Aberrant Behaviour and Neural Activity in the 5xFAD Mouse Model of Alzheimer's Disease: Exploring Behavioural and Psychological Symptoms of Dementia in a Mouse Model of Amyloid Pathology

by

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Dalhousie University is located in Mi'kma'ki, the
ancestral and unceded territory of the Mi'kmaq.
We are all Treaty people.

This work is dedicated to my family for inspiring me to be a better person, and for being the reason I keep moving forward.

Table of Contents

List of Tables

List of Figures

Abstract

The present work examines behavioural and psychological symptoms of dementia (BPSD)-like behaviours in the 5xFAD mouse model of Alzheimer's disease (AD)-related amyloid pathology. In particular, this work reviews BPSD-like behaviours exhibited by transgenic mouse models of AD-related amyloid and tau pathology and suggests that apathy—particularly social withdrawal—and depression may be related to neuropathology, while aggression and sleep/wake disturbances appear to be present even at early ages. Similarly, an examination of social investigation and aggression in 5xFAD mice identified that 6-month-old 5xFAD females and males exhibit reduced social investigation compared to wild-type controls, and that altered arousal may underlie these effects; these effects are not mediated by the genotype of a novel conspecific. Additionally, cages housing only transgenic 5xFAD males are more likely to exhibit injuries arising from home-cage aggression than cages housing only wild-type males or both wild-type and transgenic males even though transgenic 5xFAD males do not exhibit an overt increase in aggression relative to wild-type controls in either novel or familiar environments. The present work also outlines the design, assembly, and function of a novel, open-source olfactometer that can be used with tethered or untethered rodents. This olfactometer was used to examine neural activity in the form of local field potential (LFP) in the medial prefrontal cortex (mPFC) of awake, behaving 5xFAD mice in response to social and non-social olfactory stimuli, identifying altered baseline and task-dependent neural activity in this area in transgenic 5xFAD mice. Overall, these findings provide an important step towards better understanding the interplay between aberrant neural activity and behavioural changes resulting from AD-related amyloid pathology.

List of Abbreviations

A:

- Aβ: amyloid-β
- AD: Alzheimer's disease
- AICD: APP intracellular domain
- APOE: apolipoprotein E
- APOE2/3/4: apolipoprotein E ε2/3/4
- APP: Amyloid precursor protein
- ASD: Autism-spectrum disorder

B:

- BACE: beta-site APP cleaving enzyme
- BPSD: behavioural and psychological symptoms of dementia

C:

- CCK: cholesystokinin
- CNS: central nervous system
- CSF: cerebrospinal fluid

E:

- EEG: electroencephalography
- EIB: electrode interface board
- EMG: electromyography

F:

- FAD: familial Alzheimer's disease
- FDA: Food and Drug Administration
- fMRI: functional magnetic resonance imaging
- FST: Porsolt forced swim test

G:

- GPIO: general purpose input/output
- GSK3: glycogen synthase kinase 3
- GSK3α/ß: glycogen synthase kinase 3 α/ß

H:

- HDL: high-density lipoprotein
- HMW: high molecular weight

I:

- i.c.v.: intracerebroventricular
- i.p.: intreperitoneal

L:

- LDL: low-density lipoprotein
- LFP: local field potential
- LTD: long-term depression

- LTP: long-term potentiation

M:

- MAP: microtubule-associated protein
- MCI: mild cognitive impairment
- mEPSC: miniature excitatory post-synaptic current
- MI: modulation index
- mIPSC: miniature inhibitory post-synaptic current
- MMSE: Mini-Mental State Examination
- mPFC: medial prefrontal cortex
- mTOR: mammalian target of rapamycin
- MWM: Morris Water Maze

N:

- NFT: neurofibrillary tangles
- NMDA: N-methyl-D-aspartate
- NPI: Neuropsychiatric Inventory
- NPS: neuropsychiatric symptoms

O:

- ODORS: Odour Delivery Optimization Research System (olfactometer)
- OHD: olfactory habituation/dishabituation

P:

- P3: extracellular fragment cleaved from APP
- PAC: phase-amplitude coupling
- PAG: periaqueductal gray
- PET: positron-emission tomography
- PHF: paired helical fragment
- PNS: peripheral nervous system
- ppb: parts per billion
- PSEN1/2: presenilin-1/-2

R:

- REM: rapid eye movement

S:

- sAPPα/β: secreted APP-α/-β

T:

- TST: tail suspension test

V:

- VLDL: very-low-density lipoprotein
- VOC/tVOC: volatile organic compounds/total VOC

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Chapter 1. Introduction

1.1 A Brief History of Alzheimer's Disease

In 1907, Alois Alzheimer published a case study on a novel disease which would later bear his name, describing a 51-year-old woman exhibiting cognitive and behavioural impairments including memory loss, language difficulties, hallucinations, and fear or paranoia (Jarvik & Greenson, 1987). These symptoms progressed in severity over the course of 4-1/2 years before the patient ultimately passed away, at which point an autopsy revealed significant brain atrophy, along with the presence of amyloid-beta (Aß) plaques and neurofibrillary tangles (NFT, also termed paired helical filaments; PHF). These characteristics—Aß plaques and NFT concomitant with cognitive deficits, especially memory impairments—are now considered the hallmark features of Alzheimer's disease (AD; Ballard et al., 2011; Lambon Ralph et al., 2003). Today, AD is the most common form of dementia; in 2010/2011, it was estimated that approximately 96,000 community-dwelling Canadians over 65 years of age were living with AD or related dementias (Statistics Canada, 2012), and by 2022 this number was estimated at over 140,000 (Statistics Canada, 2022). By 2040, it is expected that over 80 million individuals over 60 years of age worldwide will be living with AD (Ballard et al., 2011), with approximately 11.5 million in the US alone (Hebert et al., 2013). With this projected increase, continued research into the causes and possible treatments of AD is critical to mitigating the economic and psychological costs of the disease.

1.2 Classifications of AD

AD is broadly categorized based on two factors: *1)* the age of onset, and *2)* involvement of hereditary genetic mutations. *Early-onset* AD—arising before 65 years of

age—accounts for approximately 1-2% of all cases, whereas the majority arise after 65 years of age and are considered *late-onset* (Brouwers et al., 2008). Within early-onset cases, those caused by hereditary genetic mutations are termed *familial* AD (FAD), with late-onset and the remaining early-onset cases falling under *sporadic* AD; however, the familial and sporadic forms are indistinguishable in terms of pathology (Brouwers et al., 2008). While some risk factors for sporadic AD have been identified—including lifestyle choices such as smoking, diet, and exercise, as well as several genes (Ballard et al., 2011; Sabayan & Sorond, 2017)—the etiology of this form is largely unknown.

1.3 Hypotheses of AD Etiology

Several hypotheses have been put forth for the etiology of AD. Early work indicated reduced cholinergic activity in AD patients and highlighted relationships between cholinergic activity and cognitive function in animal models, leading to the *cholinergic hypothesis* (Bartus et al., 1982); however, while Bartus *et al* suggested that reduced cholinergic activity may be a critical factor in the development of AD, they also indicated that cholinergic activity alone is likely not responsible for these changes, and there is no clear mechanism for how the disease progresses.

Shortly after the cholinergic hypothesis was posited, the Aß protein was sequenced (Glenner & Wong, 1984) and identified as neurotoxic (Yankner et al., 1989); this evidence was instrumental in the development of the *amyloid cascade hypothesis*, suggesting that Aß accumulation was responsible for tau hyperphosphorylation and formation of NFTs, and ultimately caused the neurodegeneration and cognitive deficits observed in AD patients (Hardy & Higgins, 1992). This was further supported by subsequent identification of the "Swedish" K670N/M671L amyloid precursor protein

(APP) double-mutation (Mullan et al., 1992) and a number of presenilin-1 (PSEN1) mutations—including M146L, H163R, A246E, L286V, and C410Y (Sherrington et al., 1995)—associated with FAD, suggesting that altered APP processing was linked with the hereditary form of the disease; similarly, findings that Aß accumulation affects cholinergic signaling (Auld et al., 1998) provided a mechanism by which cholinergic activity is affected in AD, and together these findings have made the amyloid cascade hypothesis the prevailing hypothesis in AD research.

While the presence of Aß plaques is a defining characteristic of AD, its specific role in AD neuropathology is debated. Hardy (2006) indicated that the original paper suggesting the amyloid cascade hypothesis (Hardy & Higgins, 1992) was inspired by a manuscript—in press at the time—written by his co-author, Gerald Higgins, reporting that transgenic mice overexpressing a C-terminal fragment of human APP exhibited AD-related neuropathology including accumulation of Aß and NFT concomitant with neurodegeneration (Kawabata et al., 1991). However, this manuscript was retracted shortly after publication amid concerns of fabricated data (Hardy, 2006), and subsequent work with transgenic mouse models of amyloid pathology has been inconsistent with regards to amyloid deposition, development of NFT, and neurodegeneration (see Chapter 2). Similarly, autopsies of humans with AD indicate that the distribution of NFT—but not amyloid plaques—corresponds with neurodegeneration (H. Braak & Braak, 1991). Additionally, while a number of risk factors have been identified—including smoking, high blood pressure, poor diet, and low levels of exercise (Sabayan & Sorond, 2017), as well as apolipoprotein E (APOE) polymorphism (Poirier et al., 1993)—research to date has not identified a causative link between Aß and sporadic AD, prompting the proposal

of alternative hypotheses. However, the amyloid cascade hypothesis was revised in the late 1990's to reflect updated research (Hardy, 1997) and has remained the prevailing theory of AD etiology since.

The *early tau hypothesis* suggests that pathogenic tau hyperphosphorylation in neurons leads to activation of glia and subsequent apoptosis (Maccioni et al., 2010). Further, tau hyperphosphorylation is a downstream effect of glial activation, and its release into the extracellular space following apoptosis acts as a damage signal that results in further glial activation and causes a positive feedback loop. Other work suggests that accumulation of Aß and NFT may be downstream effects of one or more mechanisms (Nunomura et al., 2006; Small & Duff, 2008; Sorrentino et al., 2014). The *dual pathway hypothesis* suggests that both Aß and pathogenic tau are the result of separate mechanisms driven by common upstream factors, such as the APOE ε4 (APOE4) polymorphism, glycogen synthase kinase 3 (GSK3), or the retromer sorting pathway (Small & Duff, 2008). Other hypotheses take this a step further, suggesting that upstream mechanisms are responsible for AD pathogenesis, and production of Aß and pathogenic tau is the end result of AD neuropathology rather than its driving force; these include the *oxidative stress hypothesis*—suggesting that Aß and NFT result from neuroprotective mechanisms in response to increased oxidative stress in neurons (Nunomura et al., 2006)—while Sorrentino *et al* (2014) suggest that AD is a syndrome comprised of distinct pathways that commonly result in accumulation of Aß and NFT, and the relative heterogeneity in clinical presentations is determined by the involvement of specific pathways or combinations thereof.

Overall, the hypotheses of AD etiology generally fall into four distinct schools of

thought: *(1)* pathogenic effects arise directly from Aß or NFT; *(2)* pathogenic effects arise directly from Aß and NFT, although their production is the result of common, upstream mechanisms; *(3)* production of Aß and NFT is secondary to common, pathogenic, upstream processes; and *(4)* production of Aß and NFT is secondary to common, neuroprotective, upstream processes. While the amyloid cascade hypothesis (Hardy, 1997, 2009) and early tau hypothesis (Maccioni et al., 2010) both fall into the first category, neither addresses how accumulation of one factor—either Aß or NFT—drives the production of the other, despite the former being the prevailing hypothesis in AD research for over 25 years.

1.3.1 Dual Pathway Hypothesis

The dual pathway hypothesis suggests that production Aß and NFT occurs through common upstream mechanisms (Small & Duff, 2008). While they indicate that a number of possible pathways exist, Small and Duff focus on three main mechanisms: APOE4, GSK3, and the retromer sorting pathway.

APOE is a key protein involved in cholesterol metabolism by mediating transport and uptake into cells, particularly in the brain and cerebrospinal fluid (CSF; Mahley, 1988). APOE exists as three variants that differ in amino acids at positions 112 and 158: APOE e3 (APOE3; the parent and most common form) contains cysteine at position 112 and arginine at position 158, whereas APOE e2 (APOE2) contains cysteine at both positions, and APOE4 contains arginine at both positions. These amino acid substitutions affect the conformation of the protein, leading to either decreased affinity for low-density lipoprotein (LDL) receptors (in the case of APOE2; Mahley, 1988), or altered affinity for very-low density lipoprotein (VLDL) compared to high-density lipoprotein (HDL; H. Li,

Dhanasekaran, et al., 2013). These variants are also associated with differential susceptibility to AD, with APOE4 being associated with increased Aß and NFT accumulation, while APOE2 is associated with decreased Aß accumulation (Lou et al., 2023; Marais, 2019; Small & Duff, 2008; Tiraboschi et al., 2004). While the underlying mechanisms are still unknown, evidence suggests that Aß clearance is partially mediated by APOE, and the effectiveness of this clearance may be mediated by the specific variant. Additionally, APOE4 appears to stimulate Aß production—possibly through increasing APP processing—whereas conversion of APOE3 to APOE2 can decrease Aß production in cell cultures exhibiting FAD mutations (Lou et al., 2023). The APOE4 variant has also been linked to increased tau accumulation in P301S mouse model relative to APOE2, APOE3, or knock-out mice, and APOE4 from astrocytes appears to mediate tau accumulation and phosphorylation (Lou et al., 2023). Finally, APOE regulates GSK3 activity and may exert further effects through this pathway (Small & Duff, 2008).

GSK3 is involved in axon growth and regeneration by regulating a number of proteins associated with microtubule assembly—including tau—as well as through regulation of gene expression (C.-M. Liu et al., 2012; Seira & del Río, 2014). In particular, enzymes within the GSK3 family can phosphorylate tau at a number of sites, thereby influencing cytoskeleton stability (Hanger et al., 1992; Noble et al., 2013; Seira & del Río, 2014). Moreover, GSK3α and GSK3β—two forms of GSK3—are abundantly present in the brain relative to other tissues, and tau hyperphosphorylation by these enzymes mimics the characteristics of pathogenic tau in brains of humans with AD (Hanger et al., 1992); abnormally high activity of GSK3 could then lead to the pathogenic tau hyperphosphorylation present in AD. GSK3 is regulated through a number of means

including APOE and Wnt, the latter of which has been linked with AD through T-catenin and presenilin (C.-M. Liu et al., 2012; Seira & del Río, 2014; Small & Duff, 2008). Finally, GSK3α has also been implicated in Aß production by regulating cleavage of APP by g-secretase (Phiel et al., 2003).

The final mechanism proposed by Small and Duff (2008) is the retromer sorting pathway. This pathway is involved in the recycling of mannose 6-phosphate receptors from endosomes to the trans-Golgi network and includes a number of proteins including VPS35, VPS26, and sorLA (Seaman, 2005; Small & Duff, 2008). Reduced levels of these proteins have been reported in brains of humans with AD, and animal studies have indicated that these reductions can lead to hippocampal dysfunction and increased Aß aggregation (Small & Duff, 2008). Specifically, sorLA binds to APP, and reduced VPS26 and sorLA are both implicated with increased Aß. Additionally, a genetic variant of sorLA has been linked with AD, and sorLA itself may be reduced by other factors within the retromer sorting pathway—such as reduced VPS35—suggesting the retromer sorting pathway could be implicated in AD through both direct and indirect means. Finally, the retromer sorting pathway has also been implicated in Wnt signaling, with impairments in the retromer sorting pathway leading to decreased Wnt signaling and a subsequent increase in GSK3 activity, which may ultimately lead to tau hyperphosphorylation (Small & Duff, 2008).

1.3.2 AD as a Syndrome

Sorrentino *et al* (2014) suggest that Aß and pathogenic tau arise from one or more distinct upstream pathways, leading to a syndrome which presents with common neuropathology but distinct clinical symptoms. In particular, PSEN is present in

presynaptic terminals and loss-of-function can impair neurotransmitter release and long-term potentiation (LTP), and PScDKO mice exhibit synaptic impairments prior to neurodegeneration, indicating that synaptic deficits do not arise solely due to Aß accumulation. Similarly, a shift to the amyloidogenic pathway can induce synaptic deficits through reduction of secreted APPα (sAPPα), which plays a role in LTP and long-term depression (LTD) as well as its neuroprotective role. Altered mTOR signalling is also present in AD and correlated with impaired LTP and altered GSK3 regulation, the latter of which is involved in NFT formation. PSEN and APP also play a role in neuronal survival and proliferation through Notch signalling and protein interactions at the centrosome, both of which play a role in cell cycle progression and microtubule development, while sAPPα and secreted APPß (sAPPß) are involved in cell growth and differentiation and the C-terminal fragment and APP intracellular domain (AICD) can regulate gene expression and the stability of transcription factors. Overexpression of AICD in transgenic mice also recapitulates some AD-related mechanisms—such as GSK3ß activation, accumulation of pathogenic tau, and cognitive deficits—and can cause impaired adult neurogenesis independent of Aß production. Overall, this suggests that altered APP processing may drive AD-related pathology through means other than production of Aß.

Sorrentino *et al* (2014) also point to evidence impaired intracellular trafficking in AD, including autophagic vacuoles in axons and dendrites within Aß plaques, as well as the role of PSEN in mediating function of lysosomes through delivery of vacuolar-type ATPase. Similarly, Wnt receptor complexes are incorporated into late endosomes, and altered Wnt signalling has been identified in cells lacking PSEN, suggesting that PSEN

mediates intracellular trafficking through multiple means. APP gene triplication is also required for endosomal development in cells, while gene duplication can result in impaired retrograde transport of nerve growth factor.

In line with recent evidence suggesting that APOE is involved with clearance of Aß (Lou et al., 2023), Sorrentino *et al* (2014) indicate that APOE levels increase after CNS and PNS injury and suggest that APOE4 may be associated with increased risk of AD due to reduced neuroprotective aspects of the variant. However, Lou *et al* also suggest that APOE4 may promote Aß accumulation, and conversion of APOE3 to APOE2 reduces Aß production in cell cultures exhibiting FAD mutations, suggesting that the effects of APOE variants are not purely amyloid-independent. Despite this, APOE has also been implicated in a number of aspects of neuronal cell proliferation and survival, including synaptogenesis, neurite outgrowth, dendritic arborization, synaptic plasticity, neurogenesis, and neuroinflammation (Sorrentino et al., 2014).

Overall, this hypothesis suggests that AD arises through a number of upstream pathways that are common to—but distinct from—Aß and NFT formation; while Aß and NFT may contribute to neurodegeneration, the primary pathology arises from the upstream processes, and the specific pathways involved determine the overall presentation of AD, thereby explaining the relative heterogeneity of the disease despite the generally consistent neuropathology (Sorrentino et al., 2014).

1.3.3 Oxidative Stress Hypothesis

The oxidative stress hypothesis suggests that Aß and hyperphosphorylated tau though neuroprotective mechanisms downstream of AD pathology (Nunomura et al., 2006). In particular, risk factors—such as smoking, high calorie intake, diabetes, and

hypercholesterolemia—are associated with increased production of reactive oxygen species and/or decreased antioxidant capacity; similarly, APOE variants and FAD-associated genetic mutations in APP, PSEN1, and PSEN2 are associated with increased oxidative stress in cell lines, transgenic mice, and tissue samples from AD patients, and APOE4, APOE3, and APOE2 are associated with the highest, middle, and lowest levels of oxidative stress in brain tissue of AD patients. Similarly, oxidative damage has been observed in AD even without the presence of risk factors (Nunomura et al., 2006): increased oxidative stress is present in patients with lower levels of Aß deposition or shorter disease durations; oxidative damage precedes Aß deposition in individuals with Down's syndrome; and individuals with MCI or mild AD exhibit increased lipid peroxidation and decreased antioxidant capacity. This is further corroborated through cell culture and animal experiments in which oxidative stress not only precedes Aß deposition but can actually induce production of Aß and hyperphosphorylated tau, and improving antioxidant abilities can reduce production of Aß. Finally, a large proportion of older, cognitively healthy individuals exhibit Aß and NFT deposition in the brain at a level that would be associated with definite (and in some cases advanced) AD diagnosis, and there is a poor correlation between Aß deposition and neuropathology in animal models and humans with AD; instead, evidence suggests that Aß exhibits antioxidant properties, and increased Aß deposition is linked with decreased levels of oxidative stress in brain tissue from patients with AD or Down's syndrome. Oxidative stress also precedes formation of NFT and may even drive it through activation of kinases such as GSK3, and the presence of NFT has been associated with reduced oxidative damage.

Critically, Nunomura *et al* (2006) indicate that diagnosis of AD is performed

through association of clinical symptoms with Aß and NFT, and the presence of either one alone is not sufficient. While Aß- and NFT-related pathology is typically interpreted as the cause of clinical symptoms, they point to a number of sources highlighting that Aß and NFT pathology is mediated by oxidative stress responses and the presence of Aß in many cognitively-healthy individuals, suggesting that Aß and NFT arise as neuroprotective mechanisms in response to oxidative stress rather than being responsible for neuropathology and that therapeutic interventions should target oxidative stress rather than Aß and NFT accumulation specifically. Recent evidence with disease-modifying monoclonal antibody treatments has demonstrated that removal of Aß will slow—but not halt—disease progression (Cummings, 2023), further supporting that neuropathology is primarily driven by mechanisms linked with Aß accumulation but not Aß deposition itself, although it is unclear whether upstream mechanisms are neuroprotective or pathological.

1.3.4 APP Processing

While the upstream mechanisms are currently debated, evidence indicates that Aß is produced during APP processing (H. Braak & Braak, 1991; Claeysen et al., 2012; Glenner & Wong, 1984; Hardy, 1997; Hardy & Higgins, 1992; Khachaturian, 1985; Sherrington et al., 1995; Yankner et al., 1989). APP processing occurs through one of two pathways: *(1)* a *non-amyloidogenic pathway* via a- and g-secretases that results in the formation of sAPPα, P3 (an extracellular fragment), and the AICD; and *(2)* an *amyloidogenic pathway* involving ß- and g-secretases that forms sAPPß, a 37-43 amino acid (commonly 40-42 amino acids) Aß fragment, and the AICD (Claeysen et al., 2012; Sorrentino et al., 2014). APP point mutations—such as the Swedish, Florida (I716V)

(Eckman et al., 1997), London (V717I) (Goate et al., 1991; Suzuki et al., 1994), and Indiana (V717F) (Suzuki et al., 1994) mutations—can lead to increased amyloidogenic processing by decreasing activity of a-secretase, increasing activity of ß-secretase, or modifying the subsequent C-terminal fragment cleavage by g-secretase (Claeysen et al., 2012; Hardy, 1997); similarly, point mutations in PSEN1—a component of the g-secretase complex—can also affect C-terminal fragment cleavage leading to increased production of the Aß fragment (Claeysen et al., 2012; Sherrington et al., 1995). Further, these effects are additive, and combinations of two or more APP mutations—or one or more APP mutations with one or more PSEN1 mutations—lead to increased Aß production over any single APP mutation (Borchelt et al., 1997; Citron et al., 1998; Eckman et al., 1997; Sturchler-Pierrat et al., 1997; Suzuki et al., 1994). Curiously, conditional knockout of PSEN1 and PSEN2 also leads to impaired memory and synaptic plasticity and production of hyperphosphorylated tau despite causing reduced Aß accumulation (Beglopoulos et al., 2004; Saura et al., 2004).

1.3.5 Tau Processing

The presence of NFT is another hallmark of AD, and the location and amount of NFT in the brain correlates well with neuropathological staging (Ballard et al., 2011; H. Braak & Braak, 1991). Tau belongs to the class of microtubule-associated proteins (MAPs) which promote assembly and stabilization of microtubules (Tucker, 1990). MAPs are largely—but not exclusively—located within central nervous system (CNS) neurons, with further separation of MAP subtypes within the neurons themselves; for example, tau is largely localized within axons, whereas high-molecular weight (HMW) MAP2 is primarily localized within dendrites. However, receptor dendrites within the

olfactory epithelium contain tau, not MAP2, and these dendrites are characterized by a lack of branching, suggesting that MAP subtype affects microtubule branching (Tucker, 1990).

Juvenile, lower molecular weight versions of tau and MAP2 are also present in developing mammalian brains; however, despite conservation of tau across species, developing amphibian brains exhibit the adult tau form along with relatively low levels of juvenile tau, suggesting that adult tau exhibits enough flexibility for normal neurogenesis (Tucker, 1990). Stability of both juvenile and adult forms appears to be mediated through phosphorylation, which leads to increased rigidity; in general, phosphorylation is elevated during development relative to the adult brain, and increased phosphorylation can lead to cytoskeleton instability (Noble et al., 2013; Seira & del Río, 2014; Tucker, 1990). As a result, pathogenic tau hyperphosphorylation observed in AD impairs microtubule stability and may lead to accumulation of tau into NFT within the neuron that ultimately leads to neurodegeneration (Alonso et al., 1997; Kopke et al., 1993; Noble et al., 2013; Tucker, 1990).

1.4 Neuropathology of AD

While the exact pattern of Aß and NFT deposition and associated neurodegeneration differs across individuals with AD, disease staging is dependent on the general distribution of neuropathology throughout the brain. Evidence suggests that NFT correlate better with AD staging than Aß deposition (H. Braak & Braak, 1991). The overall deposition of NFT can be divided into 6 stages covering either mild or moderate involvement across three major regions: the transentorhinal cortex (stages I and II); the limbic system (stages III and IV); and the isocortex (stages V and VI). NFT deposition in

affected regions increases with each successive stage, in addition to involvement from other regions. Stage I is characterized by NFT in the pre-α layer of the transentorhinal cortex, while stage II includes further NFT in the pre-α layer of the transentorhinal and entorhinal cortices as well as some involvement of the CA1 pyramidal cells and antero-dorsal nucleus of the thalamus. Stage III expands to the pri-α layer of the entorhinal cortex, as well as the basal magnocellular complex and the amygdala. Stage IV includes the pre-ß layer of the entorhinal cortex, CA4 non-pyramidal cells, parastriate and association areas of the isocortex, striatum, nucleus reuniens of the thalamus, and tuberomammillary nucleus of the hypothalamus. Stage V includes the pyramidal cells of CA3 and CA4, subiculum, striate cortex, claustrum, and the reticular nucleus of the thalamus. Stage VI—the final stage—is characterized by pervasive NFT throughout the entire brain.

Although NFT are better correlated with disease staging than amyloid plaques, there are three broad stages that can be characterized by amyloid deposition (H. Braak & Braak, 1991). In stage A, amyloid deposition is present in the basal areas of the frontal, temporal, and occipital lobes, with weak involvement of the parvocellular layer of the presubiculum and the pre-ß and pre-γ layers of the entorhinal cortex. With the exception of the primary sensory and motor cortices, all isocortical areas exhibit amyloid deposition in stage B, as well as mild involvement of the hippocampus. Stage C is characterized by further isocortical involvement, including the primary sensory and motor cortices.

Neurodegeneration itself can also be used to define stages. During early stages of the disease, noradrenergic neurons in the locus coeruleus and cholinergic neurons in the basal forebrain are particularly susceptible to Aß toxicity, and the loss of these neurons

has been linked with memory deficits consistent with those observed during early stages of AD (Bilkei-Gorzo, 2014; Easton et al., 2002; Sara, 2015). The entorhinal cortex, CA1, and subiculum are also affected during early stages, although neurodegeneration becomes more pronounced and widespread throughout disease progression (H. Braak & Braak, 1991; Geula, 1998; Lehéricy et al., 1989; Whitehouse et al., 1982). As noradrenaline also acts as an anti-inflammatory agent, the early impairment of noradrenergic signaling can compound neuroinflammation at later stages of the disease, thereby contributing to the widespread neurodegeneration observed in late stages (Bilkei-Gorzo, 2014; Heneka et al., 2006).

