction task in Experiment 1 (see Section 2.2.2.6.1). The apparatus consisted of a 38 x 38 x 40 cm box, with the floor raised 5 cm off of the base. Three of the walls were made of plywood and painted beige, and the fourth wall was made of clear acrylic to allow for recording from the side. The floor was made of clear acrylic to facilitate cleaning between animals; a sheet of black paper was placed immediately below the acrylic to make it appear solid.

3.2.2.4.2 Procedure

The Free Social Interaction task was performed similarly that described in Section

2.2.2.6.2; however, subjects performed one session on each of two separate days (see Section 3.2.2.1). The protocol was identical for each session, with the exception of the genotype of the stimulus animal used; one session used a transgenic male stimulus animal, while the other used a wild-type male stimulus animal. The order of presentation of each genotype was counterbalanced across subjects.

Prior to testing, subjects were transferred to a clean holding cage containing wood-chip bedding and a food pellet, and a micro-isolator cover was placed on top; holding cages were used to reduce scent from home-cages in the testing room. All subjects were removed from home-cages and placed into holding cages at the same time, and were returned to the home-cage after all cage-mates had completed testing. Stimuli mice were also transferred to holding cages for testing.

The subject was placed into the apparatus and allowed to habituate by freely exploring for five minutes. At the end of this period, the subject remained in the apparatus while an unfamiliar stimulus male was introduced; the order of presentation of stimulus animal genotype was dependent on the order of presentation used for the Social Approach task (Section 3.2.2.3), and was thereby counterbalanced across subjects. The two mice were then allowed to freely interact for 10 minutes. To ensure safety of both mice, the experimenter remained in the room and observed quietly for the duration of the trial, and intervened if excessive aggression was observed. Mice were physically separated within the apparatus if continuous fighting was observed for more than 30 seconds. If fighting continued after separation, or if more than five successive attacks were performed by either mouse, the test was stopped immediately and both mice were returned to their holding cages. Both subjects and stimulus animals were returned to their

home-cage once all cage-mates had completed testing. The apparatus was cleaned between subjects using Fisherbrand Sparkleen 1 (Fisher Scientific) and room-temperature water, then thoroughly dried with paper towels.

3.2.2.4.3 Scoring and Data Analysis

Videos were manually scored for duration of investigative (follow; orofacial sniffing; anogenital sniffing), aggressive (chase; mount; attack), defensive (induced flee; defensive posture), and self-grooming behaviours. Definitions of these behaviours were adapted from Arakawa *et al* (2007b) and Grant and Mackintosh (1963), and were as follows: follow (slow speed locomotion towards the other animal that is also moving); chase (rapid locomotion towards the other animal that is rapidly moving away); orofacial sniffing (orientation of the snout towards and nearly touching the snout of the other animal); anogenital sniffing (orientation of the snout towards and nearly touching the anogenital region of the other animal); mounting (mounting the other animal from behind and thrusting the pelvis); attack (initiating biting and/or rolling); induced flee (rapid locomotion away from the other animal that is chasing it); defensive posture (standing upright on the hind limbs with the forelimbs raised up, and the head directed away from the other animal). Although Grant and Mackintosh (1963) put anogenital sniffing under mating behaviour, it is also used to determine dominance between novel mice; as a result, it was considered an investigative behaviour in the present study.

Data analysis was performed in R version 3.4.1 (R Foundation for Statistical Computing) using multilevel modelling; see Section 2.2.2.4.4 for rationale. Subject genotype was used as the between-subject factor, while stimulus genotype was used as a within-subject factor; subject was used as an error term. Analyses were performed

separately for each behaviour, and bootstrapped 95% confidence intervals were used to examine patterns of significant effects. Outliers were not removed from analysis.

3.2.2.5 Home-cage Observations

3.2.2.5.1 Apparatus

The apparatus is described in the Sections 3.2.1.2 and 3.2.2.2. Briefly, the apparatus consisted of a standard rat cage (47 x 26 x 21 cm) containing wood-chip bedding (FreshBed), two black, opaque, polymer enrichment tubes (4 cm diameter, approx. 8 cm long) held together with a nylon cable tie, and two flat-bottomed clay bowls (approx. 10 cm diameter and 4 cm tall). These bowls were located at the back of the cage (when viewed from in front of the cage rack) to prevent obstructing the view, and one contained food pellets while the other contained water. A transparent acrylic cage top with ventilation holes was used as a cover for the cage (see Figure 3.1). Cages were placed on a cage rack with the broad side visible from the front (see Figure 3.2); this allowed three cages to be placed on a shelf. Each space on the shelf corresponded with a cage number, and all videos were made with the cages in the same location on the shelf (either left, middle, or right) each time. Red lamps at each end of the shelves were used to illuminate the cages during the dark phase, while white fluorescent ceiling fixtures were used during the light phase.

3.2.2.5.2 Procedure

At weaning, subjects were pair-housed in same-sex, same-litter cages in three housing combinations: transgenic single-genotype (transgenic—transgenic), wild-type single-genotype (wild-type—wild-type), and mixed-genotype (transgenic—wild-type). Subjects remained in the colony room until just prior to 8 weeks of age, at which point they were

moved to the recording room, where they would remain for the duration of the experiment. Upon being moved to the recording room, subjects were transferred to the recording cages, and were placed on the racks. Subjects were then given two days to habituate to the new cages and the red light cycle; the overall light:dark cycle remained the same as the colony room.

Following the two-day habituation period, subjects were weighed and placed in clean cages. Clean food and water bowls were used at each cage change, although enrichment tubes remained with the subjects throughout the duration of the experiment. Enrichment tubes were cleaned at every cage change by spraying with a solution of Fisherbrand Sparkleen 1 (Fisher Scientific) and room-temperature water and then rinsing thoroughly with hot water to remove any debris. During this cage change, subjects were also checked for injuries; any animals exhibiting injuries were separated from their cage-mate and removed from the study. Cages that did not require separation during this first cage change were assigned a cage number and a location on the rack.

Subjects were recorded every second week, starting at 8 weeks of age. Recordings were made on the 1st, 3rd, and 7th day following a cage change. Recordings were made for the last 4.5 hours of the dark phase, and the first 4.5 hours of the light phase; however, recordings were started at least an hour early to prevent any behavioural changes from the experimenter entering and leaving the room.

In addition to weekly cage changes, subjects also had their food and water bowls emptied and refilled every two days (on the third, fifth, and seventh day following a cage change). After two days, food and water bowls were often full of feces and bedding. Both cage changes and food/water changes were performed during the first half of the dark

phase.

Subjects were recorded until 23 weeks of age. At 23 weeks of age, single-genotype cages were transferred to standard mouse cages and returned to the colony room. Mixed-genotype cages remained in recording cages until 24-26 weeks of age, at which point they performed the tube test of dominance.

3.2.2.5.3 Scoring and Data Analysis

Videos were manually scored on a per-cage basis for frequency of aggressive encounters, allogrooming (licking given by one animal to another), aggressive grooming (one animal holds down the other and forcibly grooms it using its teeth), huddling (the bodies of both animals touching for at least 10 seconds consecutively), and being alone (both animals at least one body length apart from each other for a minimum of 10 seconds consecutively); these behaviours were largely adapted from Arakawa *et al* (2007b) and Grant and Mackintosh (1963). Due to the nature of aggressive encounters, they often result in several instances of the aggressor chasing, attacking, and/or mounting the target, as well as the target repeatedly fleeing and/or performing defensive posture; as a result, scoring each behaviour separately could artificially inflate the number of aggressive encounters. In order to prevent this, scoring of aggressive encounters was adapted from the protocol of Williamson *et al* (2016) who scored aggressive encounters using a hierarchical scale, using the following rankings: attack > chase > mount > defensive posture > induced flee (flight). Aggressive encounters in the present study were scored by recording the highest level behaviour observed in each encounter; e.g., if one animal attacked the other, which responded by fleeing, that was counted as an attack only. Each encounter was considered as the period of time when the aggressor or target

exhibited one of these ranked behaviours, until the aggressor moved away to perform a different activity (e.g., feeding, self-grooming, etc).

Scoring of behaviours was performed by time-sampling. A 30-sec period was scored every 10 minutes for the number of instances of the above behaviours, for a total of six periods per hour. Videos were scored from 4.5 to 0.5 hours before onset of the light phase, and from 0.5 to 4.5 hours after the onset of the light phase; this resulted in a total of four hours of video per phase.

Data analysis was performed in R version 3.4.1 (R Foundation for Statistical Computing) using multilevel modelling; see Section 2.2.2.4.4 for rationale. Counts of each behaviour were analyzed individually, as well as the total number of aggressive behaviours (attack, chase, mount), defensive behaviours (defensive posture, induced flee), and all aggressive encounters (both aggressive and defensive behaviours). Housing condition (transgenic single-genotype, wild-type single-genotype, or mixed-genotype) was used as the between-subjects factor, while age (in weeks) and light phase were used as within subjects factors; the effect of individual cages were used as the error term. A preliminary examination indicated that the data exhibited a Poisson distribution due to a nearly 1:1 covariance of means and standard deviations for each group and behaviour. As a result, analyses were performed using a generalized linear model with a Poisson distribution. Due to limited data, the effect of day could not be properly modelled, and had to be excluded from analysis. Bootstrapped 95% confidence intervals were used to examine patterns of significant effects. Outliers were not removed from analysis.

In addition to instances of behaviours, the number of cages separated and the reason for separation was tracked. Due to limited data, bootstrapped 95% confidence intervals

were used to estimate mean proportion of cages still group-housed for each genotype at each age. Due to one wild-type single-genotype cage being removed from testing for stereotypic behaviour rather than aggression, an adjusted wild-type survival number was calculated without that cage to examine aggression-related separations only.

3.2.2.6 Tube Test of Dominance

3.2.2.6.1 Apparatus

The apparatus was based on that described by Lindzey *et al* (1961); it consisted of two clear acrylic "goal boxes" (approximately 15 x 10 x 10 cm), connected by a clear acrylic tube (approximately 3 cm diameter and 30 cm long) and mounted to a section of particle board. The goal boxes were covered by hinged flaps containing ventilation holes; this prevents subjects from escaping the apparatus before or after a trial. Sections of clear acrylic could be inserted into the side of each goal box, adjacent to the entrance to the tube, in order to prevent subjects from re-entering the tube following a trial.

3.2.2.6.2 Procedure

This test was only performed with cage-mates from mixed-genotype cages, resulting in transgenic—wild-type pairings for each trial. Due to the short duration of the test and both subjects coming from the same cage, subjects were transported to the behaviour room in their home-cages. Each subject was then given a brief habituation trial to the apparatus; subjects were placed in a goal box and were allowed to freely explore the tube and both goal boxes for two minutes. If a subject did not enter the tube within the first minute, the experimenter encouraged them to do so by opening the lid of the goal box and putting their hand inside; if that did not work, the experimenter would guide the subject's head towards the tube and lightly pull on their tail. By the end of the two

minutes, all subjects had traversed the length of the tube at least twice. Subjects were returned to their home-cage following habituation.

Test trials were performed approximately 5 minutes after habituation. For the test trial, both subjects were removed from their home-cage and lowered into opposing goal boxes without releasing their tails. Once in the goal boxes, subjects were guided towards the tube and encouraged to enter; when both subjects were inside the tube, their tails were lightly pulled to encourage them to move forward, and they were released simultaneously. The first mouse to back out of the tube completely and enter their starting goal box with all four limbs was considered the loser of the pairing, while the one that continued straight through the tube and into the opposite goal box was considered the winner. If neither mouse backed out after 2 minutes, the match was considered a draw and no winner was declared.

After testing, mice were returned to their home-cage. The apparatus was cleaned before and after each habituation and testing trial by spraying the goal boxes and tube with a solution of Fisherbrand Sparkleen 1 (Fisher Scientific) and room-temperature water, rinsing thoroughly with warm water, and then drying the apparatus with paper towels.