1.5 Cognitive and Behavioural Effects of AD

1.5.1 Cognitive Effects

Late-stage AD includes impairments in a number of cognitive domains, although mild cognitive impairment (MCI) has been proposed as a prodromal stage of AD (Lambon Ralph et al., 2003). MCI is characterized by impaired episodic memory and deficits on the Token Test—a task aimed at identifying individuals with aphasia—as well as impairments of tasks involving complex or fine motor skills (De Renzi & Faglioni, 1978; Kluger et al., 1997; Lambon Ralph et al., 2003). However, impaired performance on narrative recall, verbal fluency, and visuospatial tasks are good predictors of conversion from MCI to mild AD, the latter of which is characterized by impairments in the Mini-Mental State Examination (MMSE), semantic memory, language abilities (particularly in letter fluency, spelling to dictation, and the Test for the Reception of Grammar), the Rey-Osterrieth Complex Figure test (perception and attention), and further impairments in complex motor skills (Artero et al., 2003; Kluger et al., 1997; Lambon

Ralph et al., 2003). Subsequent progression to moderate AD is characterized by additional impairments in attention and perception, and to a lesser extent episodic memory (reverse digit span) and language (reading exception words), while severe AD leads to further impairments in episodic memory (forward digit span) and language (reading normal words and the National Adult Reading Test; Lambon Ralph et al., 2003).

1.5.2 Behavioural and Psychological Symptoms of Dementia

Although memory impairments are a hallmark of AD, many patients also exhibit behavioural and psychological symptoms of dementia (BPSD). While these symptoms have been assessed through various means, the Neuropsychiatric Inventory (NPI) was developed as a stand-alone method to broadly characterize these symptoms—referred to as neuropsychiatric symptoms (NPS) in the NPI—over 10 behavioural domains (delusions, hallucinations, agitation/aggression, anxiety, elation/euphoria, apathy/indifference, disinhibition, irritability, and aberrant motor behaviour) and 2 neurovegetative domains (sleep and night-time disturbances, and appetite and eating disorders; Cummings, 1997; Cummings et al., 1994). Approximately 80% of community-dwelling individuals with dementia exhibit at least one BPSD after the onset of cognitive symptoms, compared to ~50% of individuals with MCI (Lyketsos et al., 2002). Additionally, ~75% of individuals with dementia exhibit at least one BPSD within the month prior to testing, compared to 43% of individuals with MCI and 16% of the general population (Lyketsos et al., 2002; Steffens et al., 2005). Agitation/aggression, irritability, depression, anxiety, and apathy—including social withdrawal—are most common, with each symptom occurring in ~36-49% of individuals with AD, and apathy can arise even prior to diagnosis (Chow et al., 2002; Chung & Cummings, 2000; Frisoni et al., 1999;

Jost & Grossberg, 1996; Lyketsos et al., 2002; Steffens et al., 2005; Zhao et al., 2016).

It is important to note that BPSD are not simply a secondary symptom to cognitive deficits, but play a major role in the lives of caregivers, families, and individuals with AD. Certain BPSD—such as apathy, agitation/aggression, and depression—are correlated with age, disease progression, or cognitive impairment, and aggressive behaviours are more prevalent in males than females (Lövheim et al., 2009; Zhao et al., 2016). Being diagnosed with AD can isolate individuals and caregivers, whether voluntarily or not (Chenoweth & Spencer, 1986; Katsuno, 2005; Sørensen et al., 2008). The frequency and severity of BPSD also affect caregiver distress, with agitation and aggression being "severely distressing" for caregivers; behavioural disturbances—more so than cognitive or functional decline—are a leading factor in institutionalization (Chenoweth & Spencer, 1986; Chow et al., 2002; C. A. Cohen et al., 1993; Craig et al., 2005; C. Steele et al., 1990). As a result, care facilities exhibit higher prevalence of behavioural disturbances in individuals with AD compared to matched community-dwelling individuals, with 50-84% of institutionalized individuals exhibiting agitation and aggression, depression, irritability, apathy, and anxiety (C. Steele et al., 1990; Wood et al., 2000). While remaining in the community can be beneficial to individuals with AD by allowing them to remain active, care facilities can exacerbate issues by minimizing opportunities for meaningful social interactions and through potential infantilization of patients by care staff (Beard & Fox, 2008; Harman & Clare, 2006; MacRae, 2011; Phinney et al., 2007). Despite the importance of BPSD, it is still unclear whether these symptoms arise from neuropathology associated with AD, or from societal factors. However, evidence from mouse models of AD suggests that apathy and depressive-like

behaviours may be related to neuropathology, and that aggression and sleep/wake and night-time disturbances are present even at early ages (see Chapter 2).

1.6 Treatments in AD

The symptoms exhibited by individuals with AD over the course of the disease can play a major role in the lives of patients and caregivers, and institutionalization and medical treatments due to comorbidities (such as increased frailty) place an additional burden on healthcare systems (Burgener & Dickerson-Putman, 1999; Chenoweth & Spencer, 1986; Clodomiro et al., 2013; Duthie et al., 2011; Mega et al., 1996; Moran et al., 2005; Santiago & Potashkin, 2021; Schubert et al., 2006; Valeriani, 2011). As a result, pharmacological interventions aimed at slowing the progression of AD neuropathology—and ideally stopping disease progression or preventing it entirely—have been a focal point in research for decades. Recent work with monoclonal antibodies has suggested promising results with regards to clearance of Aß, although some controversy remains over their use. However, several pharmacological treatments are currently in use to address the cognitive impairments of the disease, and BPSD can be treated with medication targeting specific psychiatric disorders or non-pharmacological interventions.

1.6.1 Cholinesterase Inhibitors

The cholinergic hypothesis of memory dysfunction linked the effect of cholinergic dysfunction on memory impairments in AD (Bartus, 2000; Bartus et al., 1982), and a number of early—and still prescribed—treatments were based on acetylcholinesterase inhibition. The primary aim of these drugs is treatment of cognitive dysfunction through increased acetylcholine activity via cholinesterase inhibition

(primarily acetylcholinesterase; Davis & Powchick, 1995; Qizilbash et al., 1998; Stahl, 2000). Tacrine was the first of these to be approved for use in AD, with studies indicating some improvement, although hepatotoxicity was reported in a number of individuals (Davis & Powchick, 1995; Hammel et al., 1990; Stahl, 2000) and the drug was eventually withdrawn from clinical use in the US. However, continued work led to the development of alternatives—namely donepezil, rivastigmine, and galantamine—that have demonstrated improvements in memory with reduced likelihood of serious side effects (Birks & Harvey, 2018; Dou et al., 2018; Ippoliti et al., 2023; Neha, 2023; Stahl, 2000; L. S. Steele & Glazier, 1999). Butyrylcholinesterase—an enzyme also involved in regulation of cholinergic signalling—has also been suggested as a biomarker of AD, and its inhibition (e.g., by rivastigmine) may provide a target for intervention (Macdonald et al., 2017; Neha, 2023; Reid & Darvesh, 2015; Stahl, 2000). However, despite the relatively common prescription of cholinesterase inhibitors in treatment of AD, there is debate over the clinical significance of these drugs as the impact of side effects on quality of life may outweigh the minimal cognitive improvements, and in some cases (particularly early stages of AD) the use of cholinesterase inhibitors may increase cognitive impairments and accelerate AD-related neuropathology (Q. Huang et al., 2022; Raschetti et al., 2007; L. S. Steele & Glazier, 1999).

1.6.2 N-Methyl-D-Aspartate (NMDA) Receptor Antagonism

Altered glutamatergic signalling through tonic (rather than phasic) activation of NMDA receptors has been reported in AD, and this continuous stimulation leads to excitotoxicity and subsequent neurodegeneration (Danysz et al., 2000; Martins et al., 2023; Neha, 2023; Reisberg et al., 2003). To address this, memantine—a reversible, non-

competitive NMDA antagonist—is currently prescribed for individuals with moderate to severe AD. However, while the drug has slowed functional decline in humans and animals, and has demonstrated neuroprotective effects in rodents, it exhibits minimal improvement in cognitive deficits relative to cholinesterase inhibitors and no improvement in BPSD (Danysz et al., 2000; Dou et al., 2018; Reisberg et al., 2003). As a result, memantine is typically prescribed in conjunction with a cholinesterase inhibitor for moderate to severe AD in order to slow progression of functional impairments and improve cognitive function (Dou et al., 2018; Martins et al., 2023; Neha, 2023).

1.6.3 Treatment of BPSD

While cognitive impairments associated with AD are treated with drugs targeting physiological changes specific to AD—namely impaired cholinergic and/or glutamatergic signaling—these drugs tend to show minimal improvements in BPSD (Dou et al., 2018; Neha, 2023; Reisberg et al., 2003); instead, BPSD are typically addressed based on the specific symptoms. Best practices suggest the use of non-pharmacological approaches to start due to the potential for side effects associated with pharmacological interventions (Corbett et al., 2012; Tible et al., 2017). These interventions may include reminiscence therapy, cognitive behavioural therapies, group and individual sessions, physical activity, sensory stimulation and music therapy, or even improved treatment of unmet needs by caregivers. Importantly, the Need-driven Dementia-compromised Behaviour Model suggests that individuals with dementia may exhibit coping behaviours in response to unmet needs such as physiological (e.g., pain, thirst), psychosocial (e.g., level of assistance), and environmental (e.g., temperature, noise) needs, that are perceived as disruptive due to impaired behavioural inhibition and/or biases or expectations of

caregivers (Kolanowski, 1999). However, it is important to note that efficacy of non-pharmacological treatments depends on both the patient and the person administering the treatment (Corbett et al., 2012; Tible et al., 2017); for the latter, this can be influenced by level of training and skill, time constraints, and ability to effectively communicate, causing the efficacy of treatments to vary based on the specific situation in which they're delivered and making it difficult to generalize results across studies.

While best practices recommend that the costs and benefits of pharmacological treatments be carefully assessed prior to use, such treatments have been relatively common due to their perceived efficacy. In particular, anti-psychotics have been a preferred treatment for psychosis, agitation, and aggression due to their efficacy in schizophrenia (Chung & Cummings, 2000; Corbett et al., 2012; Liperoti et al., 2008; Y. Ohno et al., 2019; Tible et al., 2017). Similarly, antidepressants have been used for treatment of depression, apathy and emotional withdrawal, and agitation and aggression. Agitation and aggression have also previously been treated with sedatives (such as benzodiazepines and ß-adrenergic blockers), anticonvulsants, and anxiolytics, the latter of which can also be used for anxiety.

Despite the relatively common use of pharmacological treatments for BPSD, there has been some caution against their use—particularly for antipsychotics—for over 20 years (Chung & Cummings, 2000). Of primary concern is that the bulk of these drugs are intended for use with specific disorders (e.g., schizophrenia, major depressive disorder) and their use to address symptoms in dementia is considered off-label (Y. Ohno et al., 2019; Tible et al., 2017). Moreover, the use of antipsychotics is associated with a number of side-effects, including extrapyramidal symptoms, seizures, stroke, and the possibility

of adverse interactions with anticholinergic medications, and since the 2000's both US Food and Drug Administration (FDA) and European Medicines Agency—as well as other regulatory bodies—have recommended against their use in dementia (Chung & Cummings, 2000; Corbett et al., 2012; Liperoti et al., 2008; Y. Ohno et al., 2019; Tible et al., 2017).

1.6.4 Disease-Modifying Drugs

While disease-modifying drugs have been proven elusive thus far, recent work with monoclonal antibodies has shown promising results. Specifically, the US FDA has approved two drugs—aducanumab and lecanemab—through the accelerated approval program, thereby allowing patients access to these treatments while data collection is ongoing during final clinical trials (Cummings, 2023). During trials, these drugs have demonstrated marked clearance of Aß from the brains of patients to below levels detectable by positron emission tomography (PET), resulting in ~30% slowing of cognitive decline; this equates to ~7.5 month increase in the duration of mild cognitive impairment prior to AD, and an estimated 2.5 year increase in the duration of mild AD stages (Cummings, 2023).

Despite these promising early-stage results, there are concerns over the safety and clinical significance of these drugs, and criticism has been directed at the FDA over its approval of aducanumab (G. C. Alexander et al., 2021; Cummings, 2023; Mead & Fox, 2023; Rabinovici, 2021; Reish et al., 2023). The FDA approval of aducanumab has been seen as problematic by some researchers—including those originally involved in the FDA review of the drug—as the agency granted approval under an accelerated program with alternative endpoints (based on pre-existing clinical data) after the drug failed to

meet the original approval criteria (G. C. Alexander et al., 2021; Rabinovici, 2021). Moreover, both lecanemab and aducanumab have resulted in "amyloid-related imaging abnormalities"—effectively cerebral edema and/or hemorrhage—with deaths in a few cases; individuals homozygous for APOE4 are at increased risk of complications (Cummings, 2023; Mead & Fox, 2023; Reish et al., 2023). Given the importance of APOE4 as a risk factor for AD, this suggests that a number of individuals with AD may be at increased risk of complications due to these drugs. Finally, the clinical significance of a 30% decrease in the rate of cognitive decline has been questioned (Cummings, 2023; Mead & Fox, 2023). Of concern is the mechanisms driving continued neuropathology and cognitive decline despite the ability of these drugs to clear Aß to below detectable levels, suggesting the involvement of upstream and/or downstream mechanisms. However, if these drugs demonstrate continued efficacy, even a 30% slowing in neuropathology represents a significant step forward for AD treatments and may promote further examination of mechanisms underlying the remaining neuropathology.

1.7 The 5xFAD Mouse Model of AD

A number of animal models have been developed over the years to study AD neuropathology. Early work focused on lesions and injectable cholinergic agonists/antagonists, but work suggesting that Aß might underlie cholinergic dysfunction caused a shift towards Aß-based models (Bartus, 2000, p. 200). While infusion of exogenous Aß directly into the brain minimizes off-target effects of systemic APP overexpression, transgenic mouse models allow for reproducible, progressive neuropathology in subjects without the complications associated with invasive procedures (Bartus, 2000; Frautschy et al., 1996; Hsiao et al., 1996; LaFerla & Green,

2012; Puzzo et al., 2015). Additionally, the sporadic and familial forms of the disease are indistinguishable from one another in terms of pathology (Brouwers et al., 2008), and hypotheses on the etiology of AD account for the role of genetic factors (see Section 1.3), suggesting that transgenic models exhibiting mutated human genes are a viable method for examining the role of AD-related neuropathology on cognitive, behavioural, and physiological outcomes. As a result, a large number of models—including transgenic and non-transgenic models—have been developed over the past 30 years, although a relatively small number are used consistently by multiple researchers (for an overview, see Chapter 2).

Many transgenic mouse models of AD exhibit high face validity compared to human AD due to the relatively slow and stable progression of neuropathology, although this delayed Aß accumulation requires animals be raised to advanced ages prior to testing (Bilkei-Gorzo, 2014). Thus, using these models prolongs experiments, increases costs and risk of natural death, and makes longitudinal studies difficult. The 5xFAD mouse model of AD was developed to address these concerns by exhibiting rapid, progressive neuropathology and concomitant cognitive, behavioural, and physiological deficits. While this model does not exhibit the same face validity as some other models, the rapid accumulation of Aß allows early and repeated testing to examine the effects of amyloid pathology from early to late disease stages and makes it suitable to assess physiological factors in preclinical trials (Bilkei-Gorzo, 2014; Oblak et al., 2021).

1.7.1 Amyloid Pathology in 5xFAD Mice

The 5xFAD mouse model is a double-transgenic model based on the C57BL6/SJL background that exhibits human APP and PSEN1 genes with a total of 5 mutations; of

these, three are in APP (Swedish, Florida, and London mutations) and two are in PSEN1 (M146L and L286V; Oakley et al., 2006). These mutations have been previously shown to exhibit additive effects for Aß production, with the combination of APP K670N and M671L (the Swedish double mutation), the pairing of the APP Florida (I716V) and London (V717I) mutations, and the combination of APP London (V717I) with PSEN1 M146L and L286V all leading to increased levels of Aß accumulation relative to any of individual mutations alone (Citron et al., 1992, 1998; Eckman et al., 1997). As a result, transgenic 5xFAD mice exhibit a progressive accumulation of Aß that is much more rapid than many other transgenic mouse models, with whole-brain levels of $Aß_{42}$ at ~9-21 ng/mg of protein by 2 months of age, compared to ~10 ng/mg of protein in Tg2576 mice at 16 months of age (Oakley et al., 2006).

This rate of Aß accumulation leads to early and rapid progression of pathology (Gu et al., 2018; Jawhar et al., 2012; Mabrouk et al., 2023; Oakley et al., 2006; Xiao et al., 2015): deficits in axon myelination in the prelimbic, entorhinal, and retrosplenial granular cortices and the CA1 region of the hippocampus are evident by 1 month of age, amyloid plaques and gliosis are present by 2 months of age, dystrophic neurites (primarily from axon terminals) and reduced glucose metabolism in the olfactory bulb is present by 3 months of age, and reduction in whole-brain synaptophysin is evident by 4 months of age. Widespread pathology is present in the CNS by 6 months of age, with transgenic mice exhibiting neurodegeneration of noradrenergic, cholinergic, and Layer V pyramidal neurons in the cortex, synaptic loss in the ventral horn of the spinal cord, reduced basal synaptic transmission levels in the somatosensory cortex and hippocampus, and reduced EEG power spectra between 0.5 and ~250 Hz, with further reductions in

synaptophysin, syntaxin, and PSD-95 observed by 9 months of age (Crowe & Ellis-Davies, 2014; Devi & Ohno, 2010; Eimer & Vassar, 2013; Jawhar et al., 2012; J.-M. Li et al., 2013; Crouzin et al., 2013; Oakley et al., 2006; Schneider et al., 2014).

While the 5xFAD strain overall represents a suitable model for examining amyloid-related neuropathology, there are two important points to consider: first, the 5xFAD model is available on two congenic strains (C57BL6/SJL and C57BL/6J). The original 5xFAD strain was developed on a C57BL6/SJL background, although these mice exhibit a recessive phosphodiesterase-6b retinal degeneration-1 ($Pde6b^{rd1}$) allele associated with retinal degeneration and subsequent vision loss (Brown & Wong, 2007; Oakley et al., 2006; Yassine et al., 2013). To develop a line that does not exhibit this allele, Jawhar *et al* (2012) backcrossed these mice onto a C57BL/6J background for 5 generations; however, the C57BL/6J background exhibits a trend towards reduced Aß pathology up to 6 months of age and significantly lower levels of Aß pathology at 9 months of age relative to transgenic 5xFAD mice on the C57BL6/SJL background (Valenzuela, 2012). Despite this, both lines are currently in use by researchers and are collectively referred to as 5xFAD mice. Secondly, transgenic 5xFAD mice exhibit sex differences in gene expression and disease progression in physiological and cognitive effects (Bundy et al., 2019; Devi et al., 2010; Oblak et al., 2021; O'Leary & Brown, 2022; Rae & Brown, 2015; Reid & Darvesh, 2015; Roberts et al., 2020; Roddick et al., 2014, 2016; Todorovic et al., 2020). In particular, 5xFAD females exhibit more rapid progression of neuropathology and increased upregulation of genes associated with this pathology relative to males; however, while female 5xFAD mice may exhibit increased cognitive deficits relative to males on some tasks, they may exhibit reduced impairments

on other measures (e.g., frailty, olfactory perception). As a result, care must be taken during study development and review of existing literature to account for sex and background strain differences in results.

Despite the effects of sex and background strain, transgenic 5xFAD mice overall exhibit rapid and severe neurodegeneration and impaired subcortical and cortical communication, with widespread neuropathology evident by 6 months of age. As a result, these mice provide a useful model for examining progressive cognitive, behavioural, and physiological effects of Aß pathology from pre-disease to severe neuropathology stages.

1.7.2 Physiological Impairments in 5xFAD Mice

Transgenic 5xFAD mice exhibit both visual and auditory deficits with age. In particular, the $Pde6b^{rd1}$ allele is associated with retinitis pigmentosa and can lead to progressive visual impairments through extensive degeneration of rod photoreceptors by 7 weeks of age and progressive degeneration of cone photoreceptors (Yassine et al., 2013). However, even transgenic 5xFAD mice without this allele exhibit neuropathology in the visual system, with Aß deposits evident in the retinal epithelium as early as 1.5 months of age (Hart et al., 2016; Park et al., 2014; Pogue et al., 2015); by 12 months of age Aß deposits resemble those observed in age-related macular degeneration and can reduce the integrity of tight junctions and increase the permeability of the blood-retinal barrier. Post-receptor impairments during retinal electrophysiology are evident by 3 months of age, with progressive thinning of the retinal nerve fibre layer (proceeding from inside out) and reduced retinal ganglion cell responses starting by 6 months of age (Lim et al., 2020; McAnany et al., 2021). Increased levels of cyclooxygenase-2 and C-reactive protein—associated with inflammatory neuropathology in AD—are also evident in the

retina and brain by 3 months of age (Pogue et al., 2015). Further, transgenic 5xFAD mice on the C57BL6/SJL background often exhibit albinism and pigment dilution (O'Leary & Brown, 2022); while these effects are not specifically linked to amyloid pathology in these mice, they can affect visual acuity, thereby influencing performance on visual-based tasks.

In addition to visual deficits, transgenic 5xFAD mice also exhibit deficits in auditory processing, with reduced acoustic startle response by 4 months of age, increased auditory brainstem threshold at 13 months, and increased loss of hair cells in the cochlea by 15 months of age in transgenic mice compared to wild-type controls (O'Leary et al., 2017); however, peripheral hearing loss arises after reduced acoustic startle response and increased auditory brainstem threshold, suggesting that auditory impairments likely result from an accumulation of deficits in the auditory system rather than Aß-pathology alone, and this is supported by recent evidence indicating impaired auditory responses to gaps in white noise between 2-4 months of age (Weible et al., 2020).

Unlike visual and auditory deficits, evidence indicates that transgenic 5xFAD mice do not exhibit impaired olfactory processing by 15 months of age. During an olfactory delayed matching-to-sample task—in which subjects are rewarded for making a response after two successive presentations of the same odour, but not different odours—5xFAD males and females exhibit no genotype effects in the number of errors made at 6-7 months of age, although females performed better than males overall (Roddick et al., 2014). Similarly, transgenic 5xFAD mice were not impaired in olfactory sensitivity, with no genotype effects for sensitivity to odours between 0.01 and 1000 parts per billion (ppb; Roddick et al., 2016). We have also previously reported no differences in scent

marking in response to social and non-social odour stimuli and no differences in olfactory habituation/dishabituation in response to non-social stimuli up to 12 months of age in transgenic 5xFAD females relative to wild-type controls; other work has indicated no deficits in olfactory habituation/dishabituation up to 15 months of age (Kosel, Torres Munoz, et al., 2019; O'Leary, Stover, et al., 2020). Curiously, 6-month-old transgenic 5xFAD males exhibit reduced glucose metabolism in the olfactory bulb and increased latencies to find a hidden food pellet (Xiao et al., 2015). However, these results may have more to do with motivation than olfactory or cognitive impairments, as O'Leary, Stover, *et al* (2020) indicate no genotype effects for investigation duration of conditioned odour stimuli (positive stimulus associated with a sugar reward vs a negative stimulus associated with no reward) up to 15 months of age, and retention of this memory for 90 days (between 3 and 6 months of age), with both wild-type and transgenic mice investigating the positive stimulus over 85% of the time. The role of motivation is further supported by evidence indicating that 6-month-old transgenic 5xFAD mice exhibit increased investigation of odour stimuli (as well as different odour preferences) relative to wild-type controls (Roberts et al., 2020).

Transgenic 5xFAD mice also exhibit locomotor deficits with age; while O'Leary, Mantolino, *et al* (2020) reported reduced locomotor speed at 4, 13, and 16 months of age, they did not report any differences at 7 or 10 months of age, and other studies suggest that transgenic 5xFAD mice generally exhibit no differences in locomotor speed or distance travelled up to 9 months of age (Braun & Feinstein, 2019; Jawhar et al., 2012). However, disease progression based on background strain likely plays a role in this, as transgenic 5xFAD mice on the C57BL/6J background exhibit no differences in locomotor

speed or distance in the open field at 12 months of age (Jawhar et al., 2012), whereas transgenic mice on the C57BL6/SJL background exhibit reduced distance travelled at 12 and 15 months of age compared to wild-type controls (O'Leary, Mantolino, et al., 2020) as well as reduced swim speed in the Morris Water Maze (MWM) at 12 months of age (O'Leary et al., 2018; O'Leary, Mantolino, et al., 2020). Finally, transgenic 5xFAD mice exhibit an abnormal "shuffling" gait at 12 months of age relative to wild-type controls (O'Leary et al., 2018).

Deficits in motor coordination and motor learning arise earlier than locomotor deficits, with transgenic mice exhibiting shorter latencies to fall off of a balance beam at 9-10, 12-13, and 16 months of age (Jawhar et al., 2012; O'Leary et al., 2018; O'Leary, Mantolino, et al., 2020) as well as decreased latency to fall and reduced improvement over successive days on the rota-rod task at 9, 12, and 15 months of age (Fertan & Brown, 2022; O'Leary et al., 2018; O'Leary, Mantolino, et al., 2020) compared to wild-type controls. Deficits in motor strength are also evident with increasing pathology as transgenic 5xFAD mice on the C57BL/6J background exhibit reduced latency to fall during a string suspension task at 9 and 12 months of age (Jawhar et al., 2012), while those on the C57BL6/SJL background exhibit reduced latency to fall during the grid and wire suspension tasks at 12-13 and 16 months of age and exhibited reduced rearing in the open field at 9, 12, and 15 months of age relative to wild-type controls (O'Leary et al., 2018; O'Leary, Mantolino, et al., 2020). However, transgenic 5xFAD mice exhibit no impairments in soleus muscle twitch force relative to wild-type controls at 12 months of age, indicating that motor deficits are not due to impairments in signalling at the neuromuscular junction (O'Leary et al., 2018).

In summary, transgenic 5xFAD mice exhibit progressive visual system neuropathology that could lead to visual impairments starting at 3 months of age, and auditory deficits by 4 months of age. They also display reduced glucose metabolism in the olfactory bulb by 6 months of age but do not exhibit impaired olfaction up to 16 months of age. Transgenic 5xFAD mice exhibit progressive motor impairments, with impaired strength, motor coordination, and motor learning by 9 months of age, and impaired locomotor activity by 12 months of age. Finally, transgenic 5xFAD mice exhibit reduced weight gain relative to wild-type controls starting at about 6 months of age (Gendron et al., 2021). This accumulation of physiological impairments in transgenic 5xFAD mice relative to wild-type controls results in frailty—an increased vulnerability to insult. Age in mice and AD pathology in humans is associated with increased frailty, and the level of frailty is predictive of hospitalization and mortality in AD patients (Buchman et al., 2008; Kelaiditi et al., 2016; Whitehead et al., 2014), and the differences in life expectancy for 5xFAD mice—738 days (24 months) for transgenic 5xFAD mice compared to 880 days (29 months) for wild-type controls (Rae & Brown, 2015)—may result from the progressive increase in frailty relative to wild-type controls over the course of disease progression (Gendron et al., 2021; Todorovic et al., 2020).

1.7.3 Memory Impairments in 5xFAD Mice

Much like the memory impairments observed in humans with AD, mouse models of the disease also exhibit deficits in learning and memory concomitant with neuropathology (Bilkei-Gorzo, 2014). Working memory—the ability to remember small amounts of information for immediate use, such as reading a phone number and then calling it—is key for learning and memory, and impaired working memory can affect

both short-term (e.g., decision making) and long-term (e.g., learning) processes (Baddeley, 2010). Working memory in mice can be assessed through a number of means including the Y-maze and plus-maze which are both based on the tendency for animals to preferentially explore novel areas over recently visited ones. These apparatuses consist of three (Y-maze) or four (plus-maze) equally-sized, equally-spaced arms extending from a central hub (Lalonde, 2002; Olton, 1979). A subject is placed in one of these arms and allowed to explore freely. During exploration, animals will preferentially enter a "novel" area over a previously visited one (termed *spontaneous alternation*), and a high proportion of spontaneous alternations over one or more trials indicates the ability to recall previously entered arms, whereas lower proportions suggest impaired working memory. In the 5xFAD mouse model, there are no genotype effects for the number of spontaneous alternations at 2 months of age, although transgenic mice exhibit reduced spontaneous alternations relative to wild-type controls from 4—18 months of age (Devi & Ohno, 2010; Oakley et al., 2006; M. Ohno et al., 2007). Transgenic 5xFAD mice also exhibit impaired working memory in the trace fear conditioning task at 4-6 months of age, exhibiting lower levels of freezing behaviour relative to wild-type controls in response to a tone (conditioned stimulus) presented 30 seconds before a foot shock (M. Ohno et al., 2006). Curiously, transgenic 5xFAD males and females exhibit no impairments in working memory during an olfactory delayed-matching-to-sample task at 6-7 months of age (Roddick et al., 2014). In this task, subjects are trained to respond when two identical odour stimuli are presented in succession, and to withhold response when the stimuli are different. Overall, male and female 5xFAD mice, and female wild-type mice, exhibited no difference in the number of errors made during training, but wild-

type males exhibited an increased number of errors during training. Additionally, only a single transgenic female managed to reach 80% accuracy at a 30s delay between stimulus presentations, indicating that transgenic mice exhibit no impairments in olfactory working memory relative to wild-type controls.