3.2.2.6.3 Scoring and Data Analysis

Scoring was performed by recording the winner and loser of each pairing. Data analysis was performed by calculating a bootstrapped 95% confidence interval of the genotype effect for win ratio in R version 3.4.1 (R Foundation for Statistical Computing).

3.3 Results

3.3.1 Social Approach

Transgenic 5xFAD males exhibit a lower preference ratio (χ^2 (1) = 4.76, p = .029) compared to wild-type controls for social vs non-social interactions (95% CI [-0.20, -0.01]). Subject genotype effects in preference ratio to wild-type stimuli (95% CI [-0.29, 0.05]) were more variable than in response to transgenic stimuli (95% CI [-0.17, -0.02]). Transgenic males also exhibited reduced interaction time (in seconds) with social stimuli (χ^2 (1) = 8.66, p = .003) compared to wild-type controls (95% CI [-126.67, -28.93]). There were no other effects; see Tables 3.1 and 3.2 and Figures 3.3 and 3.4.

3.3.2 Free Social Interaction

Transgenic 5xFAD males exhibit less orofacial sniffing (χ^2 (1) = 4.52, p = .033), anogenital sniffing (χ^2 (1) = 11.09, p < .001), and following (χ^2 (1) = 4.53, p = .033) behaviours (in seconds) than wild-type controls (95% CIs [-20.28, 3.42], [-29.61, -7.09], and [-12.82, -1.23] for subject genotype effect on orofacial sniffing, anogenital sniffing, and following, respectively). Although mounting and induced flee behaviours were also scored, no subjects exhibited either behaviour, and they were removed from analysis. Additionally, three testing sessions were ended early due to subjects exhibiting high levels of aggression and this data was retained for analysis by extrapolating behaviours exhibited during that period to the full 10 minute duration. All three of the sessions that were ended early were the result of two wild-type male subjects, with one subject exhibiting high levels of aggression in both session 1 and session 2. There were no other effects; see Tables 3.3 and 3.4 and Figures 3.5 – 3.8.

Table 3.1

Analysis of Deviance for Social Approach

Effect	χ^2	df	*p*
	Preference Ratio		
Genotype	4.76	1	**.029**
Stimulus	0.30	1	.582
Genotype:Stimulus	0.17	1	.678
	Total Interaction Time		
Genotype	8.66	1	**.003**
Stimulus	0.00	1	.977
Genotype:Stimulus	2.72	1	.099

Note. p-values less than .05 are shown in **bold**.

Table 3.2

95% CIs for Mean Interaction Time and Preference Ratio

	Subject Genotype		Genotype Difference
Stimulus	tg	wt	tg-wt
	Preference Ratio		
tg	[0.65, 0.79]	[0.79, 0.89]	[-0.17, -0.02]
wt	[0.53, 0.85]	[0.74, 0.85]	[-0.29, 0.05]
Overall	[0.62, 0.80]	[0.78, 0.85]	[-0.20, -0.01]
	Total Interaction Time		
tg	[50.38, 125.46]	[161.19, 241.63]	[-166.69, -56.22]
wt	[65.72, 198.29]	[137.76, 205.16]	[-119.59, 32.58]
Overall	[69.84, 149.88]	[159.98, 213.85]	[-125.03, -28.89]

Note. tg = transgenic; wt = wild-type

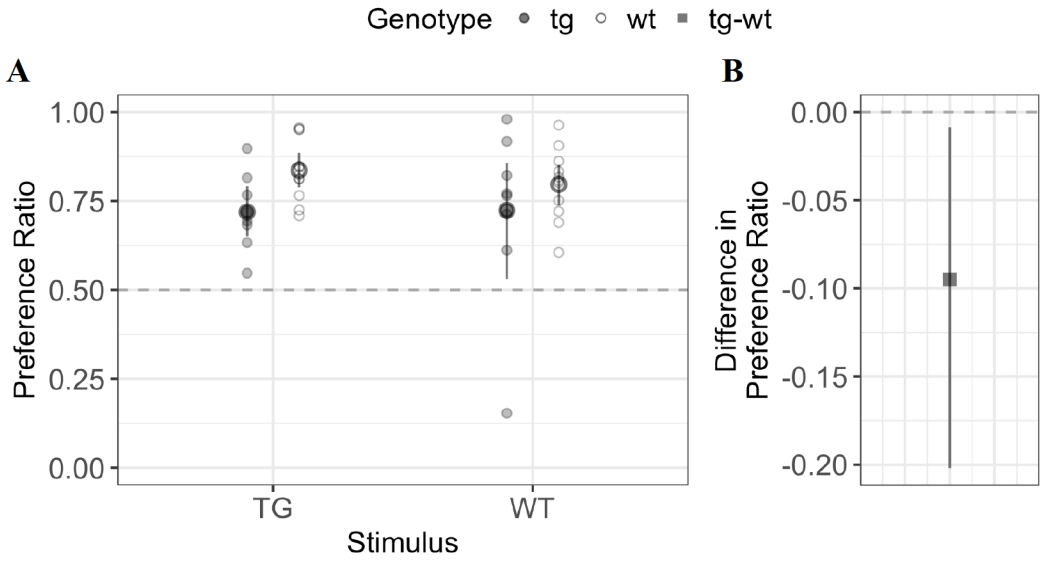

Figure 3.3. **A)** 95% CIs and raw values for preference ratio for transgenic 5xFAD males (tg) and wild-type controls (wt) in response to transgenic and wild-type stimulus males. **B)** Subject genotype effects (transgenic relative to wild-type) in overall preference ratio.

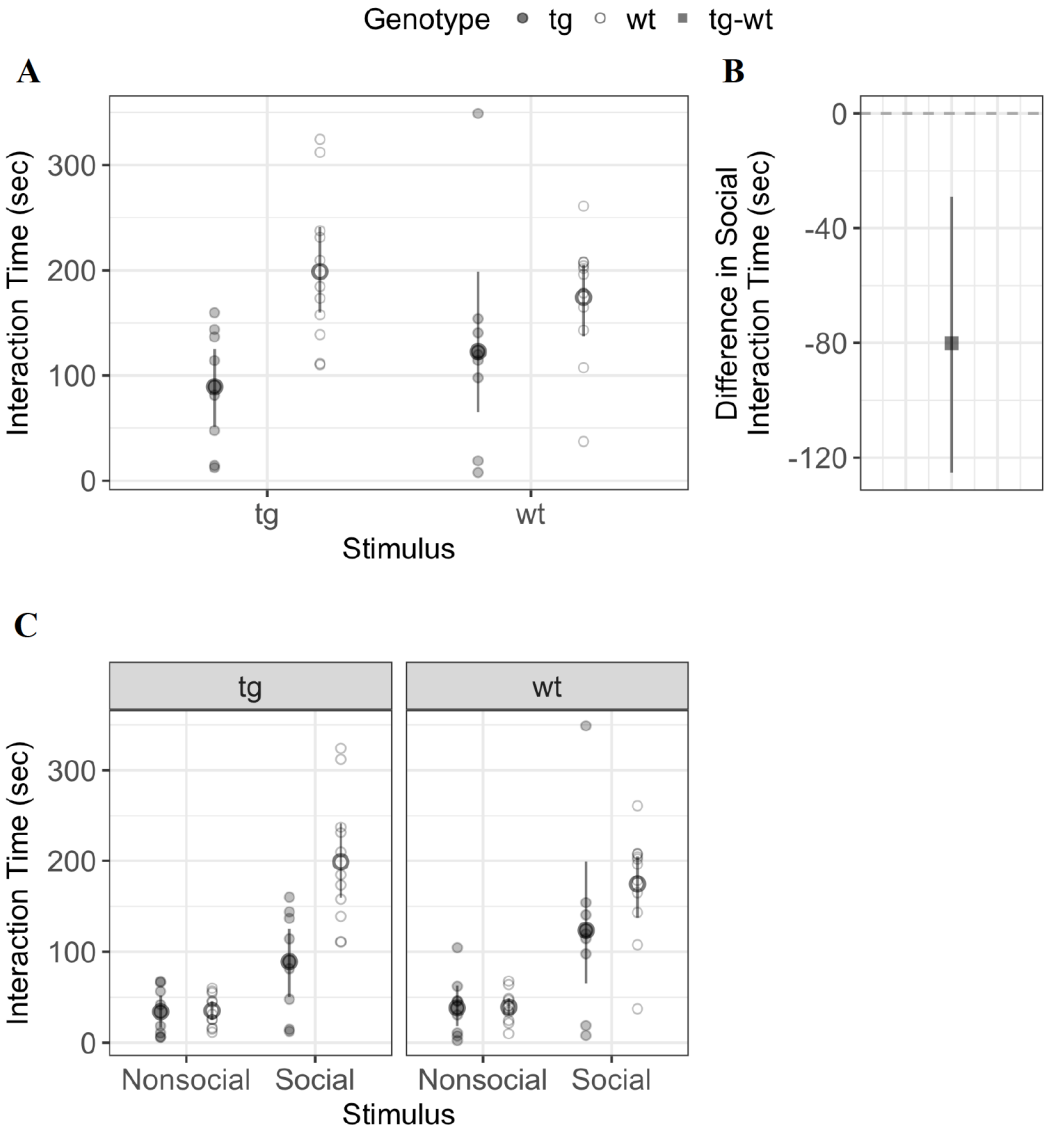

Figure 3.4. **A)** 95% CIs and raw values for social interaction time (in sec) for transgenic 5xFAD males (tg) and wild-type controls (wt) in response to transgenic and wild-type stimulus males. **B)** Subject genotype effects (transgenic relative to wild-type) for overall social interaction time. **C)** Interaction durations for each stimulus during the transgenic and wild-type stimulus trials.

Table 3.3

Analysis of Deviance for Free Social Interaction Task

Effect	χ^2	df	*p*
		Orofacial Sniffing	
Genotype	4.52	1	**.033**
Stimulus	1.01	1	.316
Genotype:Stimulus	0.79	1	.375
		Anogenital Sniffing	
Genotype	11.09	1	**.001**
Stimulus	0.59	1	.442
Genotype:Stimulus	0.67	1	.414
		Follow	
Genotype	4.53	1	**.033**
Stimulus	0.01	1	.930
Genotype:Stimulus	0.20	1	.659
		Chase	
Genotype	1.81	1	.178
Stimulus	0.91	1	.339
Genotype:Stimulus	0.64	1	.423
		Attack	
Genotype	2.34	1	.126
Stimulus	2.38	1	.123
Genotype:Stimulus	1.62	1	.203
		Defensive Posture	
Genotype	0.84	1	.358
Stimulus	1.33	1	.249
Genotype:Stimulus	0.89	1	.345
		Self-Grooming	
Genotype	0.12	1	.725
Stimulus	0.05	1	.819
Genotype:Stimulus	0.93	1	.335

Note. p-values less than .05 are shown in **bold**.