Similar to the previous tasks, the novel object recognition task allows short-term memory to be assessed through use of longer intervals. During this task, subjects are allowed to freely explore two novel objects in an open field, then are removed, one object is replaced with a third novel object, and the subjects are reintroduced after a delay. Due to the tendency of mice to preferentially investigate novel stimuli over familiar stimuli, the relative proportion spent investigating each objects represents the ability of the subject to recall previously encountered objects (Joyashiki et al., 2011). Transgenic 5xFAD mice exhibit no impairment on this task with a 10 minute delay at up to 7 months of age, and no impairments with a 1-minute delay at 8 months of age, although memory impairments are evident after a 30-minute delay at 4 and 7 months of age (Braun & Feinstein, 2019; Tohda et al., 2011).

In addition to working and short-term memory impairments, transgenic 5xFAD mice also exhibit impaired spatial memory in the MWM. In this task, subjects are placed in a large, shallow pool filled with opaque water (through the use of milk or non-toxic water-soluble paint) at a level that prevents subjects from climbing the sides. Inside one "quadrant" of the pool is a small escape platform and several extra-maze cues are in the surrounding environment (such as high-contrast shapes on the walls; Gallagher et al., 1993; Morris, 1981; Schoenfeld et al., 2017). Due to their aversion to swimming, mice will attempt to escape from the water as quickly as possible; during training, the escape

platform is visible (either by being slightly higher than the surface of the water or identified by a marker) with the goal of training subjects to locate the platform using extra-maze cues. In subsequent testing trials, the platform is hidden just below the surface of the water in the same location, moved to the exact opposite location in the pool (reversal training), or removed entirely. During testing, subjects are scored on latency and distance travelled to locate the platform, time spent in the correct zone, and proximity to the platform. Similar to results on other memory tasks, transgenic 5xFAD mice exhibit impaired spatial memory starting around 4-6 months of age with longer latencies and distances travelled to locate the platform relative to wild-type controls (M. Ohno et al., 2006; O'Leary & Brown, 2022; Xiao et al., 2015). Transgenic 5xFAD females also exhibit impaired learning at 7-9 months of age, with no improvement in performance over successive trials (Urano & Tohda, 2010).

Overall, transgenic 5xFAD mice exhibit impairments in working memory, short-term memory, and spatial memory starting at approximately 4 months of age and progressing from there. The onset of these impairments coincides with reduced levels of whole-brain synaptophysin and the progressive neuropathology observed thereafter. However, time scale, sensory modality, and task appear to play a role in these results, as transgenic 5xFAD mice exhibit impairments in working memory in the Y-maze and at a 30s delay on the trace fear conditioning task at 4 months of age, but there are no impairments evident at a 30s delay on the olfactory delayed-matching-to-sample or at a 10 minute delay on the novel object recognition task by 7 months of age.

1.8 The Present Studies

Evidence is starting to link BPSD—such as apathy, anxiety, agitation, depression,

and psychosis—with AD-related neuropathology in humans, suggesting a biological, rather than social or psychological, mechanism for these symptoms (Kolanowski et al., 2017; Mehak et al., 2023; Mendez, 2021; Pentkowski et al., 2021) The present work aims to provide further evidence for a role for amyloid neuropathology in BPSD-like behaviours using a genetic mouse model of AD. To date, a review of neuropathology and BPSD-like behaviours in AD-model mice had not been undertaken despite the usefulness of transgenic mice in the study of AD-related behavioural changes, cognitive impairments, neuropathology, and clinical trials (Bilkei-Gorzo, 2014; LaFerla & Green, 2012; Oblak et al., 2021). The progression of cognitive deficits over the course of AD is relatively consistent between individuals (Artero et al., 2003; Kluger et al., 1997; Lambon Ralph et al., 2003), and a diagnosis of probable AD can be made on the basis of cognitive impairments with subsequent confirmation made by the presence of Aß plaques and NFT during post-mortem examination of brain tissue (Ballard et al., 2011; Khachaturian, 1985). This highlights the close link between cognitive impairments and neuropathology, and this has been further supported by evidence across a number of transgenic AD-model mice (Bilkei-Gorzo, 2014; LaFerla & Green, 2012). By comparison, BPSD are present in most, but not all, individuals with AD, and apathy—the single most common symptom—is only present in approximately 50% of these individuals (Chow et al., 2002; Frisoni et al., 1999; Lyketsos et al., 2002; Steffens et al., 2005; Zhao et al., 2016), leading some researchers to suggest that BPSD are secondary to cognitive impairments—such as difficulties in communicating needs to caregivers—or caused by extrinsic (e.g., environmental, social) factors (Kolanowski, 1999; Kolanowski et al., 2017). Identifying relationships between neuropathology and BPSD in humans has

been somewhat hampered by the use of rating scales to assess BPSD and either functional neuroimaging techniques (such as fMRI or PET) to examine cortical activity or post-mortem analysis to assess neuropathology (Gilmour et al., 2019; Mehak et al., 2023; Mendez, 2021; Pentkowski et al., 2021), thereby focusing on overall correlations between general behavioural changes and gross neuropathology. Similarly, the inconsistency in operational definitions and rating scales and the limited scope of behavioural assessments—often focusing on a single symptom—have been highlighted as limitations of many studies (Kolanowski et al., 2017; Mehak et al., 2023). Overall, this points to a very coarse approach to examining the relationship between neuropathology and BPSD despite the inconsistent presentation of these symptoms, the possibility of external mediating factors, and the limitations of current assessment tools, and researchers have recently highlighted both the need to revise the classification of BPSD to better capture the nuances associated with these behaviours and the importance of translational work based on animal models to examine direct relationships between neural activity and behaviours (Gilmour et al., 2019; Volicer, 2021).

While the 5xFAD mouse model of AD exhibits some limitations for translational research—namely the overexpression of APP, rapid and early onset of disease progression, and lack of tau pathology—it has been suggested as a useful model for examining AD-related neuropathology (Bilkei-Gorzo, 2014; Oblak et al., 2021), and evidence suggests that transgenic 5xFAD mice exhibit altered behaviours related to BPSD (Braun & Feinstein, 2019; Flanigan et al., 2014; Jawhar et al., 2012; Keszycki et al., 2023; Patel et al., 2014; Sethi et al., 2015; Yamazaki et al., 2015). Similarly, I have previously reported an age-related reduction in social investigation in 5xFAD females

(Kosel, Torres Munoz, et al., 2019), suggesting that social withdrawal is related to neuropathology in transgenic 5xFAD mice. When combined with the rapid disease progression in this model, including altered neural activity in the cortex (Chen, Ma, et al., 2022; Chen, Wei, et al., 2022; Crowe & Ellis-Davies, 2014; Gu et al., 2018; Jawhar et al., 2012; Maiti et al., 2021; Oakley et al., 2006; Schneider et al., 2014; Seo et al., 2021), this makes 5xFAD mice a suitable choice for examining the relationship between AD-related neuropathology and BPSD-like behaviours.

To provide further evidence for the role of AD-related neuropathology in the development of BPSD, this thesis first describes amyloid and tau pathology and BPSD-like behaviours in transgenic mouse models of AD to provide a general overview of current knowledge on the subject (Chapter 2; see Section 1.8.1). Next, I examine how housing conditions (transgenic-only, wild-type-only, and mixed-genotype) influence home-cage aggression in 5xFAD males to assess whether these mice can be safely group-housed long enough to assess behaviours at advanced disease stages (Chapter 3; see Section 1.8.2); in this chapter, I also examine whether 6-month-old transgenic 5xFAD females and males exhibit similar social deficits to the ones I previously reported in transgenic 5xFAD females, and whether genotype of a novel social stimulus (wild-type or transgenic) affects social approach behaviour and aggression in novel environments. After this, I describe the design and assembly of a novel, open-source olfactometer for use with tethered and untethered rodents, allowing for behavioural assessments and task-dependent electrophysiological recordings during controlled exposure to social and non-social stimuli, as well as a modified version of the olfactory habituation/dishabituation task for use with the olfactometer that allows automated, accurate, reproducible

presentation of odour stimuli (Chapter 4; see Section 1.8.3). Finally, I determined neural activity in the mPFC of transgenic 5xFAD females and males in response to social and non-social odours using this olfactometer to determine whether this model exhibits altered neural activity in addition to the neuropathology and BPSD-like behaviours present in this model (Chapter 5; see Section 1.8.4).

Overall, the present work provides an important step towards understanding the relationship between neuropathology and BPSD-like behaviours in AD-model mice by examining task-dependent neural activity in response to social and non-social stimuli in the 5xFAD mouse model of AD. Further, it contributes to the growing body of knowledge regarding BPSD-like behaviours in transgenic AD-model mice by examining social behaviours in transgenic 5xFAD males and females, and addresses key gaps by examining the role of housing conditions on home-cage aggression in male AD-model mice and providing novel, freely-accessible tools and techniques for olfactory research to aid other researchers in this field and beyond.

1.8.1 Background and Aims of Chapter 2: Behavioural and Psychological Symptoms of Dementia in Mouse Models of Alzheimer's Disease-related Pathology

Although a number of studies have suggested the presence of BPSD-like behaviours in mouse models of AD, only a few studies have specifically focused on these behaviours; much of this information comes from discrete tasks as part of a test battery, or as secondary data from other tasks (e.g., locomotion in the open-field). As a result, the presence and severity of BPSD-like behaviours in transgenic mouse models of AD, their relationship to neuropathology, and the usability of these models in examining the

neurological bases of BPSD is not well described. Chapter 2 addresses this by reviewing available data related to BPSD-like behaviours in transgenic mouse models of AD to identify which symptoms may be related to neuropathology, the type of underlying pathology (amyloid, tau, or both), the severity of symptoms relative to disease progression, and the specific models exhibiting these symptoms to provide a basis for continued research in this field.

1.8.2 Background and Aims of Chapter 3: Reduced Social Investigation and Increased Injurious Behaviour in Transgenic 5xFAD Mice

My previous work had identified age-related social deficits in 5xFAD females, with transgenic 5xFAD females exhibiting reduced interest in social odours and reduced investigation of a novel conspecific when compared to performance by wild-type controls, with effects evident between 9 and 12 months of age (Kosel, Torres Munoz, et al., 2019). However, subjects had been housed in single-genotype cages (either wild-type-only or transgenic-only), and transgenic males had to be separated prior to testing at 6 months of age due to injuries sustained from home-cage aggression. While previous evidence suggested that transgenic AD-model males exhibit increased levels of aggression (see Chapter 2), and high levels of male home-cage aggression had been reported in the 5xFAD background strain (C57BL6/SJL; Kuby, 1997; Lyon et al., 1996), a thorough examination of aggression in 5xFAD males had not yet been performed. Additionally, anecdotal evidence from other labs using 5xFAD mice suggested that the genotype of group-housed males played a role in the development of home-cage aggression, with males being successfully group-housed to 12 months (and beyond) when maintained under wild-type-only or mixed-genotype (containing both wild-type and

transgenic 5xFAD males) conditions, and our own observations with wild-type-only cages reflected this. The relative lack of injuries under these conditions suggested that the C57BL6/SJL background strain was not a major factor driving aggression in these mice, and the successful group housing observed using mixed-genotype cages suggested that the increased injuries required both altered aggressive behaviours exhibited by the instigator as well as altered responses to these behaviours by the target.

The study described in Chapter 3 examines the development of aggressive, affiliative, and solitary home-cage behaviours in group-housed males from 8 weeks (2 months) to 23 weeks (5.25 months) of age under three different housing conditions: wild-type-only, transgenic-only, and mixed-genotype. The number of cages requiring separation due to home-cage injuries is also tracked to assess whether phenotype of cage-mates affects the likelihood of a mouse sustaining injuries due to home-cage aggression. Additionally, a second cohort is used to examine whether phenotype of a novel conspecific—either transgenic or wild-type—affected social investigation and aggression in novel environments (the three-chamber apparatus and an open field), and to explore whether transgenic 5xFAD males exhibit social deficits similar to those previously reported in females.

1.8.3 Background and Aims of Chapter 4: The Odour Delivery Optimization Research System (ODORS): An Open-source Olfactometer for Behavioural Assessments in Tethered and Untethered Rodents

The importance of olfaction in mice is underscored by the large number of intact olfactory receptor genes and the relatively large size of the olfactory bulb relative to other brain structures (McGann, 2017; X. Zhang & Firestein, 2002). Additionally, the role of

olfaction in rodent social behaviours is well established (Brown, 1977, 1992; Brown et al., 1987; Pearse-Pratt et al., 1999; Schellinck et al., 1992, 1993, 1995; Singh et al., 1987). Recent research with the *Cntnap2* mouse model of autism spectrum disorder has also indicated altered neural activity in the medial prefrontal cortex (mPFC) relative to wild-type controls in response to social odour cues, and altered mPFC activity is also linked to social deficits in these mice (Levy et al., 2019; Selimbeyoglu et al., 2017), suggesting that mice exhibiting altered mPFC activity related to impaired social behaviours may also exhibit altered mPFC activity in response to social odour stimuli alone, allowing them to be tested under relatively controlled conditions.

My previous work had indicated that transgenic 5xFAD females exhibited reduced social investigation of both social odour stimuli and live novel conspecifics (Kosel, Torres Munoz, et al., 2019), and it is possible that similar mechanisms underlie these behaviours, suggesting that an olfactometer could be used to explore whether social deficits in AD model mice were related to altered mPFC activity. However, the timing of stimulus presentation is important to accurately link neural activity with odour stimuli, and commercially-available olfactometers tend to be controlled by proprietary, stand-alone software; using these systems would necessitate control the olfactometer from outside of our electrophysiology recording software, making it difficult to accurately synchronize electrophysiological data with stimulus timings during analysis. Additionally, the testing chambers in these systems—as well as alternatives described in detail in previous publications—tend to use positive pressure to localize stimulus air behind an odour port, requiring subjects to actively investigate the odours in order to receive full exposure, and the testing chambers are not typically designed for use with

tethers for electrophysiological recordings. As a result, any commercially-available olfactometers would require extensive modifications to the testing chamber and valve control system to meet the needs of the study, and the resources required to adapt these units for use with our existing systems would outweigh any benefits associated with the purchase of a commercial unit. While this can be mitigated by use of an open-source design, many of these designs also require specialized components (e.g., borosilicate glass manifolds or odorant jars; custom-machined odour tube holders) that may be difficult to source commercially or require advanced fabrication capabilities.

To address these concerns, I developed an olfactometer to meet our specific needs: *1)* the ability to control the system directly from our electrophysiological suite, allowing stimulus timing to be recorded and synchronized with neural recordings and reducing CPU usage due to additional background programs; *2)* the ability to flood the chamber with odour stimuli, removing the need for subjects to actively investigate an odour port in order to receive full exposure to a stimulus; and *3)* the ability to use the apparatus with a tether for electrophysiological recordings. Additionally, sensors for volatile organic compounds (VOCs) were used at the inlet and outlet of the testing chamber to verify that odour stimuli were entering the testing chamber during stimulus presentation and were being removed from the chamber during the intertrial periods (during which clean air was pumped through the system); VOC sensors also allowed odour stimulus concentrations to be adjusted relative to baseline levels prior to testing to maintain similar stimulus presentations across subjects.

Chapter 4 describes the assembly and function of the olfactometer apparatus, as well as the testing paradigm used to verify the olfactometer function. As olfactometers

are typically composed of the same basic components—an air supply, a series of odorant vessels controlled by valves, and a testing chamber—the basic design and function of this apparatus was based on existing systems in our department (see Roddick et al., 2014); however, in addition to the specific criteria listed above, the intention is to develop a system that was open-source and accessible to labs with limited resources, and the design focused on using components that are inexpensive and can be easily sourced or produced in-house. Similarly, the VOC sensors and interface are based on an Arduino-compatible system and a Python script is used to record VOC data, allowing other potential users to adapt the apparatus to suit their needs (e.g., creating a stand-alone system controlled by the Arduino itself, adding/removing odour stimuli, adding/removing sensors).

The testing paradigm described in Chapter 4 is based on the standard olfactory habituation/dishabituation (OHD) paradigm (M. Yang & Crawley, 2009). This paradigm represents a simple, low-cost method of assessing interest in response to various odour cues, and variations of this task have been used successfully in a variety of mouse strains and models (Baum & Keverne, 2002; Kosel, Torres Munoz, et al., 2019; Oummadi et al., 2020; Wesson et al., 2010; M. Yang et al., 2012); however, the need to manually present odours makes administration of the task very labour-intensive and prone to variability. Additionally, while attempts have been made to improve throughput and automate scoring (Oummadi et al., 2020), the task has yet to be adapted for use with an olfactometer. This could be, in part, because the high cost of commercial units (~$15,000-20,000 CAD in 2020), and the limited scope of applications, has led to relatively few labs making use of this technology thereby reducing the benefit of adapting this task. However, given the usefulness of this task, the ample baseline evidence

demonstrating expected results, and the need for improved methods of administration, its adaptation would allow verification of olfactometer function against an established paradigm and provide improved methods of administering the task for future studies. When combined with the focus on open-source principles and economical components that can be readily acquired, my work improves accessibility of olfactometers to labs with limited resources and increases functionality, accuracy, and precision of the olfactory habituation/dishabituation task.

1.8.4 Background and Aims of Chapter 5: Aberrant Neural Activity in the mPFC of 5xFAD Mice in Response to Social and Non-social Stimuli

The findings from Chapter 3 (Kosel et al., 2021) indicated that transgenic 5xFAD males and females exhibit social deficits relative to wild-type controls by 6 months of age; in particular, males and females exhibited reduced investigation of novel conspecifics in the three-chamber and free social interaction tasks. Additionally, my previous work (Kosel, Torres Munoz, et al., 2019) had found that transgenic 5xFAD females exhibited reduced interest in social odours during an olfactory habituation/dishabituation task while still exhibiting normal scent marking in response to social odour cues, suggesting that transgenic females are able to differentiate between social and non-social odour cues but have less interest in the social odours than wild-type controls. A previous study suggested that altered social behaviours in transgenic 3xTg-AD mice were related to altered background synaptic activity in patch-clamp electrophysiology of the mPFC in brain slices (Bories et al., 2012), and evidence from the *Cntnap2* mouse model of autism spectrum disorder suggested that social deficits and responses to social odours are both linked with altered mPFC activity (Levy et al., 2019;

Selimbeyoglu et al., 2017). Further, transgenic 5xFAD males exhibit altered overall EEG power between 0.1 and ~250 Hz relative to wild-type controls (Schneider et al., 2014). Overall, this suggests that altered mPFC activity is linked with social deficits in AD- and ASD-model mice, that social odour stimuli are sufficient to examine altered mPFC activity in mice, and that transgenic 5xFAD mice exhibit altered cortical activity relative to wild-type controls, suggesting transgenic 5xFAD mice may also exhibit altered mPFC activity related to the social deficits observed in this model.

The findings from Chapter 4 indicate that the olfactometer is functional and can accurately deliver olfactory stimuli to the testing chamber. Additionally, they verify that the design of the testing chamber—which includes a slot along the top for a recording tether—is suitable for use with an olfactory task, and that the presence of the VOC sensors allows tracking of odour stimulus concentrations in the chamber. As a result, this apparatus was used along with a modified version of the odour presentation paradigm used by (Levy et al., 2019) in order to assess changes in local field potential (LFP) in the delta (1-4Hz), theta (4-8Hz), alpha (8-13Hz), beta (13-38Hz), low gamma (38-55Hz), and high gamma (65-150Hz) bands in response to social and non-social odours in 6-month-old male and female transgenic 5xFAD mice relative to wild-type controls; these frequency bands are commonly used in LFP studies, although the specific cut-offs were selected based on those used by (Schneider et al., 2014) in order to allow comparison of mPFC LFP with overall EEG results in 5xFAD males.

Chapter 2. Behavioural and psychological symptoms of dementia in mouse models of Alzheimer's disease-related pathology

2.0 Preamble

This work was published in Neuroscience and Biobehavioral Reviews, 2020, Vol. 112, pp. 634-647 (see Kosel et al, 2020); this version contains minor revisions (addition of C57Bl/6 sub-strain, where available) from the original. Author contributions: *Conceptualization*: F.K., J.M.S.P, and T.B.F.; *Lit. Review*: F.K.; *Writing – Original Draft*, F.K.; *Writing – Review & Editing*, F.K. and T.B.F.; *Funding Acquisition*, T.B.F.

2.1 Abstract

Transgenic mouse models have been used extensively to model the cognitive impairments arising from Alzheimer's disease (AD)-related pathology. However, less is known about the relationship between AD-related pathology and the behavioural and psychological symptoms of dementia (BPSD) commonly exhibited by patients. This review discusses the BPSD-like behaviours recapitulated by several mouse models of AD-related pathology, including the APP/PS1, Tg2576, 3xTg-AD, 5xFAD, and APP23 models. Current evidence suggests that social withdrawal and depressive-like behaviours increase with progressive neuropathology, and increased aggression and sleep-wake disturbances are present even at early stages; however, there is no clear evidence to support increased anxiety-like behaviours, agitation (hyperactivity), or general apathy. Overall, transgenic mouse models of AD-related pathology recapitulate some of the BPSD-like behaviours associated with AD, although these behaviours vary by model. This reflects the human patient population, where AD patients typically exhibit one or

more BPSD, but rarely all symptoms at once. As a result, we suggest that transgenic mouse models are an important tool to investigate the pathology underlying BPSD in human AD patients.

2.2 Introduction

Alzheimer's disease (AD) is the most common form of dementia, affecting over 35 million individuals globally, and—due to the aging population—this number is expected to rapidly increase to 80 million individuals by 2040 (Ballard et al., 2011; LaFerla & Green, 2012). The prominent features of this disease include progressive memory loss and cognitive decline, and the illness is ultimately terminal (LaFerla & Green, 2012). With this projected increase in prevalence, and the devastating nature of the symptomology, there is an urgency to study pertinent animal models of AD-related pathology to develop our understanding of the basic neural mechanisms of this disease.

2.2.1 Neuropathology in AD

The key neurological features of AD are the presence of amyloid-beta (Aβ) plaques and neurofibrillary tangles (NFT) concomitant with memory impairments (Jarvik & Greenson, 1987). The sporadic form of the disease is most common, but the etiology of this form has yet to be determined; however, there is evidence of genetic contributions leading to a hereditary form of the disease called familial AD (FAD; Eckman et al., 1997; Goate et al., 1991; Hardy, 1997; Mullan et al., 1992; Sherrington et al., 1995). While FAD accounts for less than 5% of all AD patients, this form is indistinguishable from sporadic AD with regards to pathology and symptoms (Brouwers et al., 2008). In both cases, early stages of the disease are characterized by Aβ localization in the basal forebrain, with NFT evident in the entorhinal region (H. Braak & Braak, 1991);

noradrenergic locus coeruleus and cholinergic neurons are particularly impacted during this stage (Bilkei-Gorzo, 2014; Geula, 1998; Lehéricy et al., 1989; Nardone et al., 2008; Whitehouse et al., 1982). Although the rate of progression varies across patients, Aβ spreads throughout the neocortex (apart from the primary sensory and motor regions), and NFT are present through the medial and inferior regions of the brain during subsequent stages, with late stages characterized by the presence of Aβ and NFT throughout the cortex and pronounced loss of cortical and hippocampal neurons (H. Braak & Braak, 1991).

2.2.2 Behavioural and Psychological Symptoms of Dementia in AD

While cognitive deficits—including impaired memory (Lambon Ralph et al., 2003; Perry & Hodges, 1999)—are commonly associated with AD, many patients also exhibit behavioural and psychological symptoms of dementia (BPSD) including depression, anxiety, apathy, agitation, aggression, and reduced sociability (Ballard et al., 2011; Craig et al., 2005; Frisoni et al., 1999; Steffens et al., 2005; Zhao et al., 2016). It has been reported that 80% of community-dwelling patients with dementia exhibit at least one BPSD after the onset of cognitive symptoms, compared to 50% of patients with mild cognitive impairment (MCI; Lyketsos et al., 2002). Furthermore, approximately 75% of patients with dementia exhibited at least one BPSD in the month prior to testing (Lyketsos et al., 2002; Steffens et al., 2005), compared to 43% of MCI patients and 16% of the general population (Lyketsos et al., 2002). Individuals with MCI comorbid with BPSD are also at high risk to progress to AD, with an annual conversion rate of ~21% (Rosenberg et al., 2013). Apathy, aggression, and depression are also correlated with age, disease progression, or cognitive impairment in dementia patients, and aggressive

behaviours are more common in males than females (Lövheim et al., 2009; Zhao et al., 2016). Finally, caregiver distress is more closely related to behavioural disturbances than cognitive or functional decline, and behavioural disturbances are a leading factor in institutionalization (Chenoweth & Spencer, 1986; C. A. Cohen et al., 1993, p. 199; Craig et al., 2005; C. Steele et al., 1990). Overall, the prevalence of BPSD is significantly higher in dementia patients than the normal population, particularly for symptoms such as apathy, depression, anxiety, and agitation/aggression (Lyketsos et al., 2002; M. Zhang et al., 2012). However, it is important to note that certain assessment tools (Cummings, 1997; Cummings et al., 1994) were designed to be used with dementia patients specifically and may not fully capture BPSD-like behaviours in the general population. Additionally, many studies use these assessment tools to examine prevalence of specific BPSD within populations of dementia patients, rather than making comparisons between dementia patients and the general population; as a result, the exact relationship between BPSD in AD patients relative to the general population is not clear, although it is evident that AD comorbid with BPSD is an important consideration for future research in the field.

2.2.3 Modelling AD-Related Pathology with Transgenic Mice

In order to examine AD-related pathology, its progression, and its relationship with cognitive and behavioural changes, a number of animal models—particularly those using rodents—have been utilized. The identification of amyloid precursor protein (APP) and presenilin (PSEN) gene mutations associated with FAD (Eckman et al., 1997; Goate et al., 1991; Hardy, 1997; Mullan et al., 1992; Sherrington et al., 1995) has led to the development of a vast number of transgenic mouse models of amyloid pathology (for

review, see Bilkei-Gorzo, 2014; Van Dam & De Deyn, 2011), while tau mutations associated with frontotemporal lobar degeneration (Forrest et al., 2018) have been used to induce tauopathies in mice. Despite findings that moderate overexpression of murine tau can lead to age-related tau pathology (Adams et al., 2009), and recent evidence of naturally occurring amyloid pathology in aged mice (Ahlemeyer et al., 2018), transgenic models most often use human transgenes, which may have unexpected, off-target effects relative to endogenous mouse homologues. Additionally, the use of multiple mutations in one or more transgenes allows for the acceleration of AD-related pathology (Citron et al., 1998; Eckman et al., 1997), but human AD patients with FAD do not exhibit multiple mutations and this accelerated development reduces the effects of normal senescence on AD-related pathology. As a result, these transgenic mice do not fully recapitulate the AD phenotype and can only be considered models of amyloid and/or tau pathology. However, despite these considerations, as well as limitations regarding translation of therapeutic interventions to humans, transgenic mouse models have provided valuable insight into novel therapies—such as butyrylcholinesterase inhibition (Reid & Darvesh, 2015), BACE inhibition (Luo et al., 2001), and anticoagulants (Bergamaschini et al., 2004)—as well as the mechanisms underlying AD-related pathology (for review, see (Johnson & Stoothoff, 2004; LaFerla & Green, 2012; Van Dam & De Deyn, 2011; Wisniewski & Goñi, 2015). As a result, mouse models of amyloid and tau pathology are still in use around the world to study the cognitive, behavioural, and neuropathological changes related to AD.

To date, the vast majority of research on BPSD-like behaviours in mouse models of AD-related pathology is correlative, as many models have not been specifically tested

for these phenotypes. However, we suggest that transgenic mouse models of amyloid and tau pathology can and should be used to elucidate the neural mechanisms underlying the BPSD observed in AD patients, and that further research with these models is warranted. A complete description of all transgenic models developed to date is beyond the scope of this review, so we will focus on mouse models of AD-related pathology that have reported measurements of BPSD-like behaviours. Mouse models of AD-related pathology differ in the types of pathology presented, and the progression of these pathologies. In addition, promoter-dependent expression of the transgene and the background strain are important considerations when interpreting the behavioural findings from a particular model. Thus, we have summarized this information in Table 2.1 which describes the type of pathology, gene mutations, promotors, and background strains for each model. Table 2.2 further details the presentation and progression of the neuropathologies.

This review focuses on current evidence from transgenic mouse models of AD-related pathology to suggest that BPSD are not due solely to social factors, but stem from biological mechanisms related to amyloid and tau pathology. Although the neuropathology varies between models, and no model accurately recapitulates AD pathology, models that exhibit the widespread amyloid and/or tau pathology concomitant with neurodegeneration similar to that observed at late disease stages in human AD patients—namely the 5xFAD, PS cDKO, and Tau P301S—can be considered to model early to late stages of the disease; the remaining models covered in this review—all of which exhibit minimal to no neurodegeneration—are considered models of early AD-related pathology for purposes of this review.

Table 2.1: *Type of pathology, mutations, promotors, background strains, publication numbers (Pub.), and original publication details for mouse models of AD-related pathology.*

Model	Pathology	Mutations	Promotor	Background	Pub.	Original publication
APP/PS1					1,110	
APPSwe/ PSEN1(A246E)	amyloid	APP_{Swe}, PSEN1 A246E	murine prion protein	C3H/HeJ x C57BL/6J		(Borchelt et al., 1996)
PS/APP	amyloid	APP_{Swe}, PSEN1 M146L	hamster prion protein (APP); PDGFβ2 (PSEN)	C57BL6/SJL x (Swiss Webster x B6D2F1)		(Holcomb et al., 1998)
PS/APP[1]	amyloid	APP_{Swe}, PSEN1 M146L or PSEN1 M146V	hamster prion protein (APP); PDGFβ2 (PSEN)	C57BL6/SJL x (Swiss Webster x B6D2F1)		(McGowan et al., 1999)
APP/PS1	amyloid	APP_{Swe}, PSEN1 M146L	hamster prion protein (APP); PDGFβ2 (PSEN)	C57BL6/SJL x (Swiss Webster x B6D2F1)		(Kurt et al., 2001)
TASTPM[2]	amyloid	APP_{Swe}, PSEN M146V	murine Thy-1	C57BL/6 x C3H		(Howlett et al., 2004)
APPPS1	amyloid	APP_{Swe}, PSEN L166P	murine Thy-1	C57BL/6J		(Radde et al., 2006)
Tg2576	amyloid	APP_{Swe}	hamster prion protein	C67BL6/SJL	826	(Hsiao et al., 1996)
3xTg-AD[2]	amyloid, tau	APP_{Swe}, PSEN1 M146V, MAPT P301L	murine Thy-1; murine PSEN1	129Sv x C57BL/6	534	(Oddo et al., 2003)
5xFAD					430	
5xFAD	amyloid	APP_{Swe}, APP_{Fl}, APP_{Lon}, PSEN1 M146L, PSEN1 L286V	murine Thy-1	C57BL/6J x SJL		(Oakley et al., 2006)
5xFAD	amyloid	APP_{Swe}, APP_{Fl}, APP_{Lon}, PSEN1 M146L, PSEN1 L286V	murine Thy-1	C57BL/6J		(Jawhar et al., 2012)
APP23[2]	amyloid	APP_{Swe}	murine Thy-1	C57BL/6	180	(Sturchler-Pierrat et al., 1997)
TgCRND8	amyloid	APP_{Swe}, APP_{Ind}	hamster prion protein	C3H/HeJ x C57BL/6J	167	(Chishti et al., 2001)
J20	amyloid	APP_{Swe}, APP_{Ind}	PDGF	C57BL/6J x DBA/2	75	(Mucke et al., 2000)
THY-Tau22[2]	tau	MAPT P301S, MAPT G272V	murine Thy-1	C57BL6/CBA x C57BL/6	34	(Schindowski et al., 2006)

Model	Pathology	Mutations	Promotor	Background	Pub.	Original publication
APP(V717I)	amyloid	APP_{Lon}	murine Thy-1	FVB/N	24	(Moechars et al., 1999)
PS cDKO	tau	PSEN1 conditional KO; $PSEN2^{-/-}$	CaMKIIα (Cre), PSEN1 (floxed), PSEN2	C57BL6/129	6	(Saura et al., 2004)
Tau P301S[2]	tau	MAPT P301S	murine prion protein	C57BL/6 x C3H/HeJ	5	(Yoshiyama et al., 2007)
mThy1-hAPP751[2]	amyloid	APP_{Swe}, APP_{Lond}	murine Thy-1	C57BL/6 x DBA/2	4	(Rockenstein et al., 2001)
PS2Tg2576	amyloid	APP_{Swe}, PSEN2 N141I	hamster prion protein (APP), chicken β-actin (PSEN)	(C57BL/6JJcl) x (C57BL6/SJL x 129S6.Cg)	3	(Toda et al., 2011)
PLB1-triple	amyloid, tau	APP_{Swe}, APP_{Lon}, MAPT P301L, MAPT R406W, PSEN1 A246E	CaMKIIα (APP, MAPT), murine prion protein (PSEN1)	C57BL/6J x (C3H/HeJ x C57BL/6J)	2	(Platt et al., 2011)

Note: APP_{Swe} = Swedish (K670N/M671L) mutation; APP_{Fl} = Florida (I716V) mutation; APP_{Lond} = London (V717I) mutation; APP_{Ind} = Indiana (V717F) mutation; PDGFβ2 = Platelet-derived growth factor; CaMKIIα = α-calcium-calmodulin-dependent kinase II. Names in **bold** are the common model nomenclature; specific names for variants of the APP/PS1 model are also given. Publication numbers are taken from PubMed using common model nomenclature and do not differentiate between variants of APP/PS1 and 5xFAD models. [1]The PS/APP model produced by McGowan *et al* (1999) consists of two variants, one utilizing the APP_{Swe} and $PSEN1_{M146L}$ mutations, and the other utilizing the APP_{Swe} and $PSEN1_{M146V}$ mutations. [2]The C57BL/6 strain is not identified.

Table 2.2: *Amyloid pathology, tau pathology, and neurodegeneration in mouse models of AD-related pathology.*

Amyloid pathology	Tau pathology	Neurodegeneration	Publications
	APP/PS1[1]		
Aβ deposition (6 - 10 weeks); accumulation in cerebral cortex and hippocampus (8 - 10 months); thalamus and striatum affected at advanced ages.	Hyperphosphorylated tau in proximity to Aβ deposits.	Decreased synaptic density in proximity to Aβ plaques.	(Borchelt et al., 1996; Holcomb et al., 1998; Howlett et al., 2004; Kurt et al., 2001, 2003; McGowan et al., 1999; Radde et al., 2006)
	Tg2576		
Aβ deposition in frontal, temporal, and entorhinal cortices, hippocampal formation, and cerebellum (11 months).	None.	Loss of adrenergic and cholinergic neurons.	(Apelt et al., 2002; Guérin et al., 2009; Hsiao et al., 1996; Irizarry et al., 1997)
	3xTg-AD[2]		
Aβ deposition in cerebral cortex (6 - 12 months); accumulation in cerebral cortex and hippocampus (12 - 20 months).	Hyperphosphorylation in hippocampus (12 months) and cerebral cortex (18 months).	Loss of noradrenergic neurons in locus coeruleus (8 - 15 months).	(Belfiore et al., 2019; Janelsins et al., 2008; Manaye et al., 2013; Oddo et al., 2003)
	5xFAD[3]		
Aβ plaques in deep cortical layers and subiculum (2 months); Aβ deposition in spinal cord (4 months); plaques throughout cerebral cortex and hippocampus (6 months).	None.	Reduced synaptic density (4 months); Layer 5 neurons, hippocampal cholinergic neurons, noradrenergic neurons in locus coeruleus, and synaptic loss in spinal cord (6 months).	(Crowe & Ellis-Davies, 2014; Devi & Ohno, 2010; Eimer & Vassar, 2013; Jawhar et al., 2012; Kalinin et al., 2012; J.-M. Li et al., 2013; Oakley et al., 2006)
	APP23		
Aβ deposition (6 months); plaques in cerebral cortex, hippocampus, thalamus, olfactory nucleus, and putamen (24 months).	Hyperphosphorylated tau in proximity to Aβ plaques.	Minimal in cholinergic and noradrenergic neurons.	(Boncristiano et al., 2002; Diez et al., 2003; Heneka et al., 2006; Kelly et al., 2003; Sturchler-Pierrat et al., 1997)

Amyloid pathology	Tau pathology	Neurodegeneration	Publications
	TgCRND8		
Aβ deposition in cerebral cortex (2 - 3 months); hippocampus, midbrain, brainstem, and cerebellum with age.	None.	Hippocampus (6 months); cholinergic basal forebrain neurons (7 months).	(Bellucci et al., 2006; Chishti et al., 2001; Krantic et al., 2012)
	J20		
Aβ deposition in cerebral cortex and hippocampus (5 - 6 months).	Minimal to none.	CA1 region of hippocampus (3 months).	(Mucke et al., 2000; Shankar et al., 2009; A. L. Wright et al., 2013)
	THY-Tau22		
None.	Tau aggregation in cerebral cortex and hippocampus (3 months); hyperphosphorylated tau and NFT in hippocampus and frontal cortex (6 months); age-related increase of pathogenic tau throughout hippocampus, cerebral cortex, olfactory bulb, dentate gyrus, amygdala, and thalamus.	Neurodegeneration in hippocampus (12 months); impaired synaptic transmission in hippocampus (14 - 15 months).	(Schindowski et al., 2006)
	APP(V717I)		
Aβ deposition (2 - 4 months); plaque formation in hippocampus and cerebral cortex (13 - 18 months).	Hyperphosphorylated tau in proximity to Aβ plaques (13 - 18 months).	None.	(Moechars et al., 1999)
	PS cDKO		
Decreased Aβ deposition.	Tau hyperphosphorylation (6 months).	Neurodegeneration in cerebral cortex (4 months); neurodegeneration in hippocampus (9 months).	(Saura et al., 2004; Wines-Samuelson et al., 2010)

Amyloid pathology	Tau pathology	Neurodegeneration	Publications
	Tau P301S		
None.	NFT in hippocampus, amygdala, and spinal cord (6 months); NFT in cortex (8 months).	Loss of neurons in hippocampus (8 months); amygdala and cerebral cortex (12 months).	(Yoshiyama et al., 2007)
	mThy1-hAPP751		
Aβ deposition in frontal cortex (3 - 4 months); accumulation in cerebral cortex, hippocampus, thalamus, and cerebellum with age.	None.	Decreased synaptic density in frontal cortex (6 months).	(Rockenstein et al., 2001, 2005)
	PS2Tg2576		
Aβ deposition (2 - 3 months); plaques in cerebral cortex and hippocampus (4 - 5 months).	None.	-	(Toda et al., 2011)
	PLB1-triple		
Aβ deposition in cerebral cortex and hippocampus (5 - 6 months); plaque formation (12 months).	Tau hyperphosphorylation in cerebral cortex and hippocampus (6 months).	None.	(Platt et al., 2011)

Note: Aβ = amyloid-β; NFT = neurofibrillary (tau) tangles. [1]APP/PS1 neuropathology is given as overall information across models; individual models may vary in presentation. [2]Aβ accumulation in transgenic 3xTg-AD mice appears dependent on exposure to sex hormones during development, with females exhibiting higher levels of cortical Aβ at 6 months of age relative to transgenic males (Carroll et al., 2010). [3]Transgenic 5xFAD mice on the C57BL/6J background exhibit delayed pathology relative to those on the C57BL/6J x SJL background, with significant differences in levels of pathogenic Aβ at 9 months of age (Valenzuela, 2012); however, mice on the SJL background can exhibit retinal degeneration and vision impairments due to a recessive phosphodiesterase-6b retinal degeneration-1 mutation which can ultimately affect performance on tasks requiring vision (Pittler & Baehr, 1991; Wong & Brown, 2006).

2.3 BPSD-Like Behaviours in Transgenic Mouse Models of AD-Related Pathology

2.3.1 Aggression and Agitation

The resident-intruder task is considered the standard for assessing aggression in mice (Crawley, 1999), and has been successfully used to assess aggressive behaviours in a number of mouse strains. This behavioural paradigm involves a period of social isolation housing, followed by the introduction of a novel same-sex conspecific (the intruder) into the home-cage of the subject (the resident), typically resulting in an attack by the resident against the intruder (Brain & Parmigiani, 1990).

In general, amyloidogenic and tauogenic models exhibit increased aggression relative to wild-type controls (see Table 2.3). While the social isolation housing associated with the resident-intruder task has been found to generally increase aggression in mice relative to group-housing (Goldsmith et al., 1976; Z.-W. Liu et al., 2019), transgenic males typically exhibit decreased attack latency, increased number of attacks, and increased total attack duration relative to wild-type controls tested under the same conditions (G. Alexander et al., 2011; Moechars et al., 1998; Pugh et al., 2007; Vloeberghs et al., 2006; Yan et al., 2013). Additionally, transgenic mouse models of AD-related pathology may also exhibit increased aggressive behaviours even under group-housing conditions, as transgenic mice overexpressing human APP exhibit increased home-cage aggression (Moechars et al., 1998), and transgenic 5xFAD males exhibit increased injuries resulting from home-cage aggression when housed with other transgenic, but not wild-type, males (Kosel et al., 2021). Treatment of human APP-overexpressing transgenic mice with three different drugs that act on the serotonergic

Table 2.3: *Aggression in mouse models of AD-related pathology.*

Age	Sex	Task	Result	Publication
		APP/PS1		
2, 5	Female + Male	Resident intruder	=	(Pugh et al., 2007)
10	Female + Male	Resident intruder	↑	
		Tg2576		
7	Male	Resident intruder	↑	(G. Alexander et al., 2011)
		3xTG-AD		
12, 18	Female + Male	Free social interaction	=	(Bories et al., 2012)
		5xFAD		
2-5.5	Male	Home-cage (aggressive encounters)[1]	=	(Kosel et al., 2021)
	Male	Home-cage (injuries)[2]	↑	
6	Male	Free social interaction	=	
		APP23		
6, 12	Male	Resident intruder	↑	(Vloeberghs et al., 2006)
		APP(V717I)[3]		
2.5 - 3	Male	Resident intruder	↑	(Moechars et al., 1998)
Unknown	Female + Male	Home-cage	↑	
		PS cDKO		
2, 6, 11	Male	Resident intruder	↑	(Yan et al., 2013)

Note: [1]Aggressive encounters refers to the number of instances of aggressive encounters between cage-mates (Kosel et al., 2021). [2]Injuries refers to the number and severity of injuries observed in mice (Kosel et al., 2021). [3]In addition to the APP(V717I) model, mice overexpressing the human wild-type APP or human APP bearing the Swedish mutation were also used in this study (Moechars et al., 1998).

system—risperidone, buspirone or 8-OH-DPAT—reduced aggression in the resident-intruder task by increasing latency to attack and decreasing the total number of attacks, suggesting that aggression observed in these models is due to altered monoaminergic signalling (Moechars et al., 1998) which may be downstream of amyloid pathology. Curiously, the environment may also play a role in the exhibition of aggressive behaviours, as transgenic 3xTg-AD males and females and transgenic 5xFAD males did not exhibit increased aggression during free social interaction with a novel conspecific in a novel environment (Bories et al., 2012; Kosel et al., 2021).

Hyperactivity can represent agitation and restlessness in mice, and activity levels are often assessed during tasks examining anxiety-like behaviours—such as the open-field and elevated plus maze (Carola et al., 2002; Lister, 1987)—or short-term and working memory, such as the Y-maze (Olton, 1979). Overall, results for activity levels in transgenic mouse models of AD-related pathology are mixed, and there is no clear link between AD-related pathology and activity level (see Table 2.4). Differences in exploration or anxiety-like behaviours likely contribute to this, although results for activity levels do not necessarily concur with expression of anxiety-like behaviours (see also "Anxiety-like behaviours" section below), suggesting that differences in anxiety-like behaviours are not necessarily driving a consistent change in activity level. However, when tested in familiar environments (such as a home-cage) or allowed to habituate to an environment for extended periods (such as 24 hours), several models (e.g., APP/PS1, Tg2576, APP(V717I), PS2Tg2576) exhibit increased activity levels relative to wild-type controls (Moechars et al., 1999; Pugh et al., 2007; Toda et al., 2011).

In summary, it appears that aggression and agitation in mouse models of AD-related pathology is dependent on the model and the environment, although several amyloidogenic models exhibit increased home-cage aggression against both intruders and cage-mates, even at early stages of amyloid pathology. There is no clear relationship between agitation (as measured by activity levels) and the severity of AD-related pathologies, but it is important to note that activity levels are often measured on tasks of anxiety-like behaviours, and it is unclear whether these models would show similar results when tested in more familiar environments.

Table 2.4: *Hyperactivity in mouse models of AD-related pathology.*

Age	Sex	Task	Result	Publication
		APP/PS1		
3	Female + Male	Y-maze	↑	(Holcomb et al., 1998)
2, 5, 10	Female + Male	EPM	=	(Pugh et al., 2007)
2	Female + Male	LMA (24 hr)[1]	=	
5	Female	LMA (24 hr)[1]	=	
	Male	LMA (24 hr)[1]	↑	
10	Female + Male	LMA (24 hr)[1]	↑	
2, 5	Female + Male	LMA (30 min)[2]	↓	
10	Female	LMA (30 min)[2]	↓	
10	Male	LMA (30 min)[2]	=	
4, 11, 15	Male	Wheel running (24 hr)	=	(Duncan et al., 2012)
		Home-cage (24 hr)	=	
5, 8	Female + Male	Open field	=	(Radde et al., 2006)
6.5	Male	LDB	↑	(D. Cheng et al., 2013)
5-7	Female + Male	Open field	=	(Arendash et al., 2001)
15-17	Female + Male	Open field	↑	
5-7, 15-17	Female + Male	Y-maze	↑	
		EPM	=	
6	Male	Open field	↑	(Filali et al., 2011a)
		EPM	↑	
7, 11, 15, 24	Female + Male	Open field	=	(Webster et al., 2013)
10.5, 12.5	Female + Male	EPM	↑	(Verma et al., 2015)
13	Male	Open field	↓	(L. Liu et al., 2002)
24	Male	Open field	=	(H. Huang et al., 2016)
		Tg2576		
2-3, 4-5, 6-7	Female + Male	Home-cage	↑	(Toda et al., 2011)
		Open field	=	
3	Female + Male	Y-maze	=	(Holcomb et al., 1998)
5, 9	Male	Home-cage	↑	(Bedrosian et al., 2011)
7-12	Unknown	Y-maze	↑	(Ognibene et al., 2005)
		Open field	↑	
		EPM	=	
8-15	Male	Wheel running	=	(Wisor et al., 2005)
17	Female	Wheel running	=	
12-14	Female + Male	Activity monitor	↑	(Bardgett et al., 2011)
18	Male	Home-cage	↑	(Fernández-Fernández et al., 2012)
		Tg2576		
23	Male	Open field	↑	(Deacon et al., 2009)

Age	Sex	Task	Result	Publication
		3xTg-AD		
2-4, 6-8, 12-14	Female + Male	Y-maze	=	(Carroll et al., 2010)
6-7	Female + Male	Open field	=	(Pietropaolo et al., 2009)
		Y-maze	=	
7	Male	Open field	=	(Shruster & Offen, 2014)
6, 12	Female	EPM	=	(Pietropaolo et al., 2014)
		Open field	↓	
6	Male	EPM	=	(Pietropaolo et al., 2008)
12	Male	EPM	↑	
7.5-11	Female	Open field	=	(Sterniczuk, Antle, et al., 2010)
		EPM	=	
		Viewing jar[3]	↑	
12	Male	Open field	↓	(García-Mesa et al., 2012)
		Hole-board	↓	
12-14	Female	Open field	↓	(Filali et al., 2012)
12, 18	Female + Male	Open field	↓	(Bories et al., 2012)
		5xFAD		
4-7	Male	Open field	=	(Tohda et al., 2011)
8	Male	Novel object recognition	=	(Braun & Feinstein, 2019)
9	Female + Male	Savings test[4]	↑	(Flanigan et al., 2014)
		Open field	=	
9, 14		EPM[5]	↑	
9, 12	Female	Open field	=	(Jawhar et al., 2012)
12	Female	Three-chamber	=	(Kosel, Torres Munoz, et al., 2019)
6, 15-18	Unknown	Y-maze	=	(Devi & Ohno, 2010)
15-18	Unknown	Y-maze	=	(M. Ohno et al., 2007)
		APP23		
10	Female	Open field	↑	(Heneka et al., 2006)
24	Female	Open field	=	(Lalonde et al., 2005)
		TgCRND8		
5	Female	Open field	=	(Görtz et al., 2008)
		J20		
3-4	Male	Open field	↑	(I. H. Cheng et al., 2007)
		Y-maze	↑	
4, 6	Male	Open field	↑	(A. L. Wright et al., 2013)
5-8	Male	LDB	↑	(Fujikawa et al., 2017)
		EPM	↑	
		THY-Tau22		
6	Female + Male	EPM	=	(Schindowski et al., 2006)

Age	Sex	Task	Result	Publication
		THY-Tau22		
12	Male	Home-cage	↑	(Van der Jeugd et al., 2013)
		APP(V717I)[6]		
2	Female + Male	Home-cage	↑	(Moechars et al., 1999)
1-2, 3-4, 5-12	Female + Male	Open field	↓	
		PS cDKO		
6	Male	Open field	↑	(Yan et al., 2013)
		mThy1-hAPP751		
6	Male	Open field	↑	(Faizi et al., 2012)
		Activity monitor[7]	↑	
		PS2Tg2576		
2-3, 4-5, 6-7	Female + Male	Home-cage	↑	(Toda et al., 2011)
		Open field	=	
		PLB1-triple		
5	Female + Male	Home-cage	=	(Platt et al., 2011)
12	Female + Male	Home-cage	↑	

Note: EPM = Elevated plus maze; LDB = Light-dark box; LMA = locomotor activity. [1]24 hour LMA was measured using the LABORAS^{TM} system (Pugh et al., 2007). [2]30 min LMA was assessed using activity monitors. [3]The viewing jar consists of an inverted clear glass beaker placed over the mouse to allow observation without direct contact (Irwin, 1968). [4]The savings test assesses changes in activity level over successive trials using activity monitors. [5]Subjects were tested with full whiskers and trimmed whiskers at 14 months of age due to indications of vibrissae hypersensitivity. [6]In addition to the APP(V717I) model, mice overexpressing the human wild-type APP or human APP bearing the Swedish mutation were also used in this study; results are similar for all lines. [7]The activity monitor was a smaller version of the open field used in the same study.

2.3.2 Depressive-like Behaviours

In mice, depressive-like behaviours are typically modelled by behavioural despair and anhedonia. Behavioural despair is characterized by a cessation of escape-oriented behaviours in response to an unpleasant and/or stressful situation, and is most commonly measured in the forced swim test (FST) or tail suspension test (TST; (Crowley et al., 2004). In both tasks, subjects are placed in a situation from which there is no escape—either swimming a high-sided, water-filled cylinder in the FST, or suspended by the tail from a raised bar in the TST—and trials are scored for the total time spent immobile

(including passive floating or swinging). These tasks have been shown to be sensitive to anti-depressants (Crowley et al., 2004), with longer periods of immobility indicating higher levels of depressive-like behaviours. Anhedonia is the inability to find pleasure in a normally pleasurable stimulus, and can be measured in mice by quantifying sucrose consumption (Pfeffer et al., 2018).

Experiments investigating depressive-like behaviours in amyloidogenic and tauogenic mouse models suggest that behavioural despair and anhedonia increases with progressive AD-related pathology (see Table 2.5). The PS cDKO and J20 models exhibit increased depressive-like behaviours with age (Iascone et al., 2013; Yan et al., 2013), and the 3xTg-AD and THY-Tau22 models exhibit increased depressive-like behaviours at advanced ages (Romano et al., 2015; Van der Jeugd et al., 2013). Additionally, intracerebroventricular injection of Aβ oligomers into 3-month-old Swiss mice led to increased depressive-like behaviours which were then rescued by administration of fluoxetine antidepressant or Neuropeptide Y (dos Santos et al., 2013; Ledo et al., 2013). Curiously, despite exhibiting aggressive amyloid pathology, conflicting results have been observed in the 5xFAD mouse model: while Patel *et al* (2014) indicated increased immobility in the FST at 4.5 – 5 months of age—indicative of increased depressive-like behaviours—Yamazaki *et al* (2015) reported decreased immobility in the FST and TST at 6 months of age, suggesting a decrease in depressive-like behaviours relative to wild-type controls. Although Yamazaki *et al* (2015) do not comment on this discrepancy, their study included injections prior to behavioural testing that could have resulted in increased agitation (and therefore reduced immobility) in transgenic mice. Additionally, the use of the C57BL6/SJL background by Yamazaki *et al* (2015) compared to the C57BL/6J

Table 2.5: *Depressive-like behaviours in mouse models of AD-related pathology.*

Age	Sex	Task	Result	Publication
			3xTg-AD	
18	Male	TST	↑	(Romano et al., 2015)
		FST	↑	
		Anhedonia	↑	
			5xFAD	
4.5-5	Male	FST	↑	(Patel et al., 2014)
6	Unknown	FST	↓	(Yamazaki et al., 2015)
		TST	↓	
			APP23	
1.5, 4, 7	Female	Anhedonia	=	(Pfeffer et al., 2018)
3, 6, 12	Male	FST	↓	(Vloeberghs et al., 2007)
3, 12	Male	TST	=	
6	Male	TST	↓	
3, 6, 12	Male	Anhedonia	=	
			J20	
5-7	Female + Male	TST	=	(Iascone et al., 2013)
13-15	Female + Male	TST	↑	
			THY-Tau22	
12	Male	TST	↑	(Van der Jeugd et al., 2013)
		Anhedonia	↑	
			PS cDKO	
2, 6, 11	Male	FST	=	(Yan et al., 2013)
2	Male	Anhedonia	=	
2	Male	TST	=	
6	Male	Anhedonia	↑	
6, 11	Male	TST	↑	

Note: TST = Tail suspension test; FST = Porsolt forced swim test.

background used by Patel *et al* (2014) may also be a contributing factor.

Overall, results suggest that depressive-like behaviours in transgenic mice are related to severity of AD-related pathology. While the APP23 model exhibits no or reduced depressive-like behaviours, it only recapitulates early stages of amyloid pathology; by comparison, more advanced neuropathology or direct intracerebroventricular injection of Aβ oligomers is correlated with increased depressive-

like behaviours, suggesting that amyloid pathology has a direct effect on depressive-like behaviours.