Table 3.4

95% CIs for Mean Durations and Genotype Differences (in sec) of Behaviours in the Free Social Interaction Task

	Mean Duration		
	Subject Genotype		Genotype Difference
Stimulus	tg	wt	tg-wt
		Orofacial Sniffing	
tg	[25.65, 46.07]	[37.86, 64.76]	[-32.58, 1.73]
wt	[37.27, 58.13]	[39.02, 57.58]	[-14.68, 13.36]
Overall	[33.64, 50.19]	[41.76, 58.48]	[-20.28, 3.42]
		Anogenital Sniffing	
tg	[11.88, 28.02]	[32.74, 58.75]	[-42.12, -12.20]
wt	[19.67, 43.64]	[32.06, 47.20]	[-22.52, 5.93]
Overall	[17.54, 33.62]	[35.62, 51.70]	[-29.61, -7.09]
		Follow	
tg	[4.46, 13.22]	[11.87, 24.54]	[-18.24, -2.54]
wt	[5.55, 15.40]	[7.10, 19.76]	[-11.09, 5.00]
Overall	[5.77, 12.76]	[11.34, 20.73]	[-12.82, -1.23]
		Chase	
tg	[0.00, 0.00]	[0.46, 3.00]	[-2.98, -0.46]
wt	[0.00, 0.00]	[0.00, 4.56]	[-4.56, 0.00]
Overall	[0.00, 0.00]	[0.55, 3.21]	[-3.20, -0.57]
		Attack	
tg	[0.00, 0.00]	[0.68, 9.04]	[-8.98, -0.68]
wt	[0.00, 0.00]	[0.40, 40.09]	[-40.09, -0.40]
Overall	[0.00, 0.00]	[1.51, 22.40]	[-22.76, -1.62]
		Defensive Posture	
tg	[0.00, 0.00]	[0.00, 0.00]	[0.00, 0.00]
wt	[0.00, 0.00]	[0.00, 15.77]	[-15.77, 0.00]
Overall	[0.00, 0.00]	[0.00, 7.36]	[-7.36, 0.00]
		Self-Grooming	
tg	[16.68, 31.18]	[13.24, 35.34]	[-13.94, 13.14]
wt	[15.69, 49.08]	[7.04, 41.77]	[-16.72, 33.04]
Overall	[18.50, 37.92]	[13.73, 34.02]	[-9.92, 18.46]

Note. tg = transgenic; wt = wild-type

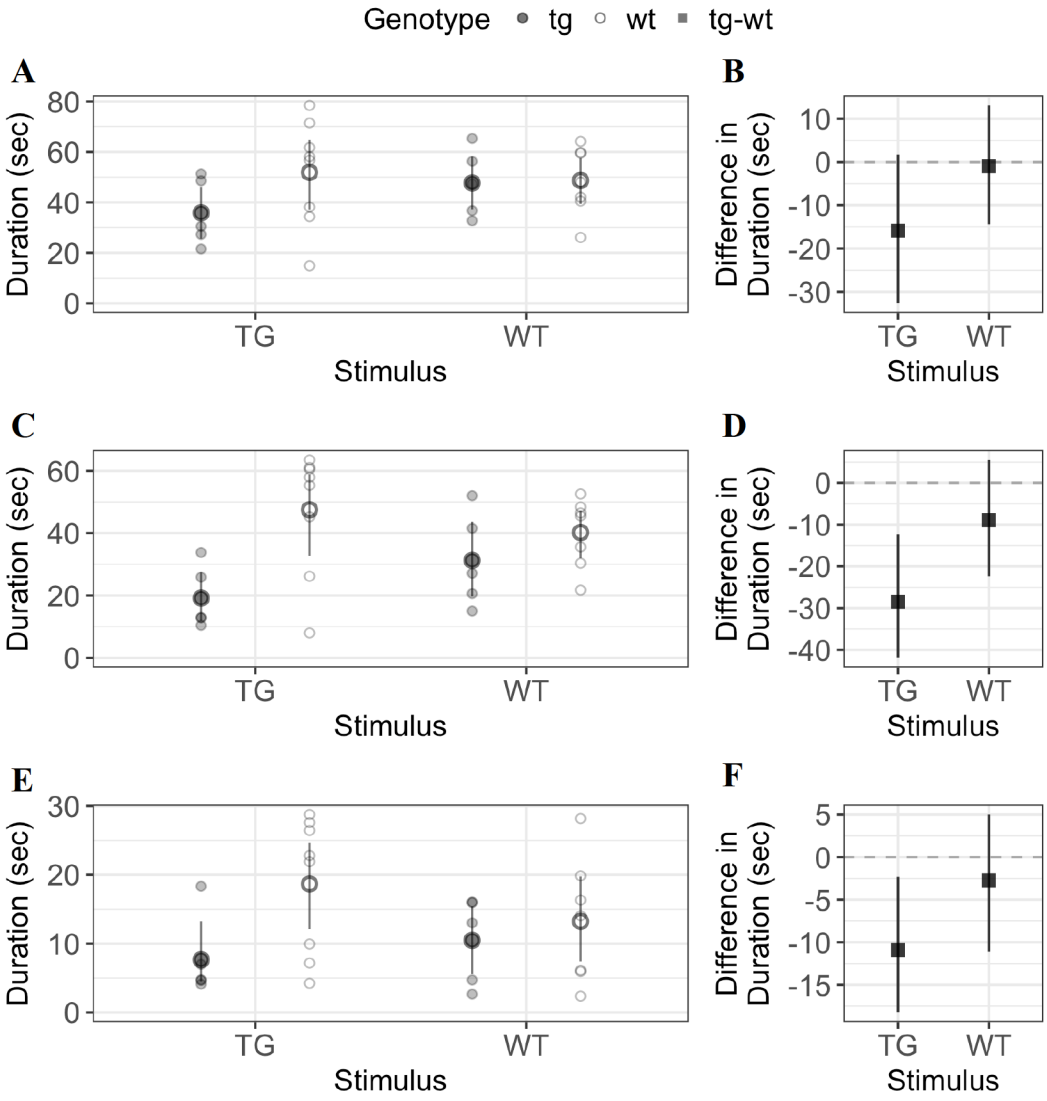

Figure 3.5. Raw data and 95% CIs for mean duration (in sec) of **A)** orofacial sniffing, **C)** anogenital sniffing, and **D)** following behaviours for transgenic 5xFAD males (tg) and wild-type controls (wt) in response to transgenic (TG) and wild-type (WT) stimulus males. 95% CI for mean subject genotype effect (in sec) on **B)** orofacial sniffing, **D)** anogenital sniffing, and **F)** following behaviours.

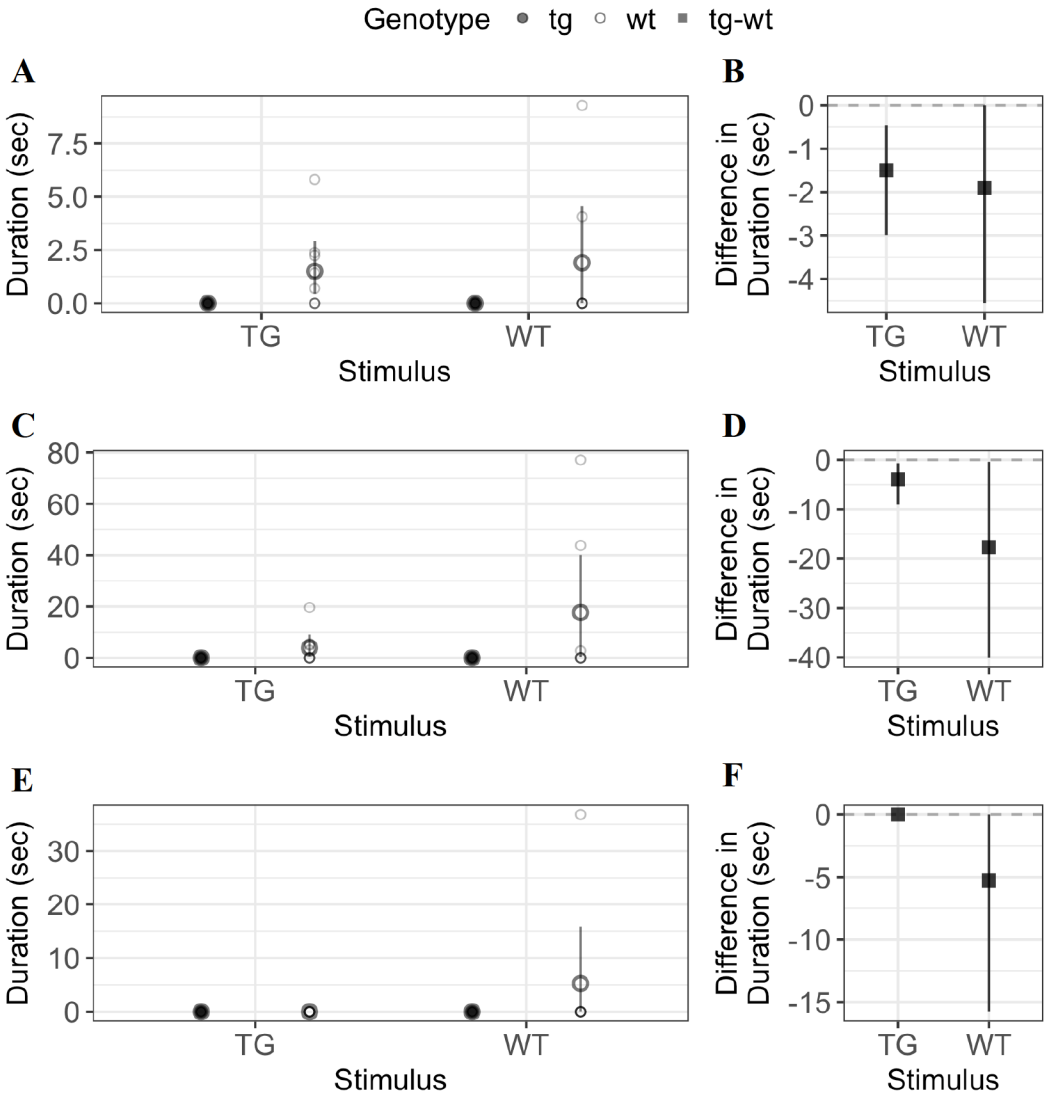

Figure 3.6. Raw data and 95% CIs for mean duration (in sec) of **A)** chase, **C)** attack, and **E)** subordinate behaviours for transgenic 5xFAD males (tg) and wild-type controls (wt) in response to transgenic (TG) and wild-type (WT) stimulus males. 95% CI for mean subject genotype effect (in sec) on **B)** chase, **D)** attack, and **F)** subordinate behaviours.

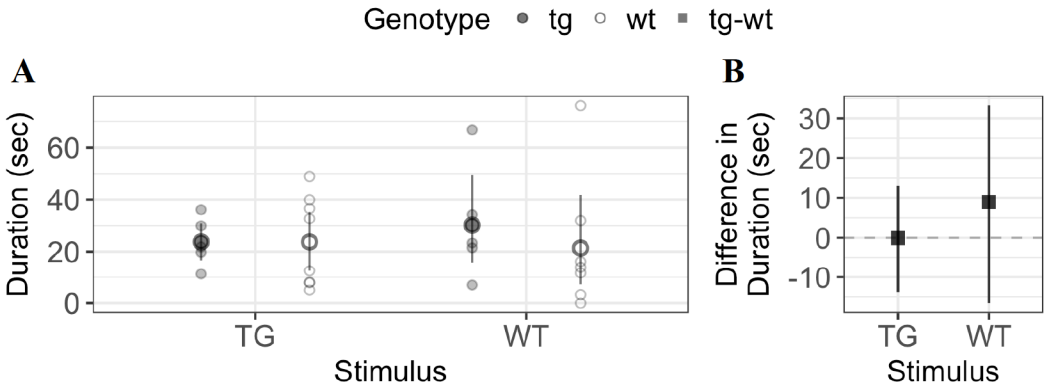

Figure 3.7. **A)** Raw data and 95% CIs for mean duration (in sec) of self-grooming behaviours for transgenic 5xFAD males (tg) and wild-type controls (wt) in response to transgenic (TG) and wild-type (WT) stimulus males. **B)** 95% CI for mean subject genotype effect (in sec) on self-grooming behaviours.