2.3.3 Anxiety-like Behaviours

Evidence regarding anxiety-like behaviours in mouse models of amyloid and tau pathology is inconsistent, with some results suggesting either an increase, decrease, or no change between transgenic subjects and wild-type controls (Table 2.6). Anxiety-like behaviours in mice are commonly measured using three tasks: the open-field, the elevated plus maze, and the light-dark box. These tasks are based on the aversion of mice to open areas and light and are typically scored for time spent in exposed vs protected areas, number of entries into exposed or protected areas, overall locomotor activity, and number of fecal boli (Bourin & Hascoët, 2003; Carola et al., 2002; Lister, 1987). Studies with amyloidogenic and tauogenic mice indicate that some models (e.g., APP/PS1, Tg2576, PS2Tg2576) exhibit genotype effects in one test of anxiety-like behaviours but not another (D. Cheng et al., 2013; Toda et al., 2011). Further, results can vary across studies even when testing a model using the same paradigm, such as 6-month-old APP/PS1 and 3xTg-AD males in the elevated plus maze (D. Cheng et al., 2013; Filali et al., 2011a; Pietropaolo et al., 2008, 2009); it is unclear whether these discrepancies are due to subject variability, methodological differences, or other factors. Additionally, anxiety-like behaviours in amyloidogenic and tauogenic mouse models do not appear to be related to severity of AD-related pathology, as there is no consistent trend between models presenting minimal neuropathology (e.g., APP/PS1, Tg2576, APP23) compared to models with advanced pathology (e.g., 5xFAD, PS cDKO; see Table 2.6). Thus, there is insufficient evidence to determine whether AD-related

Table 2.6: *Anxiety-like behaviours in mouse models of AD-related pathology.*

Age	Sex	Task	Result	Publication
		APP/PS1		
2	Female + Male	EPM	=	(Pugh et al., 2007)
5	Female	EPM	=	
5-10	Female	EPM	↓	
10	Male	EPM	↓	
5, 8	Female + Male	Open field	=	(Radde et al., 2006)
5-7, 15-17	Female + Male	EPM	=	(Arendash et al., 2001)
6	Male	Open field	=	(Filali et al., 2011a)
		EPM	=	
6.5	Male	LDB	=	(D. Cheng et al., 2013)
		EPM	↑	
7, 11, 15, 24	Female + Male	Open field	=	(Webster et al., 2013)
8	Unknown	EPM	↓	(Filali et al., 2011b)
10.5, 12.5	Female + Male	EPM	↓	(Verma et al., 2015)
24	Male	Open field	↑	(H. Huang et al., 2016)
		EPM	↑	
		Tg2576		
2-3, 4-5, 6-7	Male	EPM	↑	(Toda et al., 2011)
		Open field	=	
5	Male	LDB	=	(Bedrosian et al., 2011)[1]
9	Male	LDB	↑	
7-12	Unknown	EPM	=	(Ognibene et al., 2005)
18	Male	EPM	=	(Fernández-Fernández et al., 2012)
21	Male	Open field	↓	(Deacon et al., 2009)
		3xTg-AD		
6	Male	LDB	↑	(España et al., 2010)
6	Male	EPM	=	(Pietropaolo et al., 2009)
	Female	EPM	↑	
6, 12	Female	EPM	↑	(Pietropaolo et al., 2014)[2]
		Open field	↑	
6	Male	EPM	↓	(Pietropaolo et al., 2008)
12	Male	EPM	=	
7	Male	Emergence test[3]	↓	(Shruster & Offen, 2014)
		EPM	↓	
		Open field	↓	
7.5-11	Female	Open field	↑	(Sterniczuk, Antle, et al., 2010)
		EPM	↑	
12	Male	LDB	↑	(García-Mesa et al., 2012)

Age	Sex	Task	Result	Publication
		3xTg-AD		
13	Female	Open field	=	(Filali et al., 2012)
		EPM	=	
18	Male	Open field	=	(Romano et al., 2015)
		EPM	=	
		5xFAD		
6, 9, 12	Female	EPM	↓	(Jawhar et al., 2012)
8	Male	Open field	↓	(Braun & Feinstein, 2019)
9	Female	Open field	=	(Flanigan et al., 2014)[4]
		LDB	=	
14	Female	EPM	=	
		APP23		
1.5, 4	Female	EZM[5]	↓	(Pfeffer et al., 2018)
7	Female	EZM[5]	=	
3, 6	Male	EPM	=	(Vloeberghs et al., 2007)
12	Male	EPM	↑	
16	Female	EPM	=	(Lalonde et al., 2002)
		TgCRND8		
5	Female	Open field	=	(Görtz et al., 2008)
		EPM	=	
		J20		
3-4	Male	EPM	↓	(I. H. Cheng et al., 2007)
4, 6	Male	EPM	=	(A. L. Wright et al., 2013)
5-8	Male	LDB	↓	(Fujikawa et al., 2017)
		EPM	↓	
6-8	Female + Male	EPM	↓	(Chin et al., 2005)
		THY-Tau22		
6	Female + Male	EPM	↓	(Schindowski et al., 2006)
		PS cDKO		
2, 6, 11	Male	Resident Intruder	↑	(Yan et al., 2013)
		PS2Tg2576		
2-3, 4-5, 6-7	Female + Male	EPM	↑	(Toda et al., 2011)
		Open field	=	

Note: EPM = Elevated plus maze; LBD = Light-dark box; EZM = Elevated zero maze. [1]Increased anxiety in 9-month-old transgenic Tg2576 males is only observed during the later part of the dark phase. [2]Results indicated are for standard housing conditions only. [3]The emergence test is conceptually similar to the light-dark box (Shruster & Offen, 2014). [4](Flanigan et al., 2014) suggest that the decreased anxiety-like behaviour in transgenic 5xFAD females observed by (Jawhar et al., 2012) was likely due to increased sensitivity of vibrissae, thereby resulting in discomfort in the closed arms of the EPM and leading to increased time in the open arms. [5]The EZM is conceptually similar to the EPM, using a circular catwalk divided into four alternating open and closed quadrants (Pfeffer et al., 2018).

pathology (amyloid and/or tau) is positively or negatively associated with anxiety-like behaviours, if it is related at all.

2.3.4 Apathy-like Behaviours

In mice, general apathy can be assessed using object exploration (e.g., Braun & Feinstein, 2019; Filali et al., 2011a), whereas social withdrawal is examined by approach behaviour to conspecifics, either free-roaming (e.g., Bories et al., 2012; Deacon et al., 2009; Flanigan et al., 2014) or presented behind a barrier as in the three-chamber sociability test (Moy et al., 2004). While decreased exploration has been reported in young TgCRND8 mice (Görtz et al., 2008), overall results suggest that mouse models of AD-related pathology do not exhibit generalized apathy (see Table 2.7). However, a number of studies have reported decreased social investigation in APP/PS1, Tg2576, and 5xFAD mice (see Table 2.8). This decrease in sociability may be linked with AD-related pathology, as 5xFAD and Tg2576 females exhibit an age-related decrease in social investigation (Kosel, Torres Munoz, et al., 2019; Pietropaolo et al., 2012). Similarly, decreased background synaptic activity in Layer II/III pyramidal cells in the prefrontal cortex is related to decreased social investigation in transgenic 3xTg-AD mice (Bories et al., 2012). While aged Tg2576 and APP23 females exhibit no genotype effects for sociability (Deacon et al., 2009; Heneka et al., 2006), it is unclear whether the use of juvenile rather than adult conspecifics influences social investigation. Curiously, PLB1-triple mice do not show decreased sociability even with advanced neuropathology (Platt et al., 2011); this discrepancy may be due to differences (such as background strains or promotors) between this and other models, or differences in methodology between studies (such as the age of stimulus conspecifics).

Table 2.7: *General exploration in mouse models of AD-related pathology.*

Age	Sex	Task	Result	Publication
		APP/PS1		
3, 6	Female	Three-chamber	=	(Pietropaolo et al., 2012)
3, 4, 6, 8, 10	Female + Male	Novel object	=	(Howlett et al., 2004)
		Tg2576		
3, 6	Female	Three-chamber	=	(Pietropaolo et al., 2012)
12-14	Female + Male	Novel object	=	(Bardgett et al., 2011)
		3xTg-AD		
7	Male	Novel object	=	(Shruster & Offen, 2014)
		5xFAD		
8	Male	Novel object	↑	(Braun & Feinstein, 2019)
		TgCRND8		
4	Female	Novel object	↓	(Görtz et al., 2008)
7	Unknown	Hole-board	=	(Bellucci et al., 2006)

Overall, mouse models of amyloid and tau pathology appear to exhibit reduced social interest without general apathy. While transgenic APP/PS1 mice exhibit reduced social interest compared to wild-type controls even at young ages (see Table 2.8), evidence from the Tg2576, 3xTg-AD, and 5xFAD models suggests that progressive AD-related pathology mediates social withdrawal (Bories et al., 2012; Kosel, Torres Munoz, et al., 2019; Kosel et al., 2021; Pietropaolo et al., 2012), and that these effects may result from altered synaptic transmission in brain areas that mediate social interaction (Bories et al., 2012). Additionally, evidence suggests that sociability in the 5xFAD model is mediated by familiarity with the environment and conspecific (such as in the home-cage or during social novelty preference tasks; Flanigan et al., 2014; Kosel, Torres Munoz, et al., 2019; Kosel et al., 2021), and that arousal levels can affect sociability (Kosel, Torres Munoz, et al., 2019), although it is unclear whether similar effects apply to other models.

Table 2.8: *Sociability in mouse models of AD-related pathology.*

Age	Sex	Task	Result	Publication
		APP/PS1		
3, 6	Female	Three-chamber	↓	(Pietropaolo et al., 2012)
		Free social interaction	↓	
6	Male	Three-chamber	↓	(Filali et al., 2011a)
7	Male	Three-chamber	↓	(D. Cheng et al., 2013)
16	Female + Male	Three-chamber	↓	(Verma et al., 2015)
24	Male	Three-chamber	↓	(H. Huang et al., 2016)
		Tg2576		
3	Female	Three-chamber	=	(Pietropaolo et al., 2012)
6	Female	Three-chamber	↓	
		Free social interaction	↓	
23	Female	Free social interaction	=	(Deacon et al., 2009)
		3xTg-AD		
12	Female	Free social interaction	↑	(Bories et al., 2012)
	Male	Free social interaction	=	
18	Female	Free social interaction	↓	
	Male	Free social interaction	↑	
		5xFAD		
2-5.5	Male	Home-cage	=	(Kosel et al., 2021)
6	Female + Male	Three-chamber	=	
		Free social interaction	↓	
3, 6	Female	Free social interaction	=	(Kosel, Torres Munoz, et al., 2019)
9, 12	Female	Free social interaction	↓	
3, 6, 9	Female	Three-chamber	=	
12	Female	Three-chamber	↓	
9	Female + Male	Home-cage	↑	(Flanigan et al., 2014)
		APP23		
10	Female	Free social interaction	=	(Heneka et al., 2006)
		mThy1-hAPP751		
6	Male	Three-chamber	=	(Faizi et al., 2012)
		Investigation of females[1]	=	
		PLB1-triple		
5, 12	Female + Male	Three-chamber	=	(Platt et al., 2011)

Note: [1]Investigation of females was assessed during a six-trial social memory task.

Table 2.9: *Sleep-wake disturbances in mouse models of AD-related pathology.*

Age	Sex	Behavioural Changes	Publication
		APP/PS1	
4, 11, 15	Male	Delayed circadian rhythm.	(Duncan et al., 2012)
9	Female	Increased wakefulness; decreased REM and NREM sleep.	(Roh et al., 2012)
		Tg2576	
7-12	Unknown	Earlier onset of activity.	(Ognibene et al., 2005)
22	Male	Disrupted sleep EEG rhythms.	(Wisor et al., 2005)
22	Female	Decreased REM sleep; delayed sleep onset.	
		3xTg-AD	
1, 4, 6, 7.5	Male	Altered circadian rhythm (increased daytime activity; decreased nighttime activity).	(Sterniczuk, Dyck, et al., 2010)
11.5	Female	Altered circadian rhythm (increased daytime activity; decreased nighttime activity).	
		5xFAD	
4-6.5	Female + Male	Decreased average sleep bout duration.	(Sethi et al., 2015)
4-6.5	Female	Decreased total sleep.	
		TgCRND8	
3, 6-8	Male	Decreased REM and NREM sleep; increased overall wakefulness.	(Colby-Milley et al., 2015)
11	Male	Decreased NREM sleep; increased overall wakefulness.	
		Tau P301S	
9-10	Male	Decreased REM sleep.	(Holth et al., 2017)
11-12	Male	Decreased REM and NREM sleep; increased wakefulness.	
		PLB1-triple	
5, 12	Female + Male	Decreased NREM sleep; increased wakefulness.	(Platt et al., 2011)
12	Female + Male	Delayed sleep onset; increased NREM fragmentation.	

Note: REM = Rapid eye movement; NREM = Non-rapid eye movement; EEG = Electroencephalography.

2.3.5 Sleep/Wake Disturbances

A number of mouse models of amyloid and tau pathology exhibit sleep/wake disturbances, and these effects can be assessed using either physical measures—such as running wheels, infrared beams, and piezoelectric systems (Duncan et al., 2012; Sethi et al., 2015)—or electrophysiological measures, including electroencephalography (EEG) and electromyography (EMG; Colby-Milley et al., 2015; Holth et al., 2017; Platt et al., 2011; Roh et al., 2012; Wisor et al., 2005).

Several mouse models exhibit altered circadian rhythms and disrupted sleep patterns, including decreased rapid eye movement (REM) and non-REM sleep and altered EEG rhythms during sleep, and these changes appear linked with pathology (Table 2.9). Aβ levels in brain interstitial fluid have been shown to increase in parallel with the amount of time spent awake in transgenic Tg2576 mice (Kang et al., 2009), and decreased wakefulness is correlated with decreased Aβ pathology in APP/PS1 mice (Roh et al., 2014), suggesting a positive feedback loop between Aβ pathology and sleep disturbances. Sleep/wake disturbances in the Tau P301S model are also linked with tau pathology in the brainstem (Holth et al., 2017). Overall, evidence suggests that sleep-wake disturbances are consistently present in mouse models of amyloid and tau pathology, indicating that these models may be well suited to examine the neurological changes underlying these behaviours.

2.4 Conclusions

Transgenic mouse models have been successfully used to study the cognitive and behavioural deficits associated with amyloid and tau pathologies, and have provided insight for novel therapies including butyrylcholinesterase or BACE inhibition and

anticoagulants (Bergamaschini et al., 2004; Bilkei-Gorzo, 2014; LaFerla & Green, 2012; Luo et al., 2001; Reid & Darvesh, 2015; Van Dam & De Deyn, 2011). While the bulk of the literature is correlative in nature and focuses on cognitive and motor deficits, evidence of BPSD-like behaviours in mouse models of amyloid and tau pathologies is accumulating. Social withdrawal and depressive-like behaviours appear to increase with progressive AD-related pathology, and both aggression and sleep-wake disturbances have been reported even at early stages of AD-related pathology, although aggression appears to be dependent on familiarity with the environment and/or conspecific. Mouse models of AD-related pathology do not appear to exhibit generalized apathy relative to wild-type controls. Finally, there is no clear evidence to support anxiety-like or hyperactive behaviours in mouse models of AD-related pathology, as both behaviours are clearly dependent on the model and task.

Overall, transgenic mouse models of AD-related pathology mirror some of the BPSD observed in human AD patients. Much like human patients, no single model exhibits all of these behaviours, suggesting that these behaviours arise through various underlying neural mechanisms associated with AD-like neuropathology. Continued examination of BPSD-like behaviours in these mouse models may better define these mechanisms, providing avenues of investigation for therapeutic interventions.

Chapter 3. Reduced Social Investigation and Increased Injurious Behaviour in Transgenic 5xFAD Mice

3.0 Preamble

This work was published in the Journal of Neuroscience Research, 2021, Vol. 99 (see Kosel et al, 2021); this version contains minor revisions (housing conditions and habituation of stimulus mice; edits for grammar and clarity) from the original. Author contributions: *Conceptualization*: F.K. And T.B.F.; *Methodology*: F.K., J.H., and T.B.F.; *Investigation*: F.K., J.H., S.H., and V.G.; *Formal Analysis*: F.K.; *Resources*: F.K. and T.B.F.; *Writing – Original Draft*: F.K.; *Writing – Review & Editing*: F.K. and T.B.F., *Visualization*: F.K.; *Supervision*: T.B.F., F.K.; *Funding Acquisition*: T.B.F.

3.1 Abstract

Social withdrawal and agitation/aggression are common behavioural and psychological symptoms of dementia (BPSD) presented by Alzheimer's disease (AD) patients, with males exhibiting more aggressive behaviours than females. Some transgenic mouse models of AD also exhibit social withdrawal and aggression, but many of these models only recapitulate the early stages of the disease. By comparison, the 5xFAD mouse model of AD exhibits rapid, progressive neurodegeneration, and is suitable for modelling cognitive and behavioural deficits at early-, mid-, and late-stage disease progression. Anecdotal reports suggest that transgenic 5xFAD males exhibit high levels of aggression compared to wild-type controls, but to date, indirect genetic effects in this strain have not been studied. We measured home-cage behaviours in 5xFAD males housed in three different group-housing conditions (transgenic only, wild-type only,

mixed-genotype), and social approach behaviours when exposed to a novel free-roaming or restrained, wild-type or transgenic conspecific. Transgenic-only home-cages required earlier separation due to injuries arising from aggression compared to wild-type-only or mixed-genotype cages, despite no obvious increase in the frequency of aggressive behaviours. Transgenic 5xFAD males and females also spent less time investigating free-roaming conspecifics compared to wild-type controls, but they showed normal investigation of restrained conspecifics; the genotype of the conspecific did not affect approach behaviour, and there was no aggression observed in transgenic males. These findings provide evidence in an animal model that amyloid pathology ultimately leads to avoidance of novel social stimuli, and that frequent interactions between individuals exhibiting an AD phenotype further exacerbates aggressive behaviours.

3.2 Significance Statement

The present study characterizes aggression in male mice, and social investigative behaviour in male and female mice, from the 5xFAD transgenic mouse model of Alzheimer's disease (AD). Both male and female 5xFAD mice spend less time investigating a free roaming same-sex conspecific, suggesting that reduced social interest occurs as a result of AD-related neuropathology. Our findings also highlight the importance of social environment—with respect to transgenic-only, wild-type-only, and mixed-genotype group housing—for maintaining a healthy colony of 5xFAD mice, thereby aiding researchers in selecting housing conditions that maximize the longevity of their colonies.

3.3 Introduction

Alzheimer's disease (AD) is a leading cause of dementia, defined by an

accumulation of amyloid-beta (Aβ) plaques and neurofibrillary (tau) tangles concomitant with progressive memory loss and cognitive impairments (Jarvik & Greenson, 1987; LaFerla & Green, 2012). Patients also present a number of behavioural and psychological symptoms of dementia (BPSD), including apathy, anxiety, agitation/aggression, and depression (Cummings, 1997; Cummings et al., 1994). Approximately 80% of community-dwelling dementia patients exhibit at least one BPSD following the onset of cognitive symptoms, with approximately 75% of all patients exhibiting at least one BPSD in the month prior to testing, compared to 16% of the general population (Lyketsos et al., 2002). Apathy, agitation/aggression, anxiety, and depression are among the most common BPSD, and patients with mild AD exhibit a significantly higher prevalence of these compared to healthy controls (M. Zhang et al., 2012; Zhao et al., 2016). These symptoms can have a profound impact on the lives of patients, their families, and caregivers, with many patients exhibiting social withdrawal even prior to diagnosis (Chow et al., 2002; Chung & Cummings, 2000; Frisoni et al., 1999; Jost & Grossberg, 1996; Steffens et al., 2005). The frequency and severity of BPSD also affects caregiver distress (Chow et al., 2002; Craig et al., 2005), with behavioural disturbances (such as agitation/aggression) being a leading factor in institutionalization (Chenoweth & Spencer, 1986; C. A. Cohen et al., 1993; C. Steele et al., 1990). As a result, patients in care facilities, who frequently interact with other AD patients, exhibit a higher prevalence of behavioural disturbances compared to age-matched community-dwelling individuals (C. Steele et al., 1990; Wood et al., 2000), which can lead to a further reduction of meaningful social interactions.

Transgenic mouse strains have proved useful in examining cognitive and

behavioural deficits associated with AD-related neurodegeneration, and several of these strains present behaviours akin to BPSD in AD patients. Here, we focus on BPSD related to social function. Six-month-old transgenic APP/PS1 males (Filali et al., 2011a) and 18-month-old transgenic 3xTg-AD females (Bories et al., 2012) exhibit decreased sociability compared to wild-type littermates. Increased aggression has also been reported in a number of transgenic strains, including 7-month-old Tg2576 males, 6- to 12-month-old APP23 males, and 10-month-old APP/PS1 males (G. Alexander et al., 2011; Pugh et al., 2007; Vloeberghs et al., 2006), mimicking the increased aggression more often seen in male human AD patients (Lövheim et al., 2009), although increased aggression was not reported in male and female 3xTg-AD mice at 12 or 18 months of age (Bories et al., 2012). The varying age of onset presented in these models may be, in part, due to differences in the progression of the pathology. Tg2576, APP23, and APP/PS1 strains exhibit a relatively slow rate of Aβ accumulation and only recapitulate the early stages of AD (Bilkei-Gorzo, 2014). In contrast, the 3xTg-AD strain exhibits neurofibrillary tangles in conjunction with Aβ, but does not exhibit the same level of neurodegeneration characteristic of AD in humans (Bilkei-Gorzo, 2014; Janelsins et al., 2008; Manaye et al., 2013). By comparison, the 5xFAD strain exhibits rapid accumulation of Aβ, with whole-brain levels at ~9—21 ng/mg of protein by 2 months of age, compared to ~10 ng/mg of 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 have deficits in axon myelination, amyloid plaques and gliosis, and a reduction in whole-brain synaptophysin between 1 and 4 months of age (Gu et al., 2018; Oakley et al., 2006); this progresses to neurodegeneration in noradrenergic and cholinergic neurons, as well as pyramidal

neurons in Layer 5 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 by 6 months of age, and by 9 months of age there is 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 signalling (Crouzin et al., 2013; Crowe & Ellis-Davies, 2014; Devi & Ohno, 2010; Eimer & Vassar, 2013; Jawhar et al., 2012; J.-M. Li et al., 2013; Oakley et al., 2006). As a result, the 5xFAD strain provides a useful model for examining effects of early-, mid-, and late-stages of AD-related neuropathology.

Despite the suitability of the 5xFAD mouse model for examining cognitive and behavioural deficits associated with later stages of AD (Bilkei-Gorzo, 2014), relatively little is known about the BPSD-like behaviours in this strain. Transgenic 5xFAD males at 9 months of age, and males and females at 12 months of age, exhibit deficits in nest-building, a behaviour previously shown to be associated with affiliative behaviours (Devi & Ohno, 2015; Schneider et al., 2014). However, transgenic 5xFAD males and females also engage in increased home-cage social behaviours at ~11 months of age and impaired social recognition memory at 9 months of age (Flanigan et al., 2014). More recently, we found that transgenic 5xFAD females have less interest in social odours, as well as an age-related decrease in social interest in the free social interaction task (Kosel, Torres Munoz, et al., 2019); however, we were unable to test males in that study due to high levels of home-cage aggression observed in male transgenic-only cages, necessitating separation of animals due to injury and preventing us from suitably maintaining group-housing conditions.

Previous research has indicated that the 5xFAD background strain (C57BL6/SJL) 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; Lyon et al., 1996). However, we observed that high levels of home-cage aggression occurred predominantly in cages housing transgenic 5xFAD males together, whereas cages containing only male wild-type C57BL6/SJL littermates exhibited minimal injurious behaviour up to 12 months of age. Moreover, our personal observations suggested that injurious behaviour typically occurred with minimal warning, often progressing from normal home-cage aggression to injury within 24—48 hours. This pattern of rapid escalation between transgenic cage-mates, while anecdotal, suggested the possibility of *indirect genetic effects* (also termed *social genetic effects*) wherein an individual's phenotype is affected by the genotype of a social partner (Baud et al., 2017). However, this data is largely anecdotal, and to-date there are no comprehensive studies examining aggression in this strain. Additionally, while our previous study indicated an age-related decrease in social interest by transgenic 5xFAD females, it is unclear whether similar effects are observed in males, and it is unclear whether the use of mixed-genotype vs same-genotype dyads affects social approach behaviour.

Given the evidence for reduced social investigation and increased aggression in APP-overexpressing strains, as well as our personal observations of home-cage aggression in 5xFAD mice, the present study examined the influence of indirect genetic effects on home-cage behaviours, including aggression, in male 5xFAD mice. We also examined social investigation of novel same-sex mice within novel environments, and asked whether the genotype of the stimulus mouse affects exploration of that stimulus by

wild-type and transgenic, male and female 5xFAD mice. These aims were examined over two distinct experiments: *1)* home-cage observations were used to determine whether indirect genetic effects under different housing conditions (transgenic-only, wild-type only, or mixed-genotype) affected aggressive and affiliative behaviour towards cage-mates in male 5xFAD mice; and *2)* the three-chamber sociability test and free social interaction task were used to examine social behaviours in response to wild-type and transgenic stimulus mice in both male and female 5xFAD mice. Based on reports of aggression in transgenic 5xFAD males and observations in our own lab, we expected to see higher levels of home-cage aggression in cages containing only transgenic subjects, as well as increased aggression in transgenic males during the free social interaction task, particularly in response to transgenic stimulus animals. Additionally, based on our previous results in females, we expected to see decreased affiliative and social investigative behaviours in transgenic males and females in the three-chamber sociability test and free social interaction task, and reduced home-cage affiliative behaviours in transgenic male 5xFAD mice.

3.4 Methods

3.4.1 Animals

Male and female transgenic 5xFAD mice and wild-type litter-mate controls were used in this study. Mice were the progeny of male hemizygous C57BL/6J x SJL/J FN 5xFAD (B6SJL-Tg (APPSwFILon, PSEN1*M146L*L286V) 6799Vas/Mmjax) and female wild-type C57BL/6J x SJL/J F1 hybrid mice (B6SJLF1/J, JAX stock # 100012), and were bred in our colony. Male breeders were selected from existing in-house animals, while female breeders were obtained from Jackson Laboratories (Bar Harbor,

ME, USA); female F1 hybrids were used to maintain the colony on a common background based on recommended breeding practices from The Jackson Laboratory. Mice were ear-punched for identification and assigned Mouse ID numbers between P14 and P18, then weaned between P23 and P26.

Tissue samples from ear punches were used to genotype subjects for the PSEN transgene and the phosphodiesterase-6b retinal degeneration-1 ($Pde6b^{rd1}$) allele using standard PCR procedures and primers recommended by Jackson Laboratories (Bar Harbor, ME, USA). Mice based on the C57BL6/SJL strain exhibit a recessive $Pde6b^{rd1}$ allelle associated with retinal degeneration and vision loss (Brown & Wong, 2007; Yassine et al., 2013). 5xFAD mice are also available on a congenic C57BL/6J background that does not exhibit this allelle (MMRRC stock # 34848), but these mice were not used because they exhibit delayed Aβ pathology, with a trend towards reduced Aβ pathology at 2, 4, and 6 months of age, and significantly lower levels of pathogenic Aβ at 9 months of age, relative to transgenic 5xFAD mice on the C57BL6/SJL background used in the present study (Valenzuela, 2012).

Two separate cohorts of mice were used to measure home-cage behaviours (Cohort 1) and social investigation in novel environments (Cohort 2). Cohort 1 included only male 5xFAD mice because this experiment was designed to primarily assess aggression and injurious behaviours that are not observed in females. Cohort 2 included both male and female 5xFAD mice. See following sections for details.

All mice were housed on an inverted 12:12 light:dark cycle, with lights off from 09:30 – 21:30. Mice were group-housed in same-sex, same-litter cages of 2-3 animals, with standard housing conditions consisting of standard polysulfone 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), two black, opaque, polymer enrichment tubes (4 cm diameter, approx. 8 cm long), and a metal cage top containing *ad libitum* food (Laboratory Rodent Diet #5001; Purina LabDiet, St. Louis, MO, USA) and filtered tap water. Cages were topped with a micro-isolator filter to reduce the spread of airborne contaminants and diseases. Cages were changed weekly, just prior to the end of the light cycle. Note that cages used to assess home-cage behaviours differed from the standard cages indicated above; additional housing details for home-cage observations are provided in Section 3.4.2.2.