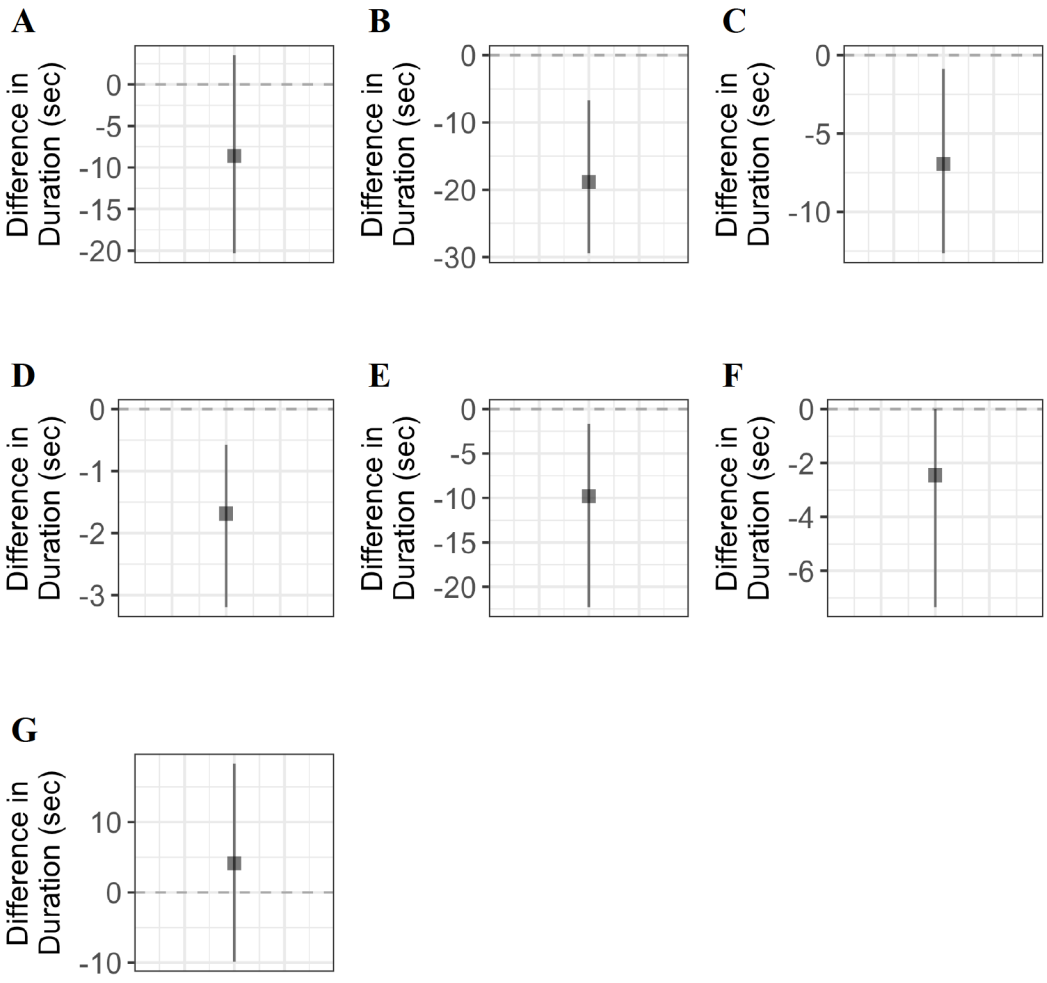

Figure 3.8. 95% CI for mean subject genotype effect (in sec) on overall **A)** orofacial sniffing, **B)** anogenital sniffing, **C)** follow, **D)** chase, **E)** attack, **F)** defensive posture, and **G)** self-grooming behaviours.

3.3.3 Home-Cage Observations

There were genotype by week by phase interactions for huddling (χ^2 (2) = 6.94, p = .031) and being alone (χ^2 (2) = 10.86, p = .004) behaviours; this is mostly driven by a difference between single-genotype transgenic cages and single-genotype wild-type cages from 18 to 22 weeks of age, with transgenic males exhibiting more instances of being alone and fewer instances of huddling than wild-type controls. There were also genotype by phase interactions for aggressive behaviours (including fight, chase, and mount; χ^2 (2) = 8.07, p = .018) and total number of aggressive encounters (all ranked behaviours listed in Section 3.2.2.5.3; χ^2 (2) = 7.70, p = .021), with transgenic males exhibiting more aggressive behaviours during the light phase, but fewer during the dark phase. Lastly, there was also a genotype effect for aggressive behaviours (χ^2 (2) = 10.71, p = .005) and total number of aggressive encounters (χ^2 (2) = 10.93, p = .004), with transgenic males exhibiting fewer aggressive behaviours overall compared to wild-type males, and wild-type males exhibiting more aggressive behaviour and overall aggressive encounters than mixed-genotype cages. See Tables 3.5 to 3.11 and Figures 3.9 – 3.12.

By 23 weeks of age, only 2 of 6 (95% CI [0, 66.67], median 33.33% of cages) of transgenic single-genotype cages were still group-housed, with 4 of 6 being separated due to aggression, while 3 of 3 (100%) of mixed-genotype cages were still group-housed. For wild-type single-genotype cages, 4 of 6 (67%) remained group-housed at 23 weeks of age; however, one was removed from testing due to stereotypic behaviour at 16 weeks of age, while the second was separated due to aggression at 18 weeks of age. The adjusted wild-type survival due to aggression only was 4 of 5 (95% CI [40.00, 100.00], median 80.00% of cages). See Table 3.12 for percentage of cages remaining at each age.

Table 3.5

Analysis of Deviance for Instances of Aggressive Behaviours during Home-Cage Observations

Effect	χ^2	df	*p*
		Fight	
Genotype	6.25	5	.283
Week	7.00	3	.072
Phase	16.00	5	**.007**
Genotype:Week	1.84	3	.605
Genotype:Phase	4.73	3	.193
Week:Phase	1.77	2	.413
Genotype:Week:Phase	0.45	2	.799
		Chase	
Genotype	0.69	2	.710
Week	3.28	1	.070
Phase	46.54	1	**.000**
Genotype:Week	1.28	2	.527
Genotype:Phase	1.99	2	.369
Week:Phase	1.19	1	.276
Genotype:Week:Phase	0.76	2	.685
		Mount	
Genotype	1.18	2	.553
Week	7.57	1	**.006**
Phase	18.95	1	**.000**
Genotype:Week	0.06	2	.970
Genotype:Phase	2.32	2	.314
Week:Phase	0.02	1	.897
Genotype:Week:Phase	0.58	2	.749

Note. p-values less than .05 are shown in **bold**.

Table 3.6

Analysis of Deviance for Instances of Defensive Behaviours during Home-Cage Observations

Effect	χ^2	df	*p*
		Defensive Posture	
Genotype	5.06	5	.408
Week	1.11	3	.774
Phase	19.84	5	**.001**
Genotype:Week	1.90	3	.594
Genotype:Phase	3.90	3	.273
Week:Phase	0.87	2	.648
Genotype:Week:Phase	0.86	2	.649
		Induced Flee	
Genotype	0.84	2	.656
Week	5.24	1	**.022**
Phase	3.51	1	.061
Genotype:Week	1.51	2	.471
Genotype:Phase	0.73	2	.693
Week:Phase	0.64	1	.425
Genotype:Week:Phase	0.23	2	.892

Note. p-values less than .05 shown in **bold**.

Table 3.7

Analysis of Deviance for Instances of Aggressive Grooming, Allogrooming, Huddling, and Being Alone Behaviours during Home-Cage Observations

Effect	χ^2	df	*p*
		Aggressive Grooming	
Genotype	1.15	2	.562
Week	2.36	1	.125
Phase	18.88	1	**.000**
Genotype:Week	0.26	2	.878
Genotype:Phase	0.31	2	.856
Week:Phase	1.03	1	.311
Genotype:Week:Phase	0.80	2	.672
		Allogrooming	
Genotype	3.73	2	.155
Week	9.53	1	**.002**
Phase	18.81	1	**.000**
Genotype:Week	2.18	2	.337
Genotype:Phase	2.39	2	.303
Week:Phase	0.16	1	.689
Genotype:Week:Phase	0.37	2	.833
		Huddle	
Genotype	3.58	2	.167
Week	24.51	1	**.000**
Phase	41.73	1	**.000**
Genotype:Week	33.61	2	**.000**
Genotype:Phase	5.43	2	.066
Week:Phase	1.40	1	.236
Genotype:Week:Phase	6.94	2	**.031**
		Being Alone	
Genotype	0.13	2	.938
Week	34.67	1	**.000**
Phase	42.16	1	**.000**
Genotype:Week	34.53	2	**.000**
Genotype:Phase	4.28	2	.118
Week:Phase	41.28	1	**.000**
Genotype:Week:Phase	10.86	2	**.004**

Note. p-values less than .05 are shown in **bold**.

Table 3.8

Analysis of Deviance for Instances of Total Aggressive Behaviours, Total Defensive Behaviours, and Overall Aggressive Encounters during Home-Cage Observations

Effect	χ^2	df	*p*
		Aggressive Behaviours, Total	
Genotype	10.71	2	**.005**
Week	1.38	1	.240
Phase	86.89	1	**.000**
Genotype:Week	1.33	2	.515
Genotype:Phase	8.07	2	**.018**
Week:Phase	4.91	1	**.027**
Genotype:Week:Phase	1.92	2	.384
		Defensive Behaviours, Total	
Genotype	2.80	2	.246
Week	1.52	1	.218
Phase	21.69	1	**.000**
Genotype:Week	0.38	2	.826
Genotype:Phase	3.10	2	.212
Week:Phase	0.00	1	.985
Genotype:Week:Phase	2.25	2	.325
		Aggressive Encounters, Total	
Genotype	10.93	2	**.004**
Week	0.13	1	.715
Phase	88.85	1	**.000**
Genotype:Week	1.05	2	.591
Genotype:Phase	7.70	2	**.021**
Week:Phase	3.04	1	.081
Genotype:Week:Phase	1.89	2	.389

Note. p-values less than .05 are shown in **bold**.

Table 3.9

95% CIs of Significant Genotype Effects for Overall Number of Instances of Behaviours during Home-Cage Observations

Cage Comparison		
tg-wt	tg-mx	wt-mx
Aggressive Behaviours, Total		
[-26.83, -0.17]	[-12.67, 1.83]	[-4.83, 20.83]
Aggressive Encounters, Total		
[-26.00, 2.17]	[-14.17, 7.50]	[-4.50, 21.33]

Note. tg-wt = transgenic single-genotype relative to wild-type single-genotype; tg-mx = transgenic single-genotype relative to mixed-genotype; wt-mx = wild-type single genotype relative to mixed genotype

Table 3.10

95% CIs of Significant Genotype by Phase Interactions for Overall Number of Behaviours during Home-Cage Observations

	Cage Comparison		
Light Phase	tg-wt	tg-mx	wt-mx
	Aggressive Behaviours, Total		
Dark	[-26.00, -3.17]	[-10.00, 0.17]	[-1.17, 20.33]
Light	[-1.83, 3.83]	[-3.33, 2.17]	[-3.83, 0.67]
	Aggressive Encounters, Total		
Dark	[-25.67, -3.00]	[-13.00, 4.50]	[-0.83, 21.33]
Light	[-1.33, 6.33]	[-2.83, 4.50]	[-4.5, 1.00]

Note. tg-wt = transgenic single-genotype relative to wild-type single-genotype; tg-mx = transgenic single-genotype relative to mixed-genotype; wt-mx = wild-type single genotype relative to mixed genotype

Table 3.11

95% CIs for Significant Genotype by Week Interactions for Overall Number of Behaviours during Home-Cage Observations

	Cage Comparison		
Week	tg-wt	tg-mx	wt-mx
		Huddle	
8	[-23.83, -2.00]	[-19.33, 2.50]	[-3.67, 12.00]
10	[-5.70, 23.80]	[-18.73, 8.60]	[-27.33, -1.50]
12	[-48.10, 8.73]	[-21.87, 29.00]	[7.33, 38.67]
14	[-15.92, 21.33]	[-29.25, 8.50]	[-20.33, -5.00]
16	[-25.40, 20.20]	[-14.00, 28.58]	[-0.13, 19.53]
18	[-55.50, -8.25]	[-56.83, -13.67]	[-12.83, 4.00]
20	[-48.75, -15.83]	[-10.33, 26.33]	[18.33, 67.42]
22	[-76.83, -24.50]	[-72.67, 3.33]	[-15.75, 46.83]
		Being Alone	
8	[1.67, 13.50]	[3.17, 11.17]	[-6.33, 5.50]
10	[-16.90, 10.10]	[-8.67, 12.67]	[-2.83, 13.50]
12	[-4.87, 43.47]	[-21.53, 21.33]	[-32.00, -6.00]
14	[-11.67, 23.00]	[-6.67, 30.00]	[-4.67, 13.67]
16	[-24.8, 7.00]	[-36.00, 9.00]	[-30.40, 21.00]
18	[7.75, 54.00]	[6.50, 53.17]	[-4.17, 1.92]
20	[-4.50, 44.50]	[-26.67, 42.67]	[-49.75, 4.00]
22	[16.33, 59.75]	[-23.67, 51.00]	[-59.75, 11.25]

Note. tg-wt = transgenic single-genotype relative to wild-type single-genotype; tg-mx = transgenic single-genotype relative to mixed-genotype; wt-mx = wild-type single genotype relative to mixed genotype

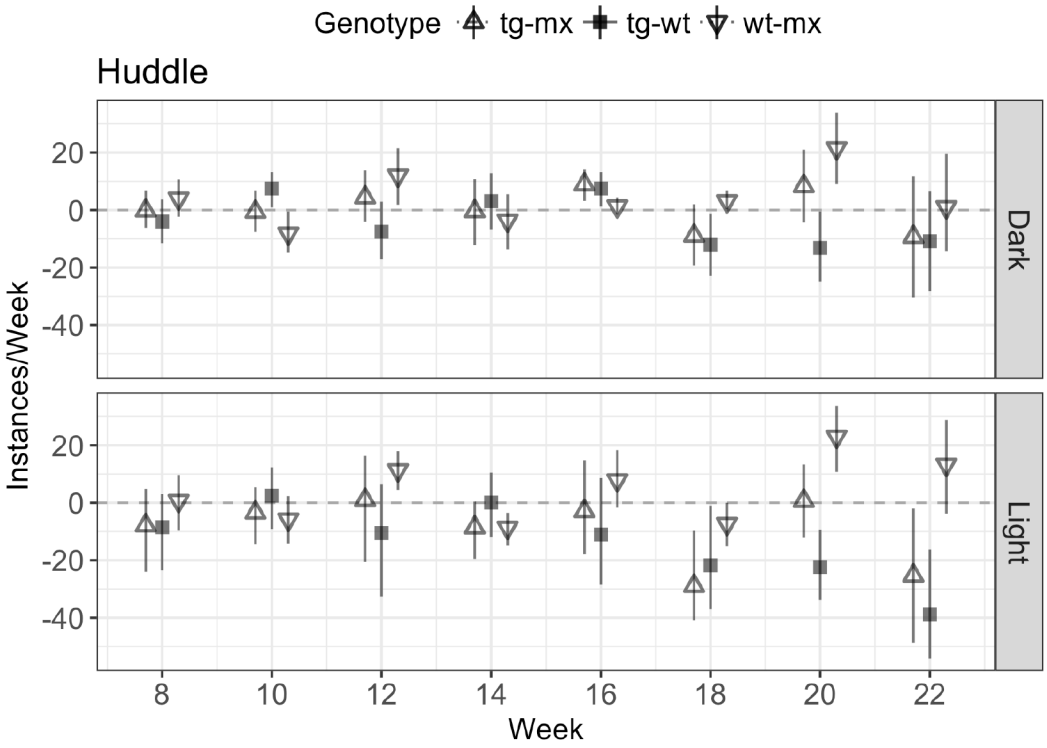

Figure 3.9. 95% CIs for cage effects for overall instances of huddling behaviour by week and phase during home-cage observations. tg-mx = transgenic single-genotype relative to mixed-genotype; tg-wt = transgenic single-genotype relative to wild-type single-genotype; wt-mx = wild-type single-genotype relative to mixed-genotype.

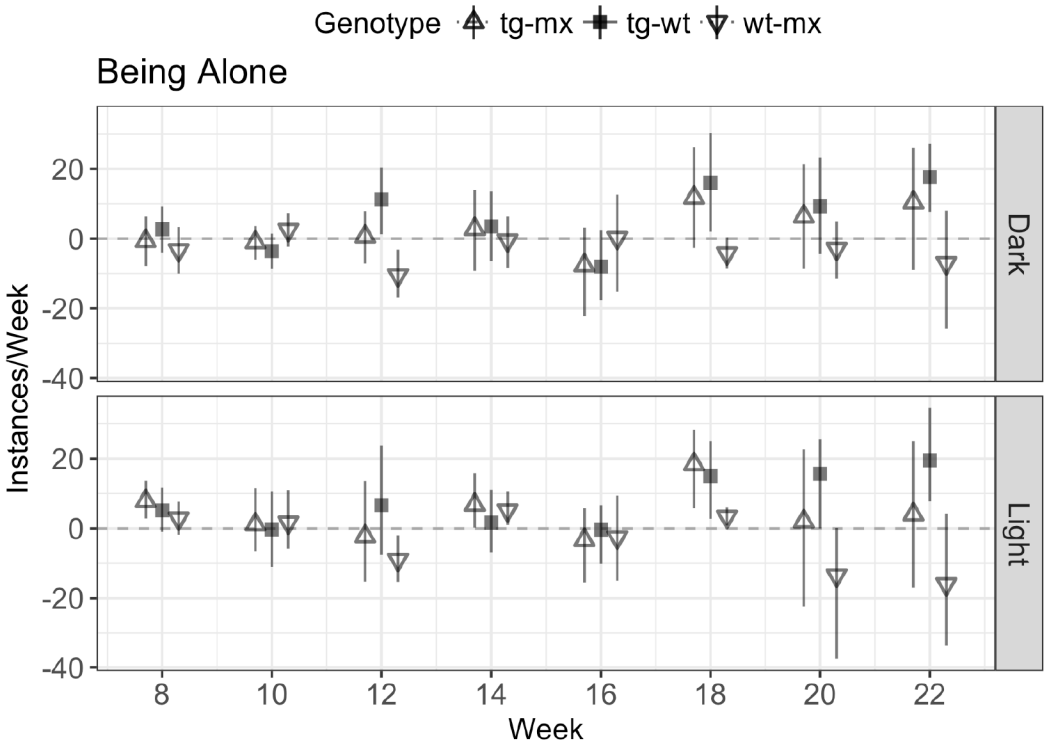

Figure 3.10. 95% CIs for cage effects for overall instances of being alone by week and phase during home-cage observations. tg-mx = transgenic single-genotype relative to mixed-genotype; tg-wt = transgenic single-genotype relative to wild-type single-genotype; wt-mx = wild-type single-genotype relative to mixed-genotype.

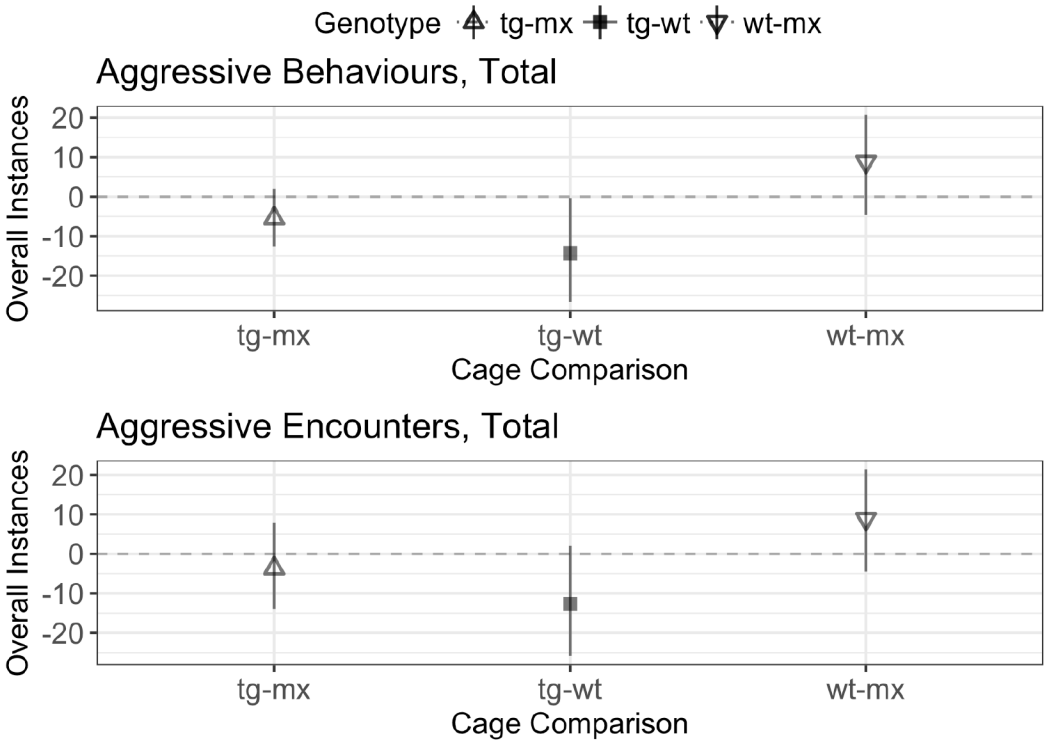

Figure 3.11. 95% CIs for cage effects for overall instances of aggressive behaviours (fight, chase, and mount) and aggressive encounters (fight, chase, mount, subordinate posture, and induced flee) during home-cage observations. tg-mx = transgenic single-genotype relative to mixed-genotype; tg-wt = transgenic single-genotype relative to wild-type single-genotype; wt-mx = wild-type single-genotype relative to mixed-genotype.

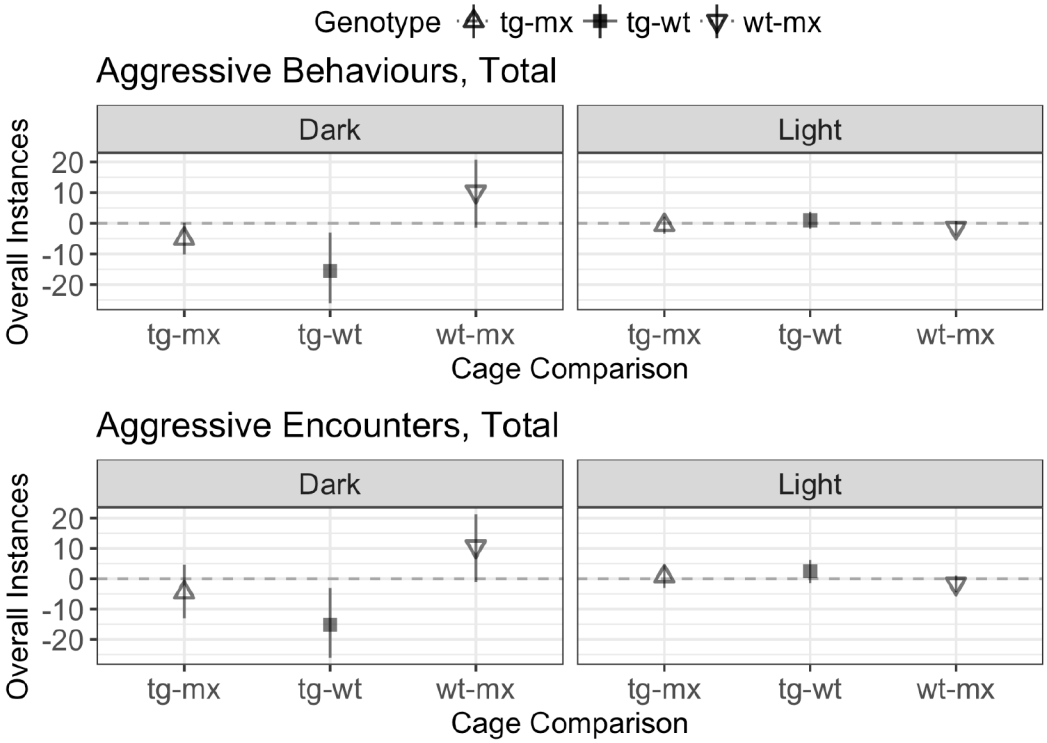

Figure 3.12. 95% CIs for cage effects for overall instances of aggressive behaviours (fight, chase, and mount) and aggressive encounters (fight, chase, mount, subordinate posture, and induced flee) by phase during home-cage observations. tg-mx = transgenic single-genotype relative to mixed-genotype; tg-wt = transgenic single-genotype relative to wild-type single-genotype; wt-mx = wild-type single-genotype relative to mixed-genotype.