Subjects homozygous for the recessive $Pde6b^{rd1}$ allele were not used to ensure that visual impairments did not affect results. Singly-housed subjects and those exhibiting stereotypic behaviour (e.g., continuously running in circles, doing backflips, jumping in one corner of the cage) were excluded from testing. Due to the possibility of home-cage aggression, subjects were monitored regularly for general health and to check for injuries; animals exhibiting signs of injury were immediately separated from cage-mates and removed from testing, although data collected up to that point was included in the final analysis. All experimental procedures were performed in accordance with the guidelines published by the Canadian Committee on Animal Care and were approved by the Dalhousie University Committee on Laboratory Animals (Protocols #16-021 and #18-102).

3.4.2 Cohort 1: Home-cage Behaviours in Male 5xFAD Mice

3.4.2.1 Subjects

Forty-eight male subjects were pair-housed in either single-genotype transgenic

(containing two transgenic males), single-genotype wild-type (containing two wild-type males), or mixed-genotype (containing one transgenic and one wild-type male) cages; a total of 9 transgenic-only cages, 8 wild-type only cages, and 7 mixed-genotype cages were used. Home-cage observations for Cohort 1 were performed between 8 weeks (2 months) and 23 weeks (5.25 months) of age; observations were stopped at 23 weeks of age due to low numbers of remaining transgenic-only cages.

3.4.2.2 Recording Conditions, Apparatus, and Schedule

At approximately 7.5 weeks of age, subjects were transferred into recording cages and moved into their own colony room, specifically used for home-cage recordings (3 x 3.5 m). This room contained two metal cage racks (1.2 x 0.5 x 1.5 m tall) against two adjacent walls, each with four shelves plus the top panel. Cages were placed with the broad side facing the front of the rack, with up to three cages per shelf. Video camcorders (Canon Vixia HF R800; Canon USA Inc, Melville, NY, USA) on tripods were used for recordings; these cameras were located 1.2 m from the rack and slightly above the cages. The room was lit by two ceiling-mounted white fluorescent lights, as well as clamp-mounted desk lamps containing red bulbs at the ends of each shelf. Red lights were on a 12:12 light:dark schedule, offset from the white light schedule by -6 hours (lights on at 15:30, lights off at 03:30), and were used to facilitate video recordings during the dark period; these lights were aimed towards the wall to avoid direct light and heat into the cages.

Recording cages consisted of a standard rat cage (47 x 26 x 21 cm; Allentown Caging Inc., Allentown, NJ, USA) containing wood-chip bedding (FreshBed), two black, opaque, polymer enrichment tubes (4 cm diameter, approx. 8 cm long), and two flat-

bottomed ceramic bowls (approx. 10 cm diameter x 4 cm high) for food (Laboratory Rodent Diet #5001) and filtered tap water; food and water were provided *ad libitum*. Enrichment tubes were held together in parallel via nylon cable ties to reduce the likelihood of tubes shifting and obscuring activity. In order to maximize visibility, metal cage tops and micro-isolator filter lids were not used, and cages were covered with clear acrylic tops containing ventilation holes.

Recordings were made every second week starting at 8 weeks (2 months) of age and ending at 23 weeks (5.25 months) of age. Subjects were recorded for 3 days per week, every second week; 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). All recordings began approximately 4.5 hours prior to the end of the dark phase, and ended approximately 30 minutes prior to the light phase, for a total of 4 hours per day. During non-recording weeks, subjects were placed in clean cages identical to the recording cages to reduce stress associated with changing housing conditions. Clean food and water bowls were also provided with cage changes, while enrichment tubes and cage covers were washed with Fisherbrand Sparkleen 1 (Fisher Scientific, Pittsburgh, PA, USA) and room-temperature water, rinsed thoroughly with hot water, and dried with paper towels.

3.4.2.3 Scoring of Behaviours

Videos were manually scored by experimenters blind to treatment on a per-cage basis. Cages were assigned ID numbers based on Mouse ID numbers; as Mouse ID numbers were assigned prior to genotyping, it was not possible to identify cage composition by ID number alone. Videos were scored for the number of instances of aggressive encounters, including attack (initiating biting and/or rolling), chase (rapid

locomotion towards another animal that is rapidly moving away), mount (mounting another animal from behind and thrusting the pelvis), defensive posture (standing upright on the hindlimbs with the forelimbs raised up, and the head directed away from the other animal), and induced flee (rapid locomotion away from another animal that is chasing it). Videos were also scored for allogrooming (all licks given by one animal to another), aggressive grooming (one animal holds the other down and forcibly grooms 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 at least 10 seconds consecutively). Due to aggressive encounters often resulting in several instances of the same behaviours, and the complementary nature of certain behaviours (such as chase and induced flee), scoring each behaviour individually could artificially inflate the number of aggressive encounters. In order to prevent this, scoring of aggressive encounters based on a hierarchical scale was adapted from (Williamson et al., 2016), using the following rankings: attack > chase > mount > defensive posture > induced flee. Aggressive encounters were scored by recording the highest-level behaviour observed during a single encounter; e.g., if one animal attacked the other, which resulted in a flee and chase, this was scored 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 or self-grooming). Due to the hierarchical scoring of aggressive behaviours, total aggressive (attack, chase, mount) and defensive (defensive posture, induced flee) behaviours were analyzed, as well as the total number of all aggressive encounters (including both aggressive and defensive behaviours), in order to assess whether the

overall number of aggressive encounters differed across cage compositions. Scoring of behaviours was performed by time-sampling, as previously discussed by (Arakawa et al., 2007); briefly, instances of the above behaviours were scored for a 30-second period every 10 minutes for the duration of the recording periods.

In addition to frequencies of behaviours, separation of cage-mates was also tracked for survival data. As aggression is expected in the formation of dominance hierarchies (Miczek et al., 2001; Williamson et al., 2016), observation of aggressive behaviours during routine checks was not considered sufficient for separation. These attacks are considered to be a typical social behaviour and are not intended to break the skin (Grant & Mackintosh, 1963), so the presence of injuries was indicative of excessive or abnormal home-cage aggression, and animals were separated immediately after any injuries were observed.

3.4.3 Cohort 2: Social Investigation Within Novel Environments in 5xFAD Mice

3.4.3.1 Subjects

Twenty-seven male (12 transgenic, 15 wild-type) and 21 female (9 transgenic, 12 wild-type) subjects were used to assess social approach behaviours in the three-chamber sociability test and free social interaction task. Mice were housed in mixed-genotype cages of 2–3 and were tested at 26 weeks (6 months) of age. Based on the results from the home-cage observations in Cohort 1, mixed-genotype housing was used to avoid aggression between males and to maintain consistent housing conditions between males and females. Stimulus conspecifics were housed under identical conditions.

3.4.3.2 Testing Conditions and Schedule

Social approach behaviour and aggression in a novel environment were measured

using the three-chamber sociability apparatus and free social interaction task. Mice were tested in a quiet 2.4 x 2.4 m room containing a desk with a computer, and a table for the testing apparatus. Tasks were performed under red light illumination and were recorded using the Biobserve Viewer 3.0 program (Biobserve GmbH, Bonn, Germany). Subjects and stimulus animals were transferred into clean, separate holding cages in the colony room, and were moved to the testing room at the time of testing. After testing, animals were returned to the colony room, and were returned to their home-cages once all cage-mates had completed testing.

Mice were tested over 11 days, with testing sessions taking place in the latter half of the dark phase. 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. Each subject underwent two testing sessions for each task in order to test approach behaviour to both wild-type and transgenic stimulus conspecifics; the presentation of each stimulus genotype was counterbalanced across sessions and subjects. Subjects were tested in order of Subject ID number.

3.4.3.3 Three-chamber Sociability Test

Apparatus: The apparatus was a three-chamber box as previously described (Kosel, Torres Munoz, et al., 2019). In brief, it consisted of a clear, acrylic box (69 x 20 x 20 cm) divided into three equal-sized compartments by two clear acrylic walls. Floor-level openings in the middle of the dividing walls allowed access between the chambers

and could be blocked by removable barriers. The floor was covered in the same wood-chip bedding as used in home-cages. Stimuli (novel same-sex transgenic 5xFAD mice and wild-type controls) were contained in two round wire cages (Galaxy Cup; Spectrum Diversified Designs Inc., Streetsboro, OH, USA) topped with white, opaque, 500 mL HDPE bottles (Nalgene Nunc International Corporation, Rochester, NY, USA) filled with water to prevent subjects from climbing on top of cages, with one Galaxy Cup in each of the two outer chambers. Stimulus mice were habituated to the apparatus and Galaxy cups for two 15 min sessions prior to testing (day before and day of testing).

Procedure: Testing was performed on two separate days. Subjects were habituated to the testing room and the testing arena simultaneously. Each day consisted of a 5-minute habituation trial, followed by a 10-minute testing trial, with a 1-minute intertrial interval. Subjects were placed into the central chamber, and both barriers were removed simultaneously to allow access to all three chambers. Subjects were allowed to freely explore for 5 minutes, at which point they were returned to the central chamber and the barriers were replaced. Subjects remained in the central chamber during the one-minute intertrial interval while a novel stimulus animal was placed under the Galaxy Cup in one of the outer chambers; the Galaxy Cup in the opposite chamber remained empty. Following the intertrial interval, the barriers separating the chambers were again removed, and subjects were allowed to explore freely for 10 minutes. Habituation was performed with no stimuli under either of the Galaxy Cups, while testing consisted of a single stimulus animal contained within a Galaxy Cup in either the left or right chamber; the genotype and location (left or right chamber) of the stimulus animal was counterbalanced across subjects and testing sessions. The apparatus was cleaned between

each subject and session using Fisherbrand Sparkleen 1 (Fisher Scientific) and room-temperature water, then dried thoroughly using paper towels.

Scoring: As Subject ID numbers were assigned prior to genotyping, and subjects were tested in order of Subject ID number, it was not possible to determine the genotype of an animal by ID number alone. All videos were only identified by Subject ID and session number (1 or 2) to ensure that experimenters were blind to the genotypes of the subject and the stimulus animals during both testing and manual scoring of videos. Videos were manually scored for the duration (in seconds) of time spent in each chamber, as well as time spent investigating the cages. Subjects were considered to have entered a chamber once their head and forelimbs had passed the threshold. Investigation of cages was defined as orientation of the snout towards the Galaxy Cups, with the snout 1 cm or less away from the Galaxy Cup. Investigation duration was used to calculate a preference ratio for the cage containing the stimulus animal over the empty cage; this was calculated by dividing the time spent investigating the social cage over the total investigation time of both cages. To ensure that preference ratios were not affected by overall differences in exploration, total investigation time in both chambers was also calculated. As investigation time is a better indicator of sociability than time spent in chamber (Fairless et al., 2011), all analyses were performed using investigation time.

3.4.3.4 Free Social Interaction

Apparatus: The apparatus was as previously described (Kosel, Torres Munoz, et al., 2019). Briefly, the apparatus consisted of a 38 x 38 x 40 cm arena with the floor raised 5 cm from the base. Three of the walls were made of plywood and painted beige, with the fourth wall being made of clear acrylic to allow for recording from the side. The

floor was made of clear acrylic to facilitate cleaning between animals, and a black, opaque sheet was placed immediately below to make it appear solid. Stimuli were novel same-sex transgenic 5xFAD mice and wild-type controls; stimulus mice were habituated to the apparatus prior to testing for two 15 min sessions (day before and day of testing).

Procedure: The free social interaction task was performed after the three-chamber sociability test. Testing was performed on two separate days. Subjects were habituated to both the testing room and the testing arena simultaneously. Each day consisted of a 5-minute habituation period, followed immediately by a 10-minute trial period. Subjects were placed in the arena at the start of habituation and were allowed to freely explore for 5 minutes; at the end of the habituation phase, subjects remained in the arena while a novel stimulus animal was introduced into the arena, and both animals were then allowed to freely explore for 10 minutes. The genotype of the stimulus animal was counterbalanced across subjects and testing sessions. The apparatus was cleaned between each subject and session using Fisherbrand Sparkleen 1 (Fisher Scientific) and room-temperature water, then dried thoroughly using paper towels. Due to the possibility for aggression during this task, the researcher remained in the room during the entire session. Animals were physically separated if fighting was observed continuously for more than 30 seconds, and the test was ended if fighting re-occurred after separation, or if 5 attacks were made by either animal.

Scoring: Experimenters were blind to the genotypes of the subject and stimulus conspecific, as described for the three-chamber sociability test. Videos were scored manually for the duration (in seconds) of investigative (follow; orofacial sniffing; anogenital sniffing), aggressive (chase; mount; attack), defensive (induced flee; defensive

posture), and self-grooming behaviours. Aggressive and defensive behaviours (as described above), and investigative and self-grooming behaviours, as previously described (Kosel, Torres Munoz, et al., 2019) were quantified. All behaviours were adapted from (Arakawa et al., 2007; Grant & Mackintosh, 1963).

3.4.4 Data Analyses

Data analyses were performed using multilevel modelling in R version 3.6.1 (R Foundation for Statistical Computing) and RStudio version 1.2.1335 "*Action of the Toes*". For home-cage observations, analyses were performed on each behaviour separately, using cages as the subjects; cage composition (transgenic-only, wild-type-only, or mixed-genotype) was used as the between-subjects factor, while week was used as the within-subjects factor. Cage survival was examined using bootstrapped 95% confidence intervals. For social approach and free social interaction, subject genotype and sex were used as the between-subjects factors, while stimulus genotype was used as the within-subjects factors. Analyses were performed separately for each behaviour in free social interaction. As females in this study, and our previous research (Kosel, Torres Munoz, et al., 2019), exhibit almost no aggressive behaviours, genotype effects for aggressive and defensive behaviours were examined for males only in order to prevent female data from skewing the results. Two cages of male subjects (n = 2 transgenic, 2 wild-type) were separated after the three-chamber sociability test, but prior to free social interaction testing, due to injuries arising from home-cage aggression. Three free social interaction sessions with male subjects were ended early due to aggressive behaviours; two of these involved the same wild-type subject (once with a wild-type stimulus conspecific, once with a transgenic stimulus conspecific), and the third involved a wild-

type subject and a wild-type stimulus conspecific. For these sessions, the length of the session was calculated as a percentage of the total session duration, and the duration of observed behaviours was extrapolated for the remaining time. With the exception of cage survival, significant effects were examined using 95% confidence intervals of Cohen's d.

3.5 Results

3.5.1 Home-cage Observations

Analyses of home-cage behaviours in males from 8 to 23 weeks (2—5.25 months) of age indicated no main effects of cage composition (genotype) or age, and no interaction, for any behaviours (see Figure 3.1 for the most prevalent home-cage behaviours). For cage survival, a median of 67% (95% CI [33.33%, 88.89%]) of transgenic-only cages survived together until 15 weeks of age, whereas wild-type-only cages did not see any separation until 18 weeks of age (median 85.71%, 95% CI [57.14%, 100.00%] for survival at 18 weeks of age; see Figure 3.2). Mixed-genotype cages did not see separation until 20 weeks of age (median 85.71%, 95% CI [57.14%, 100.00%] for survival at 20 weeks of age). By 23 weeks of age, only 33% (95% CI [0.00%, 66.67%]) of transgenic-only cages remained together, whereas 71% (95% CI [42.86%, 100.00%]) of wild-type-only and mixed-genotype cages remained together.

3.5.2 Three-chamber Sociability Test

Analyses of social approach behaviour in the three-chamber sociability test by males and females at 26 weeks (6 months) of age indicated no main effects or interactions of subject genotype, sex, or stimulus genotype for preference ratio or total investigation time of both social and non-social stimuli (see Figure 3.3). Analysis of investigation duration of each stimulus type (social or non-social) indicated a main effect

of stimulus (χ^2 (1) = 292.32, $p < .001$), with subjects exhibiting longer investigation durations for social over non-social stimuli (data not shown); there were no other main effects or interactions of subject genotype, sex, stimulus genotype, or stimulus type for investigation duration.

3.5.3 Free social Interaction

A total of four male subjects (n = 2 transgenic, 2 wild-type) were removed from the study prior to testing free social interaction due to injuries sustained from home-cage aggression. Additionally, as female mice in this study and our previous research (Kosel, Torres Munoz, et al., 2019) exhibit almost no aggressive behaviours, genotype effects for aggressive and defensive behaviours are provided for males only.

Analyses of investigative and self-grooming behaviours during free social interaction in males and females at 26 weeks (6 months) of age indicated a main effect of subject genotype for orofacial sniffing (χ^2 (1) = 12.86, $p < .001$), anogenital sniffing (χ^2 (1) = 4.77, p = .029), and following (χ^2 (1) = 4.34, p = .037) with transgenic 5xFAD mice exhibiting reduced durations of these behaviours compared to wild-type controls (see Figure 3.4). There was also a main effect of sex for anogenital sniffing (χ^2 (1) = 4.94, p = .026) and following (χ^2 (1) = 10.45, p = .001), with males exhibiting reduced durations compared to females. There were no other main effects or interactions of subject genotype, sex, or stimulus genotype for investigative or self-grooming behaviours. Analyses of aggressive behaviours during free social interaction in male 5xFAD mice at 26 weeks (6 months) of age indicated no main effects or interactions of subject genotype or stimulus genotype for aggressive (attack, chase, mount) or defensive (defensive posture, flee) behaviours.

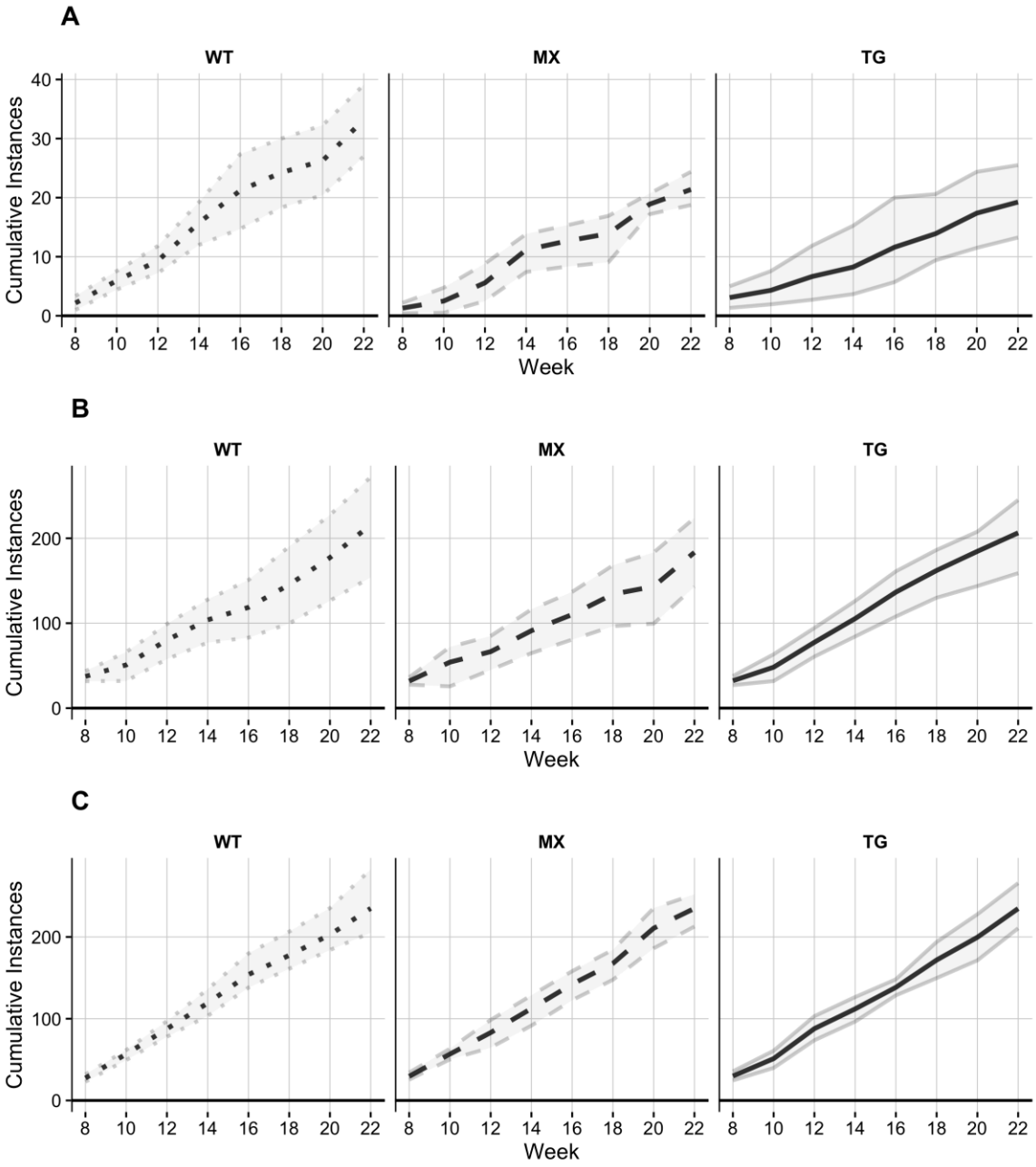

Figure 3.1: 5xFAD home-cage behaviours for wild-type-only (WT), mixed-genotype (MX) and transgenic-only (TG) cages. Median and 95% CI for cumulative incidences of ***(A)*** total aggressive encounters, ***(B)*** time alone, and ***(C)*** huddle in WT, MX, and TG pair housing. n = 7 WT, 7 MX, and 9 TG cages at start. Black lines represent median values; grey ribbon indicates 95% CI.

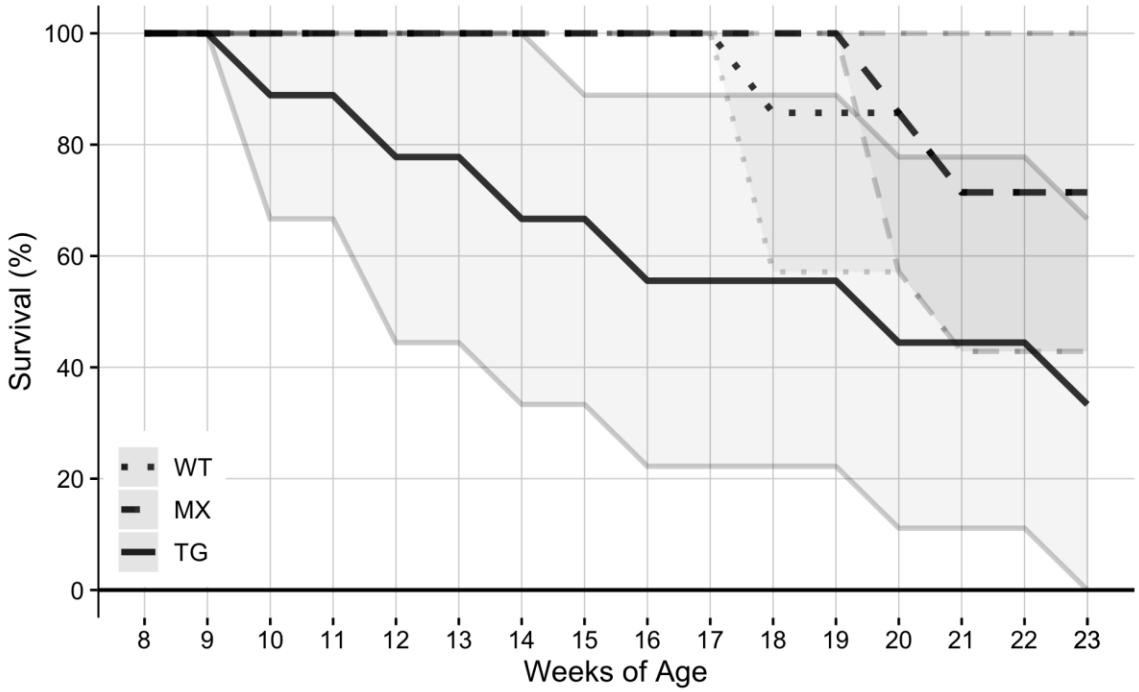

Figure 3.2: Home-cage survival per week for wild-type-only (WT), mixed-genotype (MX), and transgenic-only (TG) cages. n = 7 WT, 7 MX, and 9 TG cages at start. Black lines represent median values; grey ribbon indicates 95% CI.