Table 3.12

Percentage of Remaining Cages by Week during Home-Cage Observations

	Cage Genotype			
Age (Weeks)	tg	wt	wt-adj	mx
8	100	100	100	100
9	100	100	100	100
10	83.33	100	100	100
11	83.33	100	100	100
12	83.33	100	100	100
13	83.33	100	100	100
14	66.67	100	100	100
15	66.67	100	100	100
16	66.67	83.33	100	100
17	66.67	83.33	100	100
18	66.67	66.67	80	100
19	66.67	66.67	80	100
20	50	66.67	80	100
21	50	66.67	80	100
22	50	66.67	80	100
23	33.33	66.67	80	100

Note. Cages given as percentage from the start of recordings. tg = transgenic single-genotype; wt = wild-type single-genotype; wt-adj = wild-type single-genotype adjusted for one cage separation due to stereotypy; mx = mixed-genotype.

3.3.4 Tube Test of Dominance

There was no difference in dominance between transgenic 5xFAD males and wild-type controls, with transgenic males winning 2 of 5 pairings and wild-type controls winning 3 of 5 pairings (95% CIs [-0.80, 0.40] win proportion for transgenic males compared to wild-type controls). Although a cage of three mice (n = 1 transgenic, 2 wild-type) was also used to increase sample sizes, the results of this were counted as two separate pairings, with the transgenic male exhibiting one win and one loss against the wild-type cage-mates.

3.4 Discussion

3.4.1 Transgenic 5xFAD Males Exhibit Reduced Social Interest

Overall, transgenic 5xFAD males exhibited a preference for social over non-social interactions in the social approach task (Figure 3.3). This is similar to the overall social preference seen in other AD transgenic mouse models (e.g., 5 – 6 month old $APP^{Lond/Swe+}$, and 5 – 6 and 12 – 13 month old $PLB1_{Triple}$ males and females [Faizi et al, 2012; Platt et al, 2011]). However, findings presented here show that transgenic 5xFAD males exhibit a lower preference ratio than wild-type controls for social vs non-social interaction, as well as reduced overall investigation of the social stimulus (Figures 3.3 and 3.4). This indicates that transgenic 5xFAD males are less likely than wild-type controls to approach a novel conspecific, suggesting a reduced social interest, although these results differ from previous observations in our lab, which indicated a higher preference for social over non-social interaction (MacGowan et al, 2016). The reasons for this difference are unclear. While the previous study used a novel object in the non-social chamber (MacGowan et al, 2016), and the present study did not, prior studies did not report

neophobia in transgenic 5xFAD mice (Braun & Feinstein, 2017) making it unlikely that this difference is due to avoidance of the novel object in the previous study. The previous study also used only wild-type male conspecifics (MacGowan et al, 2016), whereas the present study used both wild-type and transgenic stimulus males to assess approach behaviour in response to both genotypes. It is possible that transgenic 5xFAD males exhibit different approach behaviours in response to wild-type vs transgenic stimulus mice, a hypothesis supported by the trend observed in the current experiment. The variance in the results of the present study is too high to make a firm conclusion at this point. Lastly, prior experience of the mice may be influencing results in this task. The previous study did not use naïve males while the mice in the current study had not been tested in any other behavioural task. Some (n = 4) had, however, been housed in cages that were larger than standard cages. Work is ongoing to clarify these findings.

The decreased, but not eliminated, social interest observed in transgenic 5xFAD males differs from findings described in other transgenic AD mouse models. Reduced preference ratios have similarly been indicated in 6-month-old APP females and APP/PS1 males and females (Filali et al, 2011a; Pietropaolo, Delage, Lebreton, Crusio, & Cho, 2012), however these mice exhibited no preference for social vs non-social interactions. Moreover, while APP and APP/PS1 females exhibit significant reductions in social interaction time (Pietropaolo et al, 2012), APP/PS1 males present similar social interaction durations but increased non-social exploration durations compared to wild-type controls (Filali et al, 2011a). This suggests that differences in overall exploration time are responsible for altered social preference in the APP and APP/PS1 strains. By comparison, Faizi *et al* (2012) reported that transgenic $APP^{Lond/Swe+}$ males do not exhibit

any genotype effects in interaction time or preference ratio for sociability. Overall, the reduced—but not eliminated—preference ratio and reduced social interaction time seen in transgenic 5xFAD males suggests that these results are due to decreased interest in social interaction.

In addition to the results of the social approach task, transgenic 5xFAD males also exhibited decreased investigative behaviours on the free social interaction task compared to wild-type controls, with reductions in orofacial sniffing, anogenital sniffing, and following behaviours (Figures 3.5 to 3.8). Flanigan *et al* (2014) had previously examined social investigation in the 5xFAD strain in a novel environment with a novel stimulus, however they report a ratio of interaction times (to assess social recognition and discrimination) rather than the interaction durations, so it is not possible to identify whether transgenic 5xFAD mice exhibited more or less social interaction with the novel conspecifics compared to wild-type controls. Previous evidence also indicates that the amount of investigative behaviour exhibited during a free social interaction task is dependent on sex, age, and strain. Transgenic Tg2576 females at 21 months of age, as well as 18-month-old transgenic 3xTg-AD males and 12-month-old transgenic 3xTg-AD females all exhibited an increase in social behaviours in response to a novel conspecific in a novel environment (Bories et al, 2012; Deacon et al, 2009). However, by 18 months of age, transgenic 3xTg-AD females exhibited a decrease in social behaviours compared to wild-type controls (Bories et al, 2012), suggesting that disease progression may result in an initial increase and a subsequent decrease in social interaction.

The differing presentation of these behaviours may be the result of differing timelines for disease progression in these models and varying presentation across time-points.

Levels of Aβ plaque load have not been directly compared between the 5xFAD and 3xTg-AD strains, however transgenic 3xTg-AD females exhibit an increase in both $A\beta_{40}$ and $A\beta_{42}$ at approximately 15 months of age (Hirata-Fukae et al, 2008) and exhibit plaques throughout the brain by 18 months of age (Mastrangelo & Bowers, 2008). By comparison, the 5xFAD strain exhibits plaques throughout the brain by 6 months of age (Oakley et al, 2006). Additionally, despite the advanced age of Tg2576 mice used in the study by Deacon *et al* (2009), 2-month-old transgenic 5xFAD mice exhibit higher levels of $A\beta_{42}$ in whole brain homogenates than 16-month-old transgenic Tg2576 mice (Oakley et al, 2006), indicating a much more rapid neuropathology in the 5xFAD strain. Overall, this suggests that high levels of sociability are indicative of early stages of AD in mouse models, whereas reduced social interaction represents later stages. This reduced social interaction in later disease stages is supported by human AD studies which indicate that apathy (and associated social withdrawal) is highly correlated with disease progression, with a higher prevalence at later stages (Mizrahi & Starkstein, 2007). However, it is not clear whether increased sociability is observed at any stage in human AD. Disinhibition may cause increased approach to unfamiliar individuals (Cummings et al, 1994), and has been shown to decrease in patients with time (Craig et al, 2005), suggesting that patients in early stages of AD may be more willing to engage in social interactions. However, disinhibition is also associated with inappropriate behaviours and comments (Cummings et al, 1994), and many studies only report the prevalence of the symptoms rather than the behaviours leading to the diagnosis of those symptoms. Additionally, evidence suggests that apathy and social withdrawal can be present even at very early stages (Jost & Grossberg, 1996). As a result, although higher levels of disinhibition may be present

during early stages of the disease, an overall increase in sociability does not appear to be characteristic of any stage of AD.

Transgenic 5xFAD males exhibited reduced social investigation in response to novel stimuli in a novel environment, and also exhibited reduced home-cage affiliative behaviour between 18 – 22 weeks of age (Figures 3.9 and 3.10). In the home-cage, this appeared as a reduction in huddling behaviour and an increase in being alone in transgenic vs wild-type single-genotype cages. My findings are supported by previous studies demonstrating that 5xFAD transgenic mice display impaired nest building, a measure of affiliative behaviour, at 9 and 12 months in males and at 12 months in females (Devi & Ohno, 2015; Schneider et al, 2014; Wesson & Wilson, 2011). In contrast to these findings, Flanigan *et al* (2014) have reported increased social interaction in transgenic 5xFAD mice around 9 months of age, although this is difficult to interpret; in their calculations of social behaviours they include mounting and tail pulling, which would be considered aggressive behaviours in the present study. Additionally, sex differences were not examined in their study, and it is possible that behaviours such as allogrooming or sniffing were more prevalent in females rather than males. Finally, a selection bias may be present in the final results. Just like in our lab, Flanigan *et al* observed high levels of home-cage aggression in transgenic males, and often had to reduce cage numbers (M.P. McDonald, personal communication, June 27, 2017); as isolated mice were not used for behavioural testing in their study, it is likely that the transgenic males that remained group-housed to 9 months of age were much less aggressive than their former cage-mates.

Though there were no subject by stimulus genotype interactions for social approach and free social interaction, it is interesting to note that these changes in behaviour seem largely driven by interactions between transgenic males. While it is likely that both cage-mates contributed to the reduced affiliative behaviour seen during home-cage interactions, anogenital sniffing, following, and social approach are all behaviours that require active approach by the subject. This suggests that transgenic males can differentiate between genotypes based on one or more phenotypic cues—whether visual, auditory, olfactory, or some other modality or combination thereof—and that this determination then influences their willingness to interact with that conspecific.

3.4.2 Transgenic 5xFAD Males are Comparably Dominant to Wild-Type Controls

Transgenic 5xFAD males in the present study were found to have a similar level of overall home-cage dominance as wild-type controls. This was found in a relatively small sample size but is comparable to results previously indicated by Flanigan *et al* (2014). This procedure was performed because differences in overall dominance may mediate levels of aggression. Although dominance and aggression are not the same behaviour (Lindzey, Manosevitz, & Winston, 1966), they are inherently linked because aggressive encounters are used in establishing dominance hierarchies (Liebenauer & Slotnick, 1996; Williamson et al, 2016), and destabilized dominance hierarchies have been associated with increased aggression in previously stable groups following introduction of a novel environmental enrichment (Howerton et al, 2008). However, results from the current study indicate that transgenic males are not more dominant overall than wild-type

controls, and that home-cage aggression is not likely to be due to increased contention for dominance.