3.6 Discussion

Anecdotal reports in our lab and others have suggested that housing conditions (specifically, the use of single- or mixed-genotype group housing) can affect home-cage aggression in male 5xFAD mice. As a result, we examined how the development of affiliative and aggressive home-cage behaviours is affected by cage composition in these mice. Contrary to our hypotheses, we found that single-genotype cages housing only transgenic males do not exhibit differences in the number of aggressive or affiliative home-cage behaviours compared to male wild-type-only or mixed-genotype cages. However, male transgenic-only cages do result in more injurious behaviour, with these cages requiring earlier separation due to injuries compared to male wild-type-only or

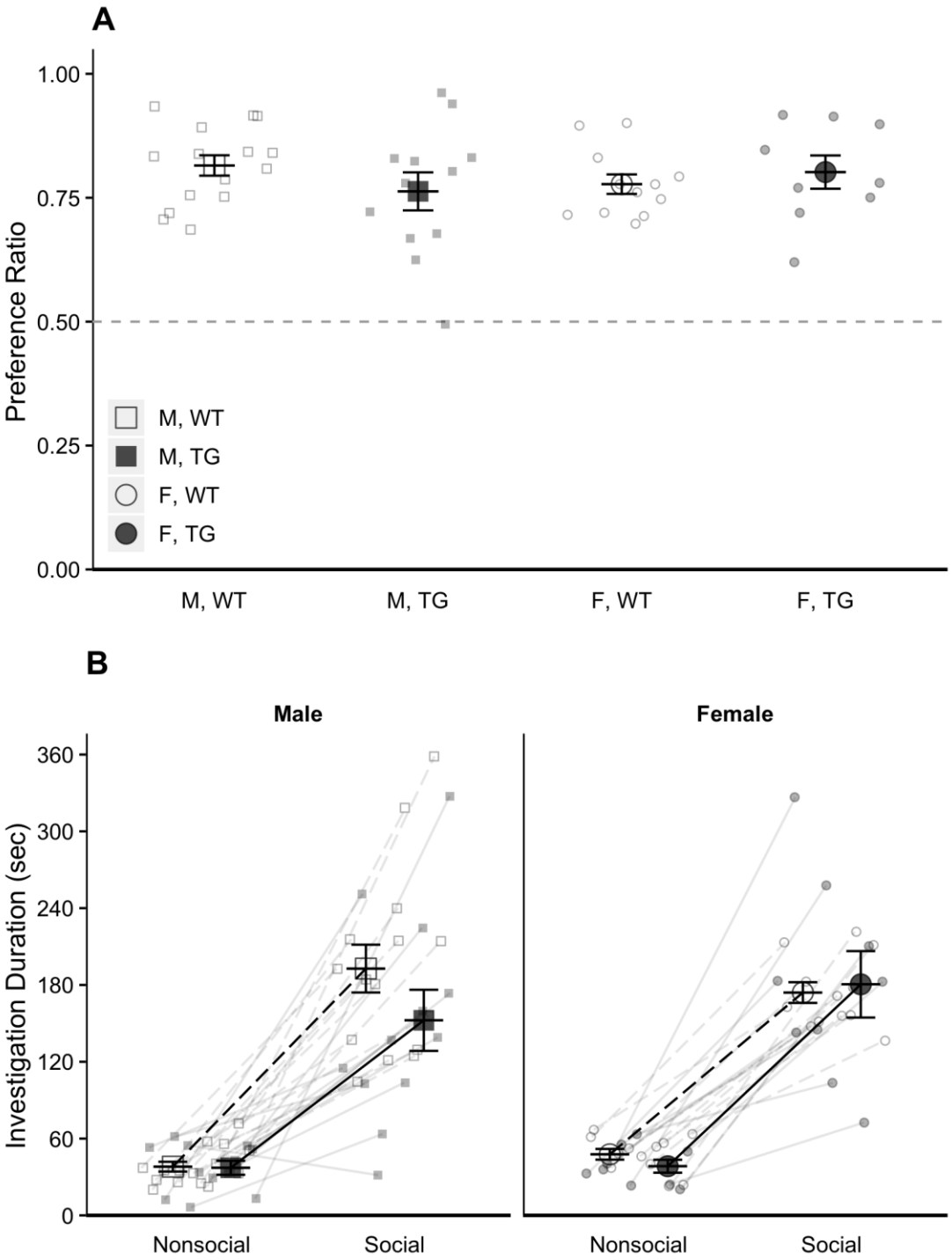

Figure 3.3: Investigation in the three-chamber sociability test for wild-type (WT) and transgenic (TG) 5xFAD males (M) and females (F). Raw data, means, and standard error for ***(A)*** preference ratio in the social approach task and ***(B)*** investigation duration (in seconds) of social and non-social stimuli. As there were no significant effects for stimulus genotype, data shown is averaged across both stimulus genotypes. Small points represent raw data; large points represent means; error bars indicate standard error. n = 15 M WT, 12 M TG, 12 F WT, 9 F TG

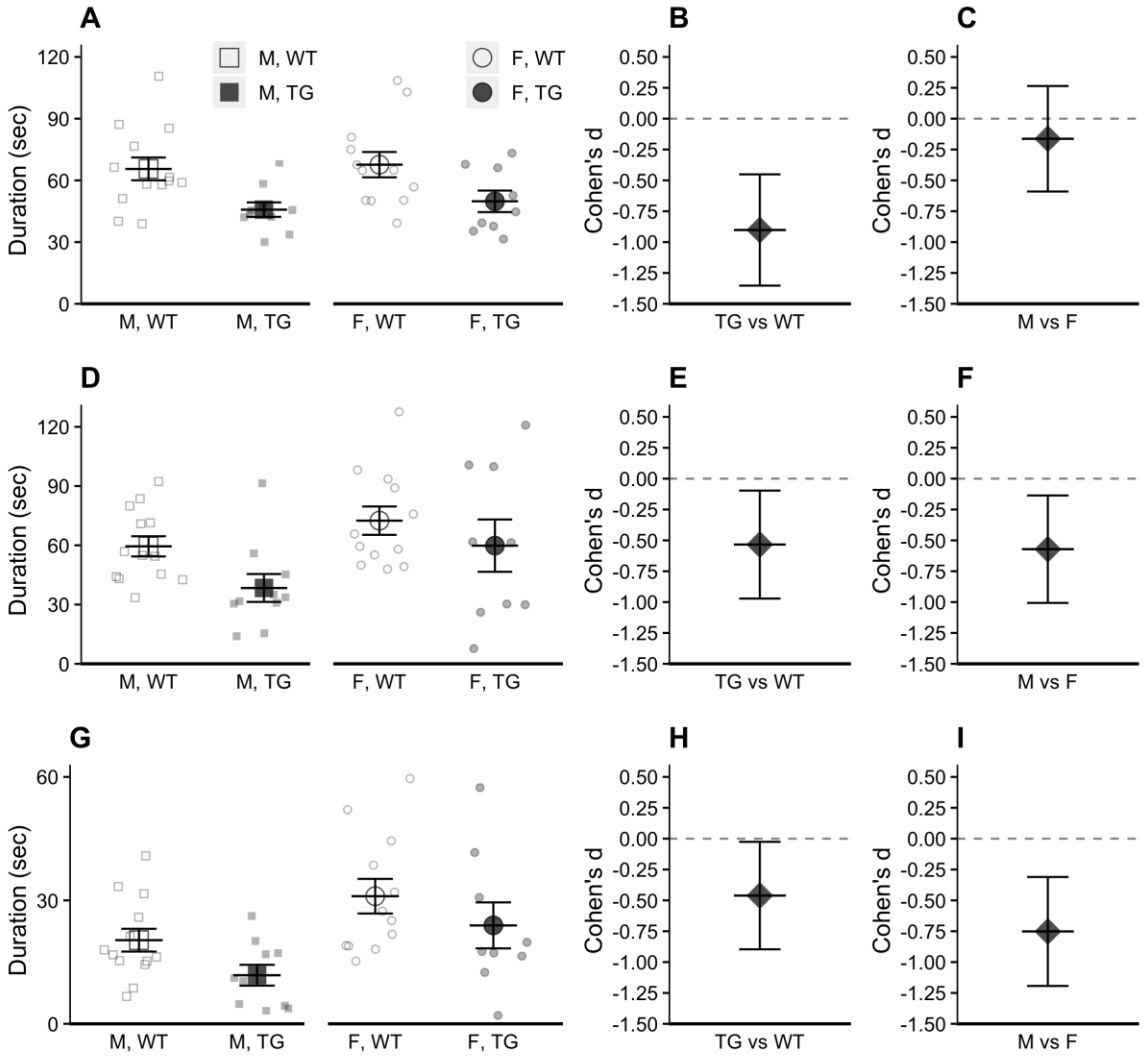

Figure 3.4: Investigation in the free social interaction task for wild-type (WT) and transgenic (TG) 5xFAD males (M) and females (F). For orofacial sniffing, ***(A)*** raw data and 95% CIs of Cohen's d for ***(B)*** genotype effects and ***(C)*** sex effects. For anogenital sniffing, ***(D)*** raw data and 95% CIs of Cohen's d for ***(E)*** genotype effects and ***(F)*** sex effects. For following, ***(G)*** raw data and 95% CIs of Cohen's d for ***(H)*** genotype effects and ***(I)*** sex effects. Due to no significant effects for stimulus genotype, data shown was averaged across both stimulus genotypes. For raw data graphs, small dots indicate raw data, large dots indicate means, error bars indicate standard error. For Cohen's d, diamonds represent median, error bars indicate 95% CI. *n* = 15 M WT, 12 M TG, 12 F WT, 9 F TG.

mixed-genotype cages, consistent with previous observations. In addition, we measured social investigation by male and female 5xFAD mice, and determined whether genotype of a stimulus conspecific plays a role in these behaviours during novel social interactions, expanding upon our previous research in female mice (Kosel, Torres Munoz, et al., 2019). While transgenic 5xFAD males show normal affiliative behaviours in the home-cage, transgenic males and females exhibited reduced social investigation of a novel social stimulus in the free social interaction task, with males exhibiting overall lower durations of anogenital sniffing and following relative to females. This decrease in social investigation was not dependent on the genotype of the social stimulus and was also not observed in the three-chamber social approach task, when the social stimulus is placed behind a barrier.

3.6.1 Transgenic 5xFAD Males Require Earlier Home-cage Separation Due to Injuries, But Do Not Exhibit More Aggressive Encounters Than Wild-type Controls

Overall, transgenic 5xFAD males did not initiate more aggressive encounters than wild-type controls. There were no differences in the number of aggressive encounters observed within transgenic-only cages compared to wild-type-only or mixed-genotype cages (Figure 3.1a), and there were no genotype effects in the number of aggressive behaviours exhibited during the free social interaction task. However, males housed in transgenic-only cages required earlier separation from cage-mates due to injuries sustained during home-cage aggression. This occurred despite no obvious increase in aggression during routine checks prior to injuries arising, indicating that the progression from normal aggression to injurious behaviour occurred within 24—48 hours.

Additionally, our findings show that being housed with a wild-type mouse mitigates the injurious behaviour observed in transgenic-only cages, suggesting that the behaviour of both the aggressor and target lead to these injuries. Overall, the involvement of two transgenic 5xFAD males and the rapid development indicates that indirect genetic effects are likely responsible for the rapid escalation of aggressive behaviours.

These results confirm previous anecdotal observations in our lab indicating that transgenic 5xFAD males cannot be reliably housed together, particularly in single-genotype cages. While characterizing the progression of social deficits in 5xFAD mice from 3—12 months of age, we attempted to house subjects in single-genotype cages (as previously reported in (Kosel, Torres Munoz, et al., 2019); however, we found that transgenic males could not be housed together past 6 months of age, with the majority of cages being separated between 3 and 5 months of age due to injuries sustained in the home-cage. Interestingly, while the results of the present study indicate that 5xFAD males housed in transgenic-only cages require early separation due to home-cage aggression, they did not exhibit an overt increase in overall aggressive encounters. Similarly, transgenic 5xFAD males did not exhibit increased aggressive behaviours towards novel stimulus males in a novel environment during the free-social interaction task, suggesting that the injuries observed in the home-cage may be due to increased intensity of aggressive encounters, rather than an increased frequency.

The heightened home-cage aggression observed in transgenic 5xFAD mice may also be contributed to by their background strain, as high levels of home-cage aggression have been reported in the C57/B6SJL strain (Kuby, 1997; Lyon et al., 1996). However, high levels of male-male and male-female home-cage aggression have also been reported

for transgenic animals from three human APP-overexpressing lines (wild-type, London, and Swedish mutations) on the FVB/N background strain (Moechars et al., 1998), and wild-type 5xFAD littermates did not show the same level of injurious behaviour, suggesting that amyloid pathology—rather than background strain—may be the critical factor in increased aggression in these animals. The onset of injurious behaviours also correlates with the approximate presentation of neurodegeneration in transgenic 5xFAD mice, with a reduction in whole-brain synaptophysin evident by 4 months of age, and neurodegeneration evident in cholinergic neurons, as well as pyramidal neurons in Layer V of the cerebral cortex, by 6 months of age (Crowe & Ellis-Davies, 2014; Devi & Ohno, 2010; Oakley et al., 2006).

Further evidence for increased aggression in transgenic AD model mice has been found in the resident-intruder paradigm; increased aggression was observed in 7-month-old transgenic Tg2576 males (G. Alexander et al., 2011), 6- and 12-month-old APP23 transgenic males (Vloeberghs et al., 2006), and 2.5- to 3-month-old males from APP-overexpressing lines (wild-type, London, and Swedish; (Moechars et al., 1998). While the present results do not fully recapitulate the increase in aggressive behaviours observed in other strains, it is important to note that methodological differences may play a key role. Aggression in mice is commonly seen between two unfamiliar conspecifics, and typically serves two purposes (Brain & Parmigiani, 1990; Miczek et al., 2001; Williamson et al., 2016): *1)* to establish a dominance ranking between two conspecifics; and *2)* to drive an intruder out of an owned territory. The resident-intruder paradigm is designed to create the second situation, wherein an unfamiliar male (the intruder) is introduced into the owned (familiar) territory (the home-cage) of the resident.

Additionally, the social isolation housing required for the resident intruder paradigm creates a chronic stress condition that itself increases aggression, in addition to causing a number of behavioural and physiological changes (Goldsmith et al., 1976; Koike et al., 2009; Matsumoto et al., 2005). By comparison, the present study examined aggression between familiar conspecifics in a familiar environment (home-cage observations), and novel conspecifics in a novel environment (free social interaction), and used group-housed animals. Under similar novel conditions, (Bories et al., 2012) examined social interactions using unfamiliar 3xTg-AD mixed-genotype dyads in a novel environment, and reported no aggressive behaviours in males or females at 12 or 18 months of age.

Overall, our findings suggest that indirect genetic effects—phenotypic changes arising from the genotype of a social partner—are responsible for the development of injurious home-cage behaviour in transgenic 5xFAD males. The rapid escalation and mitigating effects of wild-type cage-mates suggest that this behaviour is due to environmental factors driven by their transgenic cage-mates, and is not the result of increased aggression *per se* arising from AD-related pathology. This is further supported by the finding that transgenic 5xFAD males do not exhibit increased aggression in novel environments relative to wild-type controls. However, because transgenic 5xFAD single-genotype cages require separation due to injury earlier than wild-type-only or mixed-genotype cages, laboratories using single-genotype housing conditions for 5xFAD mice must be aware of a possible selection bias that leads to surviving cages being only a particular sub-group of the transgenic 5xFAD mice.

3.6.2 Transgenic 5xFAD Mice Exhibit Reduced Investigation of Novel Free-roaming, But Not Restrained, Conspecifics

Compared to wild-type controls, 6-month-old transgenic 5xFAD males and females exhibited decreased investigation time of novel conspecifics in the free social interaction task, exhibiting shorter durations of orofacial sniffing, anogenital sniffing, and following (Figure 3.4). However, this deficit was a specific response to a freely-roaming social stimulus, as transgenic males and females exhibited no differences in preference ratio in the three-chamber sociability task when the social stimulus is contained (Figure 3.3B). Because both the three-chamber sociability test and the free social interaction test use novel conspecifics in a novel environment, we propose that the differing effects between the two tasks is due to the presence of free-roaming vs restrained conspecifics, which likely impact the arousal levels of the experimental animal. Additionally, the genotype of the stimulus mouse had no effect on investigative behaviour, further suggesting that the behaviour of the stimulus mouse has little effect on the behaviour of the experimental mouse in either task, and that differences in arousal levels of the stimulus mouse are not a contributing factor.

Results presented here mirror those previously observed in 5xFAD females, where transgenic females at nine months of age exhibit reduced orofacial sniffing, anogenital sniffing, and following behaviours in the free social interaction task, but no difference in preference ratio or social investigation time in the three-chamber apparatus (Kosel, Torres Munoz, et al., 2019). Moreover, previous findings in transgenic females also exhibited a strong trend towards reduced investigative behaviours during the free social interaction task at six months of age, similar to the effects observed in the present

study, suggesting that these behaviours can be observed even earlier than previously reported. The previous study (Kosel, Torres Munoz, et al., 2019) used same-genotype housing conditions, rather than the mixed-genotype housing conditions used in the present study; similar results from 5xFAD females in either housing conditions suggest that indirect genetic effects related to home-cage social environments do not play a role in social approach behaviour to a novel conspecific in a novel environment.

The contribution of altered olfaction to the social deficits observed in transgenic 5xFAD mice here cannot be entirely ruled out because transgenic 5xFAD males exhibit decreased glucose metabolism in the olfactory cortex by three months of age and increased latency to locate a buried food pellet by six months of age (Xiao et al., 2015). However, transgenic 5xFAD males and females exhibit no differences in olfactory sensitivity and no impairments during an olfactory delayed matching-to-sample task relative to wild-type controls (Roddick et al., 2014, 2016). The differing results of these two studies may have arisen due to variations in reward motivation and the tasks used (i.e., food restriction for 3 days in Xiao *et al* (2015) and water restriction for 10 days in Roddick *et al* (2014, 2016)). Additionally, while our previous study indicated reduced investigation of social odours by transgenic 5xFAD females relative to wild-type controls, transgenic females were able to detect non-social odours similarly to wild-type mice, and were also able to appropriately increase their scent-marking in response to either social or non-social odour cues similarly to wild-type controls (Kosel, Torres Munoz, et al., 2019). Overall, the evidence suggests that transgenic 5xFAD males and females do not exhibit olfactory deficits, and that reduced social investigation in transgenic females is not due to an inability to identify social odours.

Despite evidence of altered social interaction in other transgenic mouse models of AD (Bories et al., 2012; Faizi et al., 2012; Filali et al., 2011a), no consistent trend in social investigation across transgenic mouse models of AD has been indicated, and it is unclear how AD pathology specifically contributes to these behaviours. While this is consistent with the varying presentation of social withdrawal in human AD patients (Lyketsos et al., 2002; M. Zhang et al., 2012; Zhao et al., 2016), it is important to note that transgenic mouse models of AD recapitulate differing aspects of AD pathology (Bilkei-Gorzo, 2014; LaFerla & Green, 2012), although transgenic Tg2576 females similarly exhibit reduced social investigation during free social interaction but normal behaviour in the three-chamber apparatus at 3 months of age (Pietropaolo et al., 2012). Together with the present study, these findings further highlight the differing responses in these two behavioural tests, and suggest that the free social interaction task may be more sensitive to differences in social interest than the three-chamber sociability test. We suggest that transgenic 5xFAD mice are able to recognize the relative safety of investigating a mouse behind a barrier but are unwilling to cross a certain arousal threshold in order to investigate a freely roaming mouse. Further work is required to identify the contribution of specific AD neuropathology to these deficits, including pinpointing the onset and progression of these deficits relative to AD pathology, and if they expand to include abnormal behaviours in the three-chamber sociability at more advanced stages, as previously shown in transgenic Tg2576 mice (Pietropaolo et al., 2012).

3.7 Conclusions

The present study indicates that cages containing only transgenic 5xFAD males

exhibit injuries arising from home-cage aggression at younger ages than wild-type-only or mixed-genotype cages, pointing to a significant contribution of indirect genetic effects to this behaviour and highlighting the need for careful consideration of housing conditions for this strain. The presence of a wild-type conspecific delays the onset of injurious behaviour, suggesting that during transgenic-transgenic aggressive encounters both the aggressor and target mouse exhibit altered behaviours, thereby leading to an escalation that results in injury. This has important implications for researchers working with mouse models that demonstrate social deficits, as housing conditions and perhaps the social stimulus presented can have significant impact on the animal's behaviour (Kalbassi et al., 2017). Our findings also show that 6-month old transgenic 5xFAD males and females are less motivated to explore a social stimulus, reminiscent of social withdrawal presented by AD patients. This is an earlier timepoint than previously reported in transgenic 5xFAD females (Kosel, Torres Munoz, et al., 2019). Further, the reduced social exploration observed in transgenic mice is context-specific, and is limited to high arousal situations with a free-roaming social stimulus, suggesting that transgenic 5xFAD mice may exhibit particular avoidance to perceived threats from conspecifics. However, the genotype of a novel social stimulus does not appear to mediate social exploration within a novel environment, suggesting that the reduced social exploration is due to intrinsic factors rather than as a response to the behaviours of the stimulus mouse.

3.8 Other Acknowledgements

The authors would like to thank J. Renee Yang and Aimee A. Wong for their technical support, and R.E. Brown for use of their behavioural apparatus.

Chapter 4. The Odour Delivery Optimization Research System (ODORS): An Open-source Olfactometer for Behavioural Assessments in Tethered and Untethered Rodents

4.0 Preamble

This work was submitted for review to eNeuro in May, 2023, and is currently in revision. Author contributions: *Conceptualization*: F.K. And T.B.F.; *Methodology*: F.K. and T.B.F.; *Investigation*: F.K.; *Formal Analysis*: F.K.; *Resources*: F.K. and T.B.F.; *Writing – Original Draft*: F.K.; *Writing – Review & Editing*: F.K. and T.B.F.; *Visualization*: F.K.; *Funding Acquisition*: T.B.F.

4.1 Abstract

Olfaction is a key sense in animals and the dominant sensory modality in rodents. It has been used to assess behavioural phenomena including stress, learning and memory, and social investigation, and impaired olfaction has been implicated in neurological disorders including Alzheimer's disease, Parkinson's disease, and autism spectrum disorder. Paradigms such as the olfactory habituation/dishabituation (OHD) task are an important tool in assessing olfactory perception, memory, and motivation; however, these tasks require manual stimulus presentation, introducing variability and making them labour-intensive to administer and score. While olfactometers allow automated stimulus delivery, the OHD task has not yet been adapted for use with an olfactometer. Additionally, current olfactometer designs require difficult-to-obtain components or proprietary software—making them difficult to integrate with existing lab hardware and software—and commercial units are expensive. As a result, these apparatuses have not

been widely implemented. Here, we describe the design and assembly of the Odour Delivery Optimization Research System (ODORS), an economical, modular, and open-source olfactometer for use in tethered or untethered rodents, and describe a variant of the OHD task that can be automated using this apparatus. Mice performing this task in the ODORS exhibit the characteristic habituation to repeated presentations of a given odour and dishabituation to the first presentation of a novel odour, and that these effects are evident with both manual and automatic scoring methods. As a result, we suggest that the ODORS makes improved olfactory testing accessible to many labs and offers a major refinement over existing OHD testing paradigms.

4.2 Significance Statement

Olfaction is a key modality in animals, and evidence suggests some neurological disorders (e.g., Alzheimer's disease, Parkinson's disease, autism spectrum disorder) are linked with impaired olfaction. However, paradigms used to study olfaction in rodent models, including the olfactory habituation/dishabituation task, are subject to variability introduced by the experimenter and are labour-intensive to administer and score. Additionally, automated olfactometers are expensive and difficult to integrate with existing testing systems due to proprietary hardware and software. The apparatus and paradigm described herein provides an accessible alternative to existing systems and tasks, allowing labs of all sizes to contribute to this valuable field of research.

4.3 Introduction

For many animals—including mice and rats—olfactory cues are key in identifying food and toxins, the presence of predators or prey, locating potential mates, and delineating territorial boundaries. Compared to the ~350 intact olfactory receptor

genes in humans, the mouse genome contains ~1000 intact genes, (X. Zhang & Firestein, 2002), and relative to other brain structures (e.g., somatosensory cortex or occipital lobe) the mouse olfactory bulb is larger than that of humans (McGann, 2017), suggesting the importance of this organ for survival outweighs the increased metabolic cost necessitated by its size.

Rodent studies with olfactory stimuli have examined a number of behavioural phenomena, including stress responses (L. D. Wright et al., 2013), learning and memory (Roddick et al., 2014), and social investigative behaviours (Kosel, Torres Munoz, et al., 2019), and the importance of odour cues in social behaviours is underscored by electrophysiological data (Levy et al., 2019). As a result, several behavioural paradigms and apparatuses have been developed to standardize and facilitate testing with olfactory stimuli. Tasks based on habituation to repeated presentations of odour stimuli (Cleland et al., 2002; Wesson et al., 2010; M. Yang & Crawley, 2009) offer simple, low-cost methods for assessing olfactory perception, memory, and motivation to investigate specific odours. However, these tasks are time- and labour-intensive to administer, and even with attempts to improve efficiency (Oummadi et al., 2020), available methods still require manual presentation of odour stimuli and introduce variability associated with the experimenter.

Olfactometers allow for automated and precise stimulus delivery, allowing administration of tasks such as the delayed matching-to-sample and olfactory sensitivity tasks (Roddick et al., 2014, 2016) and assessment of neural activity in response to specific stimuli (Levy et al., 2019). Currently-available olfactometers for animal testing fall into one of two categories: units which flood the testing chamber with odorized air

and use an exhaust fan/pump (Levy et al., 2019); and units which use positive pressure to restrict odorized air behind an odour port (Bodyak & Slotnick, 1999; Roddick et al., 2014, 2016). However, commercially-available units are expensive, and the use of proprietary hardware and software can preclude control of these systems directly from existing lab systems (e.g., behavioural, electrophysiological, or optogenetic software and equipment). While some previous publications describe the design and function of olfactometers (Bodyak & Slotnick, 1999; Burton et al., 2019; Fox & Thiessen, 1984; Rebello et al., 2015; Slotnick & Bodyak, 2002), these designs require components that may be difficult to obtain commercially or require advanced fabrication capabilities (e.g., borosilicate glass air manifolds, custom-made odorant vessels, or custom-machined holders for odorant tubes), or may be primarily designed for use with head-fixed animals (Burton et al., 2019; Rebello et al., 2015). Finally, tasks using stimulus odour concentrations based on simple dilution (M. Yang & Crawley, 2009) can lead to presentation of unknown odorant concentrations, while calculating final odorant concentrations using liquid- and vapour-phase concentrations of stimuli (Bodyak & Slotnick, 1999) can make it difficult to consistently test novel odorants where these values are unknown, thereby limiting the ability to rapidly and consistently test subjects across various odour stimuli.

Here, we describe the design, assembly, and use of the Olfactory Delivery Optimization Research System (ODORS), a simplified, low-cost olfactometer for accurate and automated delivery of olfactory stimuli. The design is based on five principles: 1) familiar layout and function; 2) use of inexpensive and readily-available components; 3) easily integrated, modular design; 4) real-time assessment of odorant

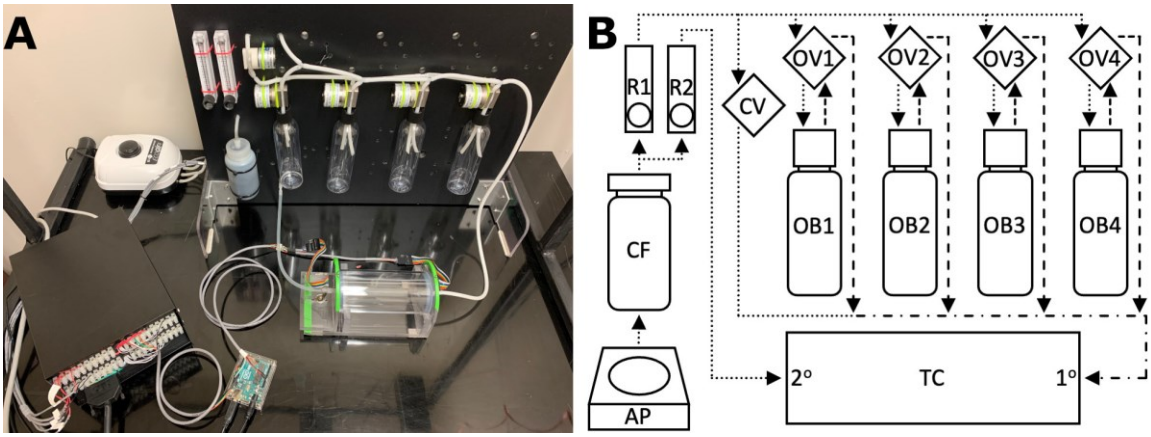

Figure 4.1: ODORS overview and air flow diagram. ***(A)*** Representative image of the ODORS, with the air pump (upper left), odorant board (upper right), testing chamber (middle right), Arduino (lower centre), and valve control box (lower left). Note that connections to/from the computer interface (valve control box and Arduino) use long cables and are located away from the testing chamber during normal use to minimize interference during tethered recordings. ***(B)*** Overall air flow diagram for the ODORS. Air from the air pump (AP) passes through the carbon filter (CF) and then splits into two rotameters (R1 and R2). Air from the primary rotameter (R1) supplies the single-tube clean air valve (CV) and the four double-tube odorant valves (OV1-4). Air through the clean air valve forms the beginning of the downstream feed to the testing chamber (TC) primary side (1˚). Air passing through the odorant valves enters the respective odorant bottle (OB1-4) where it mixes with the odour stimulus, exits the bottle, passes back through the odorant valve, and joins the downstream feed to the testing chamber. Air from the secondary rotameter (R2) connects directly to the testing chamber secondary side (2˚) to provide positive pressure. Dotted lines indicate clean air lines, dashed lines indicate stimulus air lines, and the dashed/dotted line leading to the testing chamber primary side carries both clean and stimulus air. Arrows indicate direction of air flow.

levels; and 5) the ability to test tethered and untethered rodents in optogenetic and electrophysiological experiments.

4.4 Materials and Methods

The ODORS has three major components: the odorant board, testing chamber, and computer interface (Figure 4.1). Our specific design and component choices will be discussed in detail, although components with similar specifications may provide suitable alternatives.

4.4.1 ODORS Construction

4.4.1.1 Odorant Board

Function: The odorant board consists of a 3’ x 2’ sheet of low-density polyethylene and provides a mounting surface for the air filter, rotameters, air valves, and stimulus bottles (Figure 4.2), resulting in a lightweight, compact assembly that can be portable or mounted permanently.

Air supply: Air is supplied by an EcoPlus Eco Air 4 air pump (HGC728355; Hawthorne Gardening Company, Vancouver, WA). C-Flex tubing (#06424-67; Cole-Parmer, Vernon Hills, IL) and polyethylene super-flow barbed Wye connectors (1/8” tube; #2808K127; McMaster-Carr, Elmhurst, IL) were used to combine the outlets into a single air supply line.

Air filter: A custom-made air filter composed of a 500 mL Nalgene wide-mouth high-density polyethylene bottle (#2104-0016; Nalge Nunc International, Rochester, NY) drilled and tapped in the cap and on the bottom for barbed fittings (1/8” tube by male 1/8” NPT thread; #2808K22; McMaster-Carr) was used to reduce environmental odorants. Spherical carbon beads (Matrix Carbon; Seachem Laboratories, Madison, GA) were used as filter media to maximize surface area and airflow.

Flow regulation: Filtered air is divided into two rotameters (1-10 L/min; LZQ-7; Yuyao Shunhuan Flowmeter Co., Yuyao, China) to create primary and secondary supply lines that can be regulated independently. Nickel-plated brass connectors (4mm tube by male 1/16” BSPT; #6220N104; McMaster-Carr) were used to connect air lines.