3.4.3 Transgenic 5xFAD Males Exhibit Altered Timing of Aggression, but do not Initiate an Increased Number of Aggressive Encounters Compared to Wild-Type Controls

Although transgenic 5xFAD males were hypothesized to exhibit an increased number of aggressive encounters in both the home-cage and novel (free social interaction) environments, this was not the case. Transgenic males exhibited no aggressive behaviours in the free social interaction task (Figure 3.6) and fewer aggressive behaviours (fighting, chasing, and mounting) during home-cage observations compared to wild-type controls (Figure 3.11). However, despite initiating fewer aggressive behaviours overall, transgenic 5xFAD males exhibited more aggressive behaviours than wild-type controls during the light phase and fewer during the dark phase (Figure 3.12). Previous evidence indicates that aggression in mice is subject to a circadian rhythm (Todd et al, 2018), and studies have shown that circadian rhythm is delayed in human AD patients and APP/PS1 mice (Duncan et al, 2012; Satlin, Volicer, Stopa, & Harper, 1995). Other studies have also indicated the presence of *sundowning* (increased activity relative to wild-type controls near the end of the waking phase) in the APP and 3xTg-AD strains (Bedrosian, Herring, Weil, & Nelson, 2011; Sterniczuk, Dyck, LaFerla, & Antle, 2010b), although no changes in circadian rhythm were observed in the Tg2576 strain (Gorman & Yellon, 2010), suggesting that this change is strain-dependent. As a result, this suggests that the increased aggressive behaviours exhibited during the light phase may be the result of delayed circadian rhythms, although it is possible that some other factor (such as

sudden environmental changes due to lights being turned on) may also be responsible for the altered pattern of aggression in the transgenic 5xFAD mice. Further experiments are needed to identify whether this change in aggression presentation is due to altered sleep/wake cycles, or whether an acute change in environment is sufficient to induce this behaviour.

Despite this altered temporal pattern of aggression, transgenic mice exhibit fewer aggressive home-cage encounters overall, although are about three times more likely than wild-type single-genotype cages to be separated due to injuries arising from fighting (Table 3.12). While this overall reduction in aggressive behaviours could be indicative of a selection bias, the lack of a genotype by week interaction for home-cage observations indicates that transgenic 5xFAD males did not exhibit more aggressive behaviours than wild-type controls at any age, including weeks immediately prior to separation. Additionally, of 11 mixed-genotype cages originally intended for Cohort 1, only 6 cages were able to complete all four testing sessions over the 11-day period, suggesting that the presence of one transgenic male in a cage can affect aggressive encounters between cage-mates. Of those separated cages, two pair-housed cages and a single male from a cage of three required separation prior to testing, and an additional three cages were separated following the second session of social approach (prior to free social interaction). All of the separations in Cohort 1 and 2 involving transgenic males were the result of injuries to one animal, suggesting that quality of the aggressive encounters themselves, rather than the overall number, differ between transgenic and wild-type 5xFAD mice.

Contrary to the results of the present study, increased frequency of aggression has been reported in males from several transgenic AD models, including Tg2576, APP23,

APP-PS1, and other APP-overexpressing models (Alexander et al, 2011; Deacon et al, 2009; Jager et al, 2018; Moechars et al, 1998; Pugh, Richardson, Bate, Upton, & Sunter, 2007; Vloeberghs et al, 2006). However, with the exception of Deacon *et al* (2009)—who simply indicate that transgenic Tg2576 males at 21 months of age were too aggressive to test in social tasks—observations of aggression were made in the home-cage environment, whether during the resident-intruder paradigm or under normal home-cage conditions prior to testing. By comparison, no increase in aggression was observed in 12- or 18-month-old transgenic 3xTg-AD males or females in a novel environment (Bories et al, 2012). This suggests that the aggression may be offensive in nature, aimed at getting the intruder (or perceived intruder, in the case of home-cage aggression reported by Moechars *et al*) away from the territory belonging to the subject, rather than attempting to injure them (Brain & Parmigiani, 1990); as a result, these attacks are often aimed at the back and sides of a conspecific, rather than sensitive areas such as the face or abdomen.

This pattern of offensive attacks matches that exhibited by 5xFAD males in the present study. While aggression—among other factors—is used in determining dominance in groups of mice, excessive aggression in mice is maladaptive due to the potential for injury and altered physiological function, and the establishment of a dominance hierarchy that mediates access to resources is critical to the success of the group (Howerton et al, 2008; Williamson et al, 2016). Once these hierarchies are established, aggression in the group tends to be reduced. In general, subordinate males exhibit less aggressive behaviour overall, and dominant males will typically not attack familiar males without provocation (Liebenauer & Slotnick, 1996; Nakamura, Kikusui, Takeuchi, & Mori, 2007).

Overall, the aggressive nature of the attacks mounted by 5xFAD males that lead to injury indicates that 5xFAD males are attempting to defend their territory and/or assert their dominance against a threat. Although the underlying mechanism is unclear, it is most likely associated with an accumulation of Aβ between 3 – 6 months of age. Transgenic 5xFAD males exhibit about a 5-fold increase in $A\beta_{42}$ in whole-brain homogenates between 3 – 6 months, and the same period was associated with a significant increase in plaque load in the cortex, hippocampus, and spinal cord of transgenic 5xFAD females (Jawhar et al, 2012; Oakley et al, 2006), although variations in the rate of Aβ accumulation could account for differences in timeframe from mouse to mouse. This may lead to three possible explanations for these behaviours: *1)* transgenic males are unable to remember their cage-mates, thus taking them to be an unfamiliar intruder; *2)* transgenic males are unable to modulate bite force during dominance contests; or *3)* transgenic males are unable to correctly process subordinate responses exhibited by the target.

With respect to the first case—inability to remember cage-mates—Flanigan *et al* (2014) indicated impaired social recognition memory at 9 months of age, although other studies indicated impaired working/short-term and spatial memory as early as 4 months of age (Devi & Ohno, 2010; O'Leary, 2013; Oakley et al, 2006; Ohno et al, 2006; Ohno et al, 2007; Xiao et al, 2015). This strain exhibits early loss of noradrenergic and cholinergic neurons, loss of which has been linked with memory deficits (Bilkei-Gorzo, 2014; Easton et al, 2002; Sara, 2015). Although cage-mates would likely be able to identify each other over the course of the day, it is possible that they are unable to remember each other following a period of sleep. This may lead to a situation where the

aggressor is unable to recognize a cage-mate, thereby mistaking him for an intruder and leading to a prolonged attack.

The second scenario—altered bite force during dominance contests—could likely be caused by deficits in motor control and coordination. Grant and Mackintosh (1963) indicate that biting is typically a social event and is not intended to break the skin. However, transgenic 5xFAD females exhibit an increase in Aβ accumulation in the spinal cord and Layer 5 of the cortex (Jawhar et al, 2012), suggesting possible alterations in voluntary motor control. Additionally, though abnormal clasping behaviour was characterized at 9+ months of age (Jawhar et al, 2012; O'Leary, 2013), observations with pups in our lab have indicated that this clasping behaviour may be present even within the first 3 weeks of life. While this behaviour is likely controlled by a different circuit from biting, it does suggest the presence of altered motor control even in early age.

The final scenario—an inability to correctly process subordinate postures—may prevent the aggressor from ceasing an attack once submissive behaviour is exhibited. The reduced affiliative behaviour seen in this study, as well as transgenic males at 9 and 12 months and transgenic females at 12 months (Devi & Ohno, 2015; Schneider et al, 2014), indicates a difficulty for transgenic males to exhibit cues designed to promote relationships with conspecifics. By extension, transgenic males may be unable to identify the meaning of a subordinate behaviour, ultimately leading to prolonged aggressive bouts that lead to injury.

3.4.4 Transgenic 5xFAD Males Exhibit Impaired Social Interactions

Transgenic males exhibited reduced investigative and affiliative behaviours compared to wild-type controls. They did not exhibit altered dominance or an increased number of

aggressive bouts but the timing of aggressive bouts was altered relative to wild-type controls (Figure 3.12, Table 3.10). While transgenic males did not actively avoid a novel conspecific, they did exhibit decreased interest in investigative behaviour characterized by an overall decrease in social exploration. This suggests that transgenic males exhibit a decrease in social interest, but do not show an aversion to social stimuli. However, aggressive interactions that included transgenic males resulted in an increased number of injuries compared to wild-type controls, necessitating more frequent separation of cage-mates (Table 3.12). While the mechanisms are unclear, this is most likely due to altered behaviours during the aggressive encounter itself; this may involve an inability to recognize familiar conspecifics due to memory impairments, inability to modulate bite force due to motor deficits, or an inability to correctly process subordinate behaviour exhibited by the target. These findings have important implications for continued research with mouse models of AD and AD patients, as subsequent experiments into the neurological changes underlying these behaviours could point to possible causes of altered social behaviours in AD patients. Additionally, the altered investigative, affiliative, and aggressive behaviours exhibited between transgenic males may be extended to housing of AD patients in long-term care facilities, suggesting that the use of isolated wards for AD patients could cause increased social deficits.

3.4.5 Current and Future Directions

While these findings indicate that transgenic 5xFAD males exhibit decreased social investigation in response to novel conspecifics, as well as decreased affiliative behaviours and altered aggressive encounters with familiar conspecifics, additional work is currently being performed to further investigate these changes. Additional subjects have been

added to home-cage observations in order to identify whether mixed-genotype housing results in reduced separation due to aggression, or whether the presence of at least one transgenic male is sufficient to result in increased injury due to aggression. Additionally, while results of the social approach and free social interaction tasks used mice that were previously tested in the tube test of dominance and home-cage observations, data collection is ongoing with experimentally naive mice to increase sample sizes. Female 5xFAD mice are also being tested on the same social approach and free social interaction paradigm to examine sex differences in the strain.

These results also raise a number of questions, with a major one being the cause of injury arising from aggressive encounters in transgenic male single-genotype cages. While analyses of bite force would likely require specialized equipment, it is possible to test whether transgenic mice have difficulty in remembering their cage-mate, and whether they have difficulty in identifying subordinate postures. Deficits in cage-mate identification could be determined by examining investigation of a cage-mate compared to a novel conspecific of the same genotype. This could be achieved by examining investigation during a free social interaction task (novel vs familiar stimulus in a novel environment), or by using olfactory cues (such as urine) from both the cage-mate and a novel conspecific. However, it is important to note the reduced investigative behaviour exhibited in the present study, as well as the reduced interest in social odour cues reported by MacGowan *et al* (2016), indicating that transgenic 5xFAD males may exhibit little investigation of either stimulus. Additionally, further review of home-cage videos for durations of aggressive encounters could indicate whether transgenic 5xFAD males are able to correctly identify subordinate behaviours and cease attacks accordingly.

Testing latency to first attack on a resident-intruder task, even in the home-cage following separation of cage-mates, may also indicate whether transgenic 5xFAD males also exhibit reduced aggression compared to wild-type controls in response to a novel conspecific in a familiar environment. Finally, it is unclear whether the altered timing of aggressive behaviours in transgenic 5xFAD males is the result of delayed circadian rhythm or responses to environmental changes. Examination of home-cage activity levels and sleep/wake cycles could indicate whether transgenic 5xFAD mice exhibit delays in circadian rhythm that have been observed in other AD models. Additionally, the use of a brief, rapid-onset environmental stimulus, such as a bright light or a loud noise, during otherwise peaceful periods could indicate whether aggressive behaviours seen during the light phase are a response to an environmental stimulus.

Chapter 4: Discussion

4.1 Does the 5xFAD Mouse Strain Exhibit Altered Social Interactions?

The findings of this study indicate that transgenic males and females in the 5xFAD strain exhibit social impairments predominantly related to investigation of social stimuli, although these manifest in different ways. Transgenic males exhibit an overall decrease in social approach and investigative behaviours (in response to novel same-sex conspecifics), as well as reduced affiliative behaviour, indicated by increased time spent alone and reduced huddling (in response to same-sex cage-mates), suggesting an overall reduced interest in social interaction. By comparison, transgenic 5xFAD females exhibit reduced interest in social odour cues and an age-related decrease in investigative behaviours exhibited during free social interaction, but no difference in social approach to a restricted social stimulus compared to wild-type controls, suggesting that these differences may arise from a heightened social anxiety during unrestricted and reciprocal interactions or altered sensitivity to physiological arousal. Further investigation with females is needed to determine whether the genotype (transgenic or wild-type) of the stimulus animal affects social approach and investigative behaviour similar to that which I observed in males, and whether decreased social investigation during free social interaction in transgenic females compared to wild-type controls is due to social anxiety, altered physiological arousal, or perhaps a combination of the two. Similarly, it is unclear whether transgenic males exhibit the same reduction in social approach behaviours in response to social odour cues, or whether this response is mediated by visual, tactile, or auditory senses during encounters with a live conspecific. As a result, transgenic 5xFAD males and females both exhibit altered social interactions, although these may arise by

different means, the details of which should be explored through additional experiments discussed below.

4.2 Are 5xFAD Males More Aggressive Than Wild-Type Controls?

Although observations in our lab and others have previously suggested increased aggression in transgenic 5xFAD males, the results from the present study suggest that there is no difference in the number of aggressive encounters initiated by transgenic males compared to wild-type males. Despite the similar frequency of aggressive encounters, transgenic males housed in single-genotype cages have to be separated more often due to injuries than wild-type controls. Overall, this suggests that transgenic males exhibit differences in the aggressive encounters themselves, either being unable to modulate bite force and breaking the skin more often due to increased pressure, or being unable to correctly identify and respond to subordinate behaviours resulting in longer durations of aggressive behaviours. Further exploration into the duration of aggressive encounters in transgenic males and wild-type controls could identify whether expression and identification of subordinate behaviour mediates these differential encounters in transgenic males. This could be assessed by using existing recordings of home-cage behaviours to examine the duration of each aggressive bout, with longer bouts suggesting that transgenic 5xFAD males are impaired in expressing or identifying subordinate behaviours.

4.3 Do the Results of the Present Study Align with Previous Research in the 5xFAD Strain?

Similar to previous research, transgenic 5xFAD males and females exhibited no deficits in olfactory perception or discrimination. However, novel findings presented here

show that females exhibited a reduced interest in social odours. Transgenic males and females exhibited an overall decrease in social investigation, rather than the increased investigation time indicated by Flanigan *et al* (2014) and MacGowan *et al* (2016). Transgenic 5xFAD males also exhibited reduced affiliative behaviours, particularly reduced huddling and increased time spent alone, compared to wild-type controls, and previous evidence indicates impaired nest building which may be indicative of reduced affiliative behaviours in the 5xFAD strain (Devi & Ohno, 2015; Schneider et al, 2014). Finally, although previous observations suggested that other strains of AD transgenic males were more likely to attack than wild-type controls (Alexander et al, 2011; Moechars et al, 1998; Vloeberghs et al, 2006), and previous observations have suggested increased aggression in transgenic 5xFAD males, this was not observed in the present study, with transgenic 5xFAD males initiating the same or fewer aggressive encounters compared to wild-type controls. However, my findings do suggest that 5xFAD males likely exhibit altered behaviour during an aggressive encounter, leading to increased injury of the target. The results of this study largely coincide with and extend previous evidence, although differences in social investigation may result from differences in age, sex, or testing paradigm.

4.4 Does the 5xFAD strain Exhibit Changes in Social Behaviours Similar to Those Seen in Other Animal Models of AD?

While transgenic 5xFAD males and females exhibit altered social interactions, primarily in social investigation and aggression, these are not necessarily consistent with existing mouse models of AD. In the present study, transgenic 5xFAD males and females exhibit aspects of reduced social investigation behaviours compared to wild-type controls

from approximately 6 months of age, similar to 6-month-old APP and APP/PS1 females and 18 month old 3xTg-AD females (Bories et al, 2012; Pietropaolo et al, 2012). However, evidence suggests that strain, age, and sex play a role in social behaviours, with results also often indicating similar or increased social interactions compared to wild-type controls (Bories et al, 2012; Deacon et al, 2009; Faizi et al, 2012; Filali et al, 2011a; Platt et al, 2011). Due to the accelerated rate of neuropathology in the 5xFAD strain (Oakley et al, 2006), this suggests that transgenic 5xFAD males and females may exhibit social deficits not seen until much later in other models.

With regard to aggression, transgenic 5xFAD males do not exhibit an overall increase in number of aggressive encounters compared to wild-type controls; this was evident during both home-cage observations as well as interactions with a novel conspecific in a novel environment. By comparison, previous evidence suggests that transgenic males from APP-overexpressing lines are more aggressive than wild-type controls (Alexander et al, 2011; Moechars et al, 1998; Vloeberghs et al, 2006). However, these studies examined aggression using a resident-intruder task, suggesting that the type of task plays a role in the level of aggression observed. Similarly, the environment also plays a role, as increased home-cage aggression was observed in transgenic APP-overexpressing mice (Moechars et al, 1998), whereas no differences in aggression were observed between transgenic 3xTg-AD mice and wild-type controls during a free social interaction task (Bories et al, 2012). Overall, this indicates that the task, as well as familiarity with the environment and the conspecific, play a role in mediating aggression, and it is possible that transgenic 5xFAD males may exhibit increased aggression in response to a novel

conspecific in a familiar environment (such as the resident-intruder task) without exhibiting increased frequency of aggression in other situations.

Overall, transgenic 5xFAD mice exhibit altered social behaviours that do not directly resemble other mouse models of AD. These differences may arise due to differing timelines of disease progression between strains, with the 5xFAD strain exhibiting a more rapid progression that may better reflect behaviours associated with later stages of the disease. Additionally, variations in behavioural paradigms may measure different aspects of the same behaviour, or may affect the behaviours themselves, thereby leading to variations in results between AD strains. Additional exploration is needed to examine whether other AD mouse models present a similar behavioural phenotype when run in parallel with the 5xFAD strain.

4.5 Does the 5xFAD Strain Provide a Viable Model for Studying Neurological Changes Underlying Social Symptoms in Human AD Patients?

Evidence suggests that transgenic 5xFAD mice exhibit altered social behaviours that model some of the NPS exhibited by AD patients. In addition to cognitive deficits, AD patients exhibit delusions, hallucinations, agitation/aggression, anxiety, elation/euphoria, apathy/indifference, disinhibition, irritability, and aberrant motor behaviour (Cummings, 1997; Cummings et al, 1994). Agitation/aggression, irritability, depression, anxiety, and apathy are the most common (Zhao et al, 2016), with agitation/aggression being more prevalent in men than women (Lovheim et al, 2009). Evidence from the present study and previous results suggest that transgenic 5xFAD mice exhibit apathy and social withdrawal, and aggression in males (Devi & Ohno, 2015; Flanigan et al, 2014;

MacGowan et al, 2016; Schneider et al, 2014). Although there is conflicting evidence for depressive-like behaviours in the 5xFAD strain (Patel et al, 2014; Yamazaki et al, 2015), evidence from other strains suggests that mouse models of AD exhibit depressive-like behaviours with age (Iascone et al, 2013; Romano et al, 2014), suggesting that transgenic 5xFAD mice may also exhibit depressive-like behaviours with disease progression. Additionally, transgenic 5xFAD females may exhibit increased social anxiety, although evidence suggests that transgenic 5xFAD mice do not exhibit an overall increase in general anxiety (Braun & Feinstein, 2017; Flanigan et al, 2014; O'Leary, 2013). The altered aggression observed in males is also not an overall increase in aggression, but a change in the aggressive encounters themselves. Overall, transgenic 5xFAD mice exhibit altered social behaviours that may model the apathy and social withdrawal, anxiety, and aggression in human AD patients, although additional exploration is required to examine the prevalence of depressive-like behaviours in 5xFAD mice, and whether social anxiety mediates social interactions. However, present evidence suggests that the 5xFAD strain provides a viable model for examining neurological changes underlying altered social behaviours in human AD patients, particularly social withdrawal and aggression.

4.6 Future Directions

The findings of impaired social interactions in the 5xFAD strain have raised a number of additional questions. While there are still a number of experiments that could be performed in the short term—predominantly exploring the causes of injury following aggression in transgenic males, the possible roles of arousal and social anxiety on social investigation in females, and whether transgenic 5xFAD females exhibit similar approach and investigative behaviour of transgenic conspecifics as observed in transgenic males—

the findings of this study provide some avenues for exploration of the neurological changes underlying altered social interactions in the 5xFAD strain. The age-related deficits in axon myelination observed in the mPFC and hippocampus (Gu et al, 2018), combined with the involvement of mPFC projections in social avoidance (Franklin et al, 2017), suggest that this region may be implicated in the social deficits observed in the present study. Additionally, although it is not clear whether inhibition of aggression is implicated in the injuries arising from aggressive encounters with transgenic 5xFAD males, the mPFC is involved in inhibiting intermale aggression (Takahashi, Nagayasu, Nishitani, Kaneko, & Koide, 2014), suggesting a possible cause for this change in aggression. Similarly, the higher levels of aggression observed during the light phase in transgenic 5xFAD males compared to wild-type controls in the present study, as well as the delayed circadian rhythm observed in human AD patients and the Tg2576 strain (Duncan et al, 2012; Satlin et al, 1995) and the involvement of the subparaventricular zone in regulating cycles of aggression (Todd et al, 2018) may point to a possible circuit that is driving the altered timing of aggression. In each case, electrophysiological recordings could be used to examine neural activity in these regions during these behaviours, and the results may implicate analogous brain regions affected in human AD patients.

4.7 Final Conclusions

Overall, present evidence has indicated that the 5xFAD strain exhibits altered social interactions relative to wild-type controls, although the pattern of changes suggests a differential timescale of disease progression relative to other strains. Although it is unclear what is driving the change in social investigation in transgenic 5xFAD females,

the reduced motivation to investigate social odours may reflect the apathy commonly seen early in AD progression (Craig et al, 2005), and anxiety that is commonly exhibited by AD patients (Craig et al, 2005; Zhao et al, 2016). While evidence suggests that transgenic 5xFAD mice do not exhibit an increase in general anxiety (Braun & Feinstein, 2017; Flanigan et al, 2014; O'Leary, 2013), work with the Fmr1-KO mouse model has indicated that general anxiety can be dissociated from social anxiety (Spencer et al, 2005), suggesting that 5xFAD females may exhibit increased social anxiety without concomitant general anxiety.

Although data for transgenic males was not collected longitudinally, results at three months of age suggested that transgenic males do not exhibit increased apathy or aggression relative to wild-type controls, whereas changes in behaviour were observed by six months of age. Although apathy is commonly seen early in disease progression or even prior to diagnosis (Craig et al, 2005; Jost & Grossberg, 1996), its prevalence increases with progressive decline (Chung & Cummings, 2000), indicating that the reduced investigative and affiliative behaviours exhibited by transgenic 5xFAD males is consistent with AD progression. AD patients also exhibit delayed circadian rhythms (Satlin et al, 1995), which may be reflected in the altered timing of aggression in transgenic 5xFAD males during home-cage observations, although transgenic males did not exhibit an overall increase in aggressive encounters commonly seen in male AD patients (Lovheim et al, 2009).

As discussed in Chapters 2 and 3, social anxiety, arousal levels, incorrect processing of subordinate cues, altered bite force, and impaired memory provide possible explanations for altered social behaviours in the 5xFAD strain, although further

experiments are required to identify which, if any, of these factors are responsible for these changes. However, evidence suggests that this strain exhibits a number of social changes that reflect NPS in human AD patients, and the rapid progression of neuropathology in this strain makes it a viable model for examining the neurological changes underlying these altered social interactions.

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