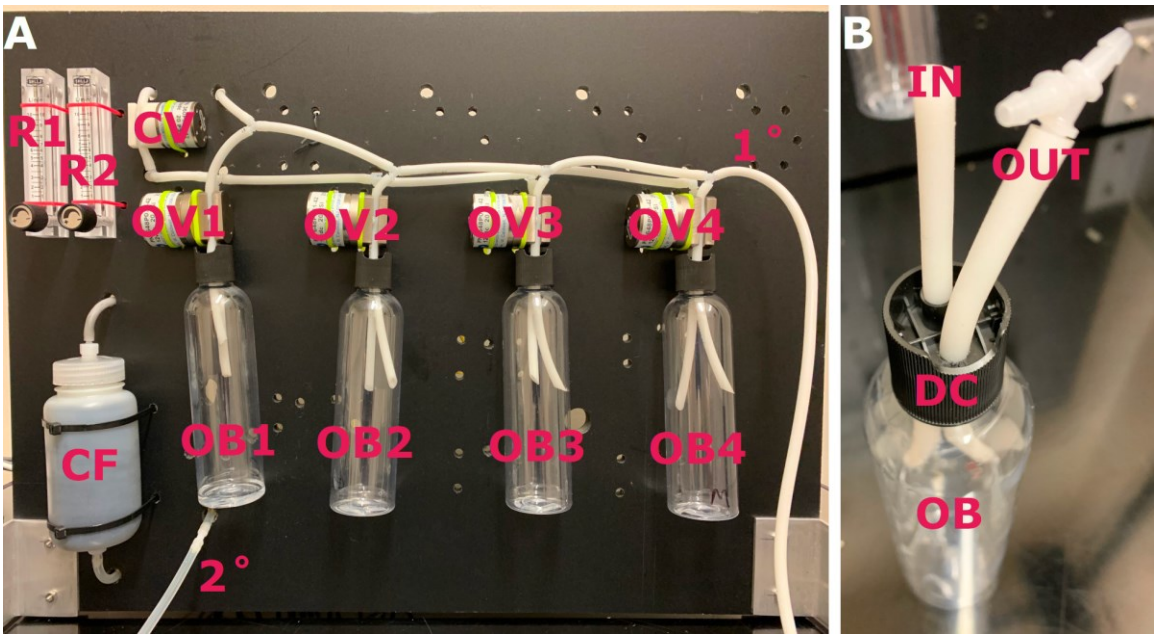

Figure 4.2: ODORS odorant board and bottle. ***(A)*** Representative image of the odorant board. Air from the air pump (not shown) passes through the carbon filter (CF) and then the rotameters (R1 & R2). The primary rotameter (R1) controls airflow through the clean (CV) and odorized (OV1-4) air valves; the secondary rotameter (R2) controls airflow through the secondary (2˚) line to provide positive pressure to the testing chamber. Odorant bottles (OB1-4) contain stimuli. The primary (1˚) air line delivers stimulus air to the testing chamber. Additional holes along the upper section of the board allow fitment of additional odorant valves/bottles. ***(B)*** Representative image of an odorant bottle (OB), including the modified 24-410 disc cap (DC). The lid from the cap has been removed, and the air line entering the bottle (IN) passes through the original 0.240" (~6.1mm) hole visible in the back. A second 6mm (~15/64") diameter hole was drilled on the opposite side of the cap (towards the front) for a second air line to exit the bottle (OUT); the barbed Wye-connector is visible on the end of the secondary line, allowing for the bottle and air lines to be easily replaced for use with different odorants.

Primary supply line: The primary supply line provides stimulus air (clean or odorized) to the testing chamber (Figures 4.1, 4.2, & 4.3). From the rotameter, serial air supply branches are created using super-flow Tee and 90-degree elbow connectors (1/8" tube; #2808K166 and #2808K115; McMaster-Carr), with one branch per valve; valves include one clean air valve (single-tube, normally-open, pinch solenoid valve; #360P021-42; NResearch Inc., West Caldwell, NJ) and four odorant valves (double-tube, normally-closed, pinch solenoid valves; #648P031-42; NResearch Inc.); odorant valves used a

double-tube design to isolate the inlet and outlet lines for each odorant bottle. Air passing through the clean air valve forms the start of a common downstream feed to the testing chamber. For odorant valves, clean upstream air passes through the valve, enters the odorant bottle to mix with odorized stimulus air, then exits the odorant bottle and passes back through the valve before joining the downstream feed. On the downstream side, air from each valve is combined into a downstream feed using Wye connectors. By using the clean air valve as the start of the downstream feed, the line is continually flushed with clean air between stimulus presentations to prevent cross-contamination of odour stimuli.

Secondary supply line: The secondary supply line is used to provide positive pressure to the testing chamber to localize stimulus air near the primary air inlet, allowing location-based scoring similar to the standard OHD task (M. Yang & Crawley, 2009) and improving clearance of odour stimuli during intertrial intervals.

Odorant bottles: Odorant bottles consist of clear 250mL polyethylene terephthalate bottles, black polypropylene disc caps (24-410 size), and C-Flex tubing (Figure 4.2). Caps are modified by removing the disc top to expose the opening (0.240" in diameter) in the base of the cap; a second hole of similar size (approximately 1/4") is drilled into the adjacent flat portion of the cap. Short sections of C-Flex tubing are threaded through each hole, with the portion at the top inserted into a solenoid pinch valve and connected to the upstream and downstream lines, allowing odorant bottles, caps, and tubing to be easily replaced as an entire unit to prevent odour cross-contamination.

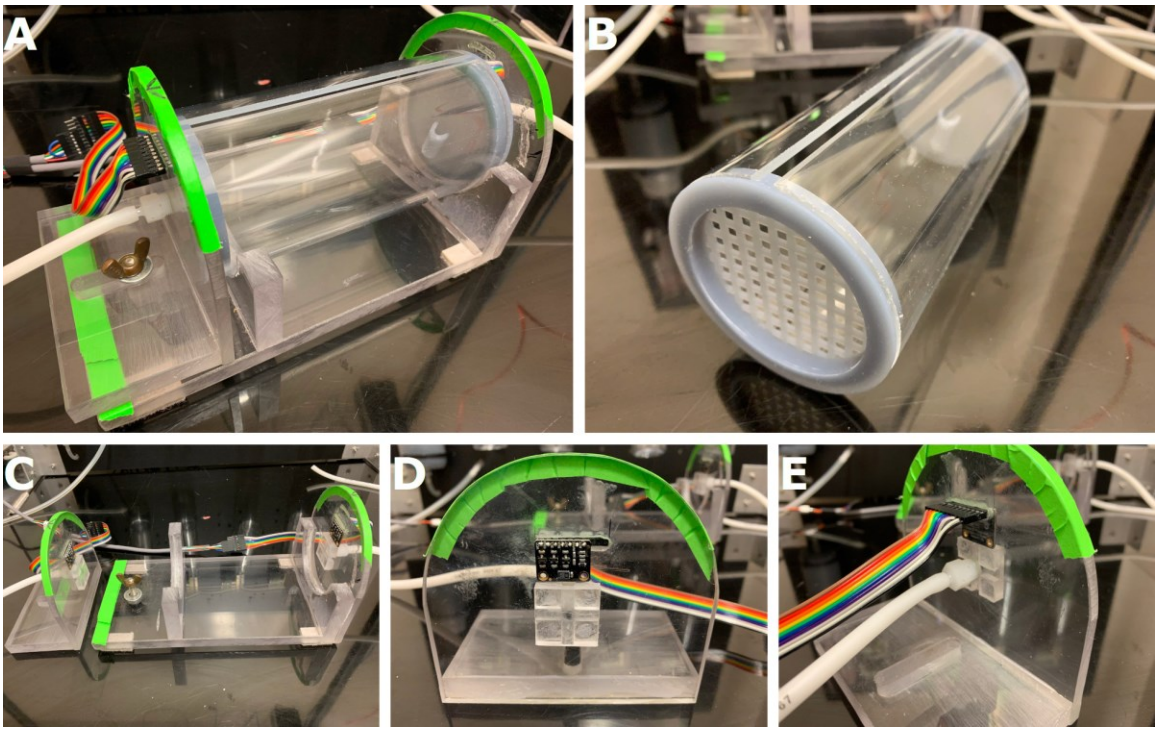

Figure 4.3: ODORS testing chamber assembly. ***(A)*** Testing chamber; secondary section is on the left, with the wingnut and adjustment slot visible. ***(B)*** Testing tube and end caps. Secondary side (perforated end cap) is at the close end, and the primary side (short odour port end cap) is at the far end. End caps are friction fit. The slot along the top allows air to escape and a tethered subject to be tested. ***(C)*** Separate primary (right) and secondary (left) sections of the holder; the screw and wingnut to attach the secondary section are visible on the left end of the primary section. ***(D)*** Inside of the secondary inlet, with the air diverter (25mm x 25mm x 6mm acrylic with 3mm wide and 3mm deep slots facing the air inlet), VOC sensor, and VOC sensor header slot (with header, behind the sensor) visible. The primary section uses the same layout. ***(E)*** Outside of the secondary inlet, with the air line connection and VOC sensor header visible.

4.4.1.2 Testing Chamber

The testing chamber is composed of the testing tube, holder, and end caps.

Testing tube: The testing tube houses the subject during testing. For mice, this consists of a clear acrylic tube (2-3/4” inner diameter, 6” long) with a 1/8” slot cut lengthwise along the top of the tube (Figure 4.3); this size allows subjects enough room to turn around, rear, and self-groom even with a tether attached, and the slot allows free movement of a recording tether and allows air to exit the testing tube. The tube is also

removable for cleaning.

Tube holder: The holder provides a stable base and localizes the air inlets and volatile organic compound (VOC) sensors at either end of the tube (Figure 4.3). A primary section contains testing tube supports and the primary air inlet and VOC sensor; a secondary section contains the secondary air inlet and VOC sensor and is used to clamp the testing tube into place. A slot in the secondary section fits over a screw mounted in the primary section, and a wingnut is used to clamp it down (Figure 4.3). Small air diverters (1/4" thick blocks with 1/8" slots cut in the shape of a cross, facing the inlets) are mounted over each air inlet to diffuse incoming air. VOC sensors (CCS811 Air Quality Sensor; DFRobot; Shanghai, China) are mounted just above the diverters using 10-pin 2.54mm pitch sockets mounted in slots (approximately 1" long and 1/8" tall). VOC sensors use a 7-pin 2.54 mm pitch male header, allowing adjustment of the sensor position above the inlet (Figure 4.3).

End caps: End caps (Figures 4.3 & 4.4) were used at each end of the testing tube to: *1)* contain the subject when inserting or removing the testing chamber from the holder; *2)* ensure that the slot along the top of the testing chamber does not pinch a recording tether; *3)* prevent subjects from damaging the VOC sensors; and *4)* to direct airflow within the testing chamber. The primary end cap is solid, with a single outlet designed to direct the airflow vertically 5° off from the slot in the top of the testing tube. The secondary end cap is perforated to allow even airflow from the secondary end of the testing tube. End caps were 3D printed with eSUN Hard-Tough Resin (Shenzhen Esun Industrial Co. Ltd, Shenzhen, China).

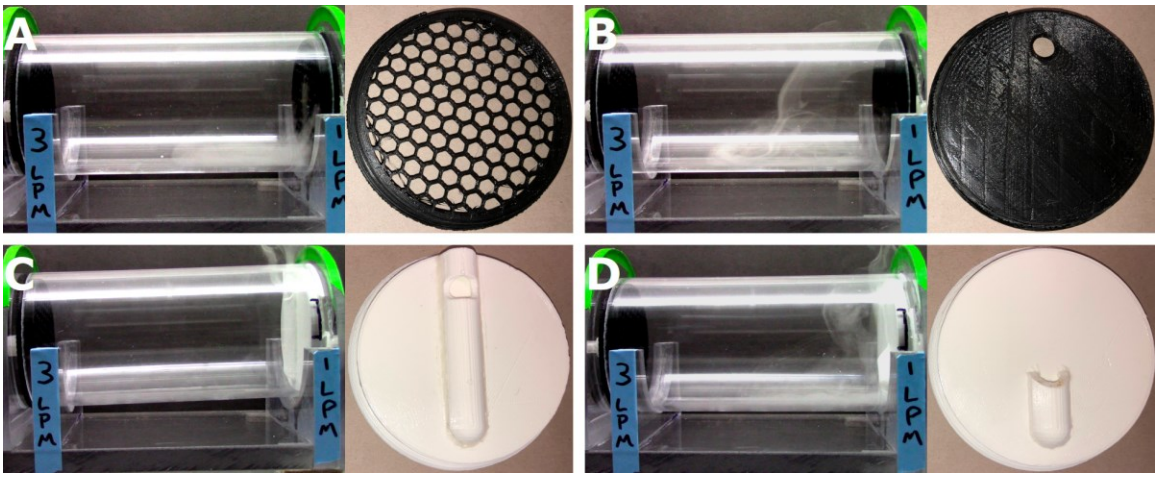

Figure 4.4: Airflow patterns in the ODORS testing chamber. Still images of testing chamber airflow patterns with glycerine-based "fog" using four different styles of end caps on the primary (right) side. ***(A)*** Perforated end cap resulting in minimal localization and diffuse airflow. ***(B)*** End cap with odour port resulting in localized source and focal stream of air. ***(C)*** End cap with long tube with odour port resulting in localized source with minimal air entering chamber. ***(D)*** End cap with short odour tube resulting in localized source and air primarily restricted to the primary side. All combinations used a perforated (Panel A) end cap on the secondary (left) side. Stills were taken 2-5 seconds after the fog entered the chamber.

4.4.1.3 Electronics Interface

The electronics interface consists of a valve control box and an Arduino Uno (Arduino, Monza, Italy). As testing systems can vary across labs, the overall design and use of these components rather than specific construction is discussed below.

Valve control box: The valve control box supplies power to activate the valves. It consists of an AC/DC power converter, a series of solid-state relays, input connections, and output connections. Input connections (in our case, a software-controlled multi-channel digital input/output connector from our electrophysiology suite) control the relays, with valves connected to the output connections. While the use of alternative devices is beyond the scope of this article, other devices that offer general-purpose input/output (GPIO) pins (such as the Arduino Uno) could be used as valve trigger

inputs.

Arduino Uno: The Uno is connected to the VOC sensors and valve inputs on the valve control box and serves as an interface to monitor total VOC (tVOC) levels and valve activation. Separate 5-connector cables are used to connect the Uno to the VOC sensors and the valve trigger inputs. A 9VDC power supply keeps the Uno constantly running to minimize warm-up time for the VOC sensors.

4.4.2 ODORS Function

Function: The ODORS delivers clean or odorized stimulus air to a testing chamber.

Air supply: Our pump is rated at ~16 L/min (253 GPH), allowing us to run the pump at sub-maximal levels to reduce noise. Rotameter needle valves allow further control of airflow through the primary (~1 L/min) and secondary (~3 L/min) lines; these flow rates were chosen based on end cap airflow patterns (Figure 4.4) and time needed to clear odorants from the testing chamber.

Valves: Valves are supplied from the primary air supply line and re-combine into a single downstream feed (Figure 4.1). The clean air valve forms the start of the downstream air supply, and each odour valve output feeds into this stream (Figure 4.1); this ensures that the downstream line is continuously flushed by clean air during habituation and inter-trial intervals to reduce odour cross-contamination. Air is only fed through a single valve at any time (i.e., either the clean air valve or one odorant valve) to keep a consistent flow rate and prevent dilution of stimulus air.

Odorant bottles: Clean, empty odorant bottles were tested for the presence of tVOCs relative to baseline air prior to use; only bottles that exhibited relatively low

tVOCs (~30-70 ppb) were used for testing. Odorant bottles were only used with a single valve and air line combination, with social odours only used with the final (fourth) odorant valve. The social stimulus bottle, cap, and air lines, as well as the final section of air line between valve 4 and the testing chamber, were all replaced when switching between male and female social odours.

End caps: End cap design was selected based on airflow pattern in the testing chamber (Figure 4.4). A visible aerosol was created using a "fog" machine and 10% mixture of glycerine and water, then captured in a 4L container and connected between the primary air supply line and the primary side of the testing chamber. Airflow patterns were assessed by assembling the testing chamber with specific end cap combinations, turning on the air pump, and recording videos of the resulting airflow. End caps tested on the primary side included: perforated; solid with a single 10mm odour port near the top; solid with a vertical odour "tube" containing a 10mm odour port near the top; and solid with a shortened vertical odour tube. Each combination used a perforated end cap on the secondary side.

VOC sensors: Air entering the testing chamber was assessed for tVOC levels relative to the clean air baseline using CCS811 environmental sensors (ScioSense B.V., Eindhoven, Netherlands). These sensors allow raw tVOC readings from 0 – 32,768 ppb at a minimum delay of 250 msec, although our maximum tVOC readings were capped at ~29,000 ppb, likely due to saturation of the sensors. However, these sensors have been discontinued and replaced by the manufacturer with ENS160 environmental sensors; assembly based on this design should consider limitations of presently available alternatives.

Arduino Uno: VOC data and valve activation was recorded using an Arduino Uno. Open-source header and source files were obtained from DFRobot and Keyestudio (Shenzhen KEYES Robot Co. Ltd.; Shenzhen, China), and a custom Arduino sketch and Python script (see *Code Availability* below) were used to record tVOC levels, valve activation, and stimulus timing in a .csv file. Header and source files and basic Arduino sketches should be available from any manufacturer producing Arduino-compatible breakout boards based on the CCS811.

4.4.3 Testing Paradigm

A modified version of the olfactory habituation/dishabituation (OHD) task was used to test functionality of the ODORS; for details on the standard OHD task, see (M. Yang & Crawley, 2009). All procedures were approved by and performed in accordance with the Dalhousie University Committee on Laboratory Animals regulations.

Subjects were 12 female and 12 male C57BL/6NCrl (Strain #027; Charles River Canada, Montreal, QC) mice at 20 weeks of age. Mice were housed in groups of 3 in a colony room on a 12:12 reversed light-dark cycle, with lights off from 09:30-21:30, in standard mouse cages (30 x 19 x 13cm; Allentown Caging Inc., Allentown, NJ, USA) containing wood-chip bedding (FreshBed; Shaw Resources, Shubenacadie, NS), a metal cage top containing *ad libitum* food (LabDiet Prolab RMH 3500; PMI Nutrition International, Arden Hills, MN) and filtered water, two black, opaque, polymer enrichment tubes (4cm diameter; ~8cm long), and nest material. Cages were topped with a micro-isolator filter. Two mice from each cage were ear-punched prior to testing to differentiate between subjects.

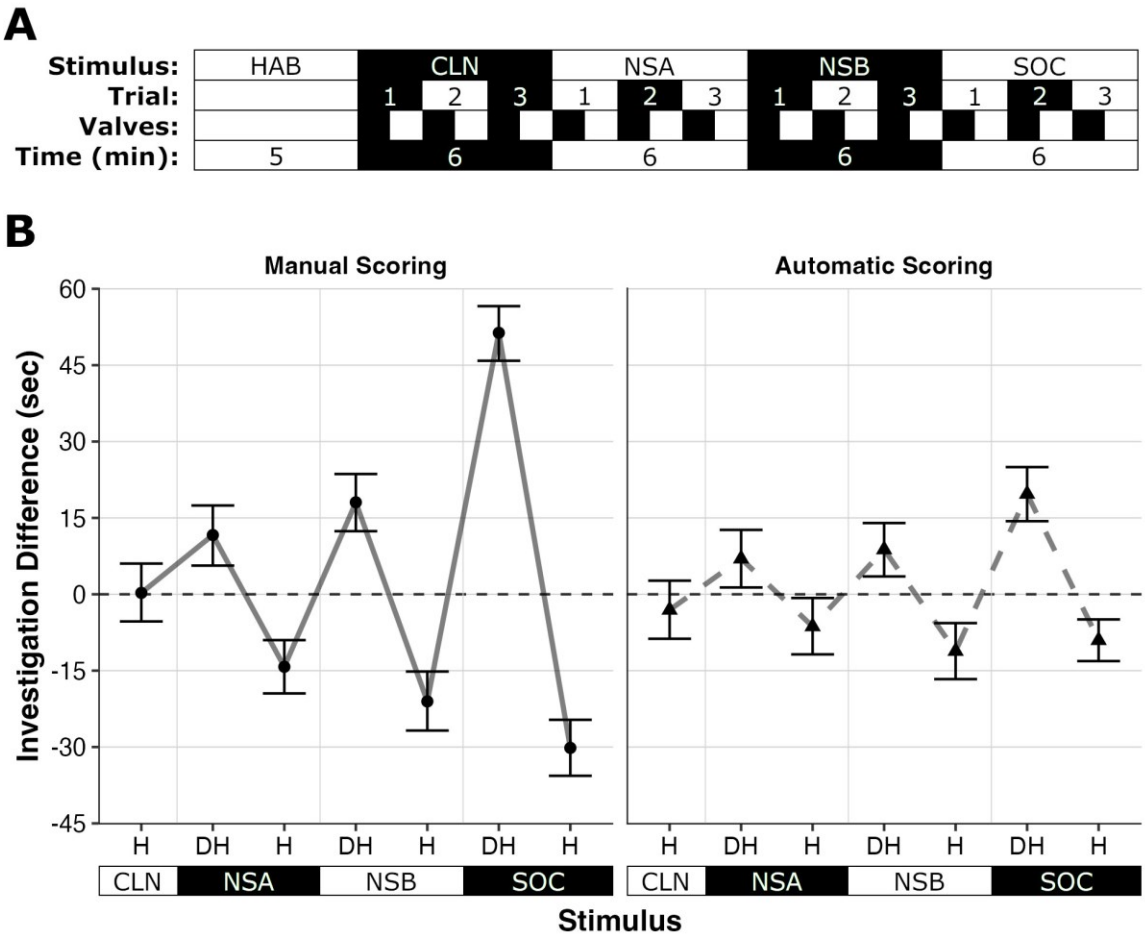

Figure 4.5: ODORS olfactory habituation/dishabituation timeline and overall results. ***(A)*** Timeline for testing sessions. Each session begins with habituation (HAB), followed by testing with the clean air control stimulus (CLN), non-social odour A (NSA), non-social odour B (NSB), and the social (same-sex urine) odour (SOC). Trials are shown below each stimulus. Valve activation during the session is shown below each trial; filled boxes indicate valve ON periods, empty boxes indicate valve OFF periods. The duration (in minutes) of each period (habituation and each of the four stimuli) is given at the bottom. ***(B)*** Automatic and manual scoring results for habituation (H; first to third presentation of one stimulus) and dishabituation (DH; third presentation of one stimulus to first presentation of the subsequent stimulus) effects (in seconds). Error bars indicate 95% CIs; points indicate median CI values. Values above 0 indicate increased investigation (dishabituation); values below 0 indicate decreased investigation (habituation). CLN = clean (control) stimulus; NSA = non-social stimulus A; NSB = non-social stimulus B; SOC = social (novel, same-sex urine) stimulus.

Two testing sessions were performed over the course of 16 days (Days 1, 2, 15, and 16). Days 1 and 15 were used for habituation, and testing was performed on Days 2 and 16; the 14-day delay was used to reduce effects from repeated testing. Mice were tested in a separate testing room under red light. Indicator lights on the computer were covered, and dark mode with minimum monitor brightness settings was used to minimize light from the monitor. A towel was placed over the odorant board to reduce noise from the valves.

For odorant presentation, we emulated the sequential, two-minute stimulus presentations from the standard OHD task by turning valves ON for 60 seconds, then OFF for 60 seconds, for each trial. When valves were ON, air passed through the respective odorant bottles to deliver stimulus air to the testing chamber; during the OFF period, clean air was supplied to flush out the lines and testing chamber. This created 2-minute trials with a rapid increase, then gradual decrease, in odorant concentration. Our paradigm utilized one clean air control stimulus (Valve 1), two novel, non-social stimuli (Valves 2 & 3), and one novel social stimulus (Valve 4). Habituation to valve noise and air flow was done using 6 trials (12 minutes total) of control valve activation with the air pump on. For testing, subjects were allowed to habituate to the testing chamber for 5 minutes with the air pump running, followed by 12 testing trials (3 sequential presentations of each of the 4 stimuli; 24 minutes total); valves were triggered in the order listed above (see Figure 4.5). The testing tube and end caps were washed using Fisherbrand Sparkleen 1 (Fisher Scientific, Pittsburgh, PA, USA) and hot water, rinsed, and dried thoroughly before and after each subject.

Odour stimuli were ethyl acetate (#270989; Sigma-Aldrich, St. Louis, MO), butyl

acetate (#287725; Sigma-Aldrich), artificial brandy flavouring (McCormick Canada, London, ON), artificial banana flavouring (McCormick Canada), and urine from novel, same-sex conspecifics. Banana, butyl acetate, and ethyl acetate odours have been used in previous olfactory studies (Bodyak & Slotnick, 1999; Kass et al., 2013; Oummadi et al., 2020; Roddick et al., 2016; M. Yang & Crawley, 2009), and ethyl acetate is present in brandy (Tsakiris et al., 2014) while butyl acetate is a component of artificial banana flavouring; as a result, ethyl acetate was paired with banana while brandy was paired with butyl acetate to retain similar odour profiles (either ethyl acetate/brandy or butyl acetate/banana) across sessions while keeping stimuli distinct within a given session. We counterbalanced these odour combinations to assess behaviour in response to both complex (e.g., banana, brandy) and monomolecular (e.g. butyl acetate, ethyl acetate) odour stimuli, and to verify that our paradigm was repeatable in the same subject using novel odours. Our undiluted urine stimuli initially measured in the range of 10,000-13,000 ppb; thus, non-social odours were diluted with purified water to peak initial VOC concentrations in that range. Final dilutions ranged between 1:200 and 1:750, and a 1 mL volume was used for each stimulus. Pooled urine stimuli were obtained from novel, sexually-naïve, same-sex conspecifics; to ensure novelty, the social stimulus used in the first session consisted of urine obtained from C57BL6/SJL mice (Strain #100012; Jackson Laboratory, Bar Harbor, ME), while the social stimulus used in the second session was obtained from an outbred strain, Crl:CD1(ICR) mice (Strain #022; Charles River Canada, Montreal, QC).

4.4.4 Data Analysis

Scoring: Manual scoring to record the duration of investigation was performed

from recorded videos; investigation was classified as any period in which the subject was facing the primary (stimulus) side of the testing chamber with its snout 2 cm or less from the end and not engaged in self-grooming. Manual scoring was performed blind as trial start, end, and stimulus were not evident from the videos. Automatic scoring was performed using Biobserve Viewer 3.0 (Biobserve GmbH; Bonn, Germany) by restricting tracking area to the testing chamber, adjusting brightness/contrast thresholds to identify the mouse and minimize background interference, and allowing the program to assess the X/Y pixel locations of the head, body, and tail on a frame-by-frame basis. Pixel to real-world distances were calibrated in the program prior to scoring (0.458mm/pixel). Raw pixel coordinates were imported into R (Version 4.2.2 "Innocent and Trusting"; The R Foundation for Statistical Computing; Vienna, Austria) using RStudio (Version 2022.12.0+353; Posit Software, PBC; Boston, MA) and used to assess location and movement direction of the subject within the testing chamber. Investigation was considered any period in which the subject's centre of mass was between 44 pixels (2cm) and 132 pixels (6cm) from the primary side of the testing chamber during or after motion towards the primary end; the subject was assumed to be turning away from the stimulus if the body was less than 2cm from the primary end of the testing chamber. Frames spent investigating were then converted to investigation time by dividing over the average number of frames per second. For manual and automatic scoring, timestamped investigation periods were compared to trial start and end times (obtained from valve onset/offset times) to determine total investigation period for each trial. VOC levels, stimulus type, and valve onset/offset were automatically recorded to CSV files at ~260ms intervals. VOC levels were recorded at both the primary and secondary inlets.

Habituation/dishabituation: Preliminary analyses of manual scoring suggested that results were similar across sexes, sessions, and odour sets (see Figure 4.6); as our goal was to validate the apparatus and associated paradigm, data was collapsed across sex, session, and odour set for manual and automatic scoring. Outliers (over 2 standard deviations above/below the mean) were removed and data analysis was performed to assess habituation and dishabituation using mixed-effects models for the effect of stimulus and presentation number; estimated marginal means were assessed *post hoc* using Holm-Bonferroni correction to examine directions of effects. Bootstrapped 95% CIs were calculated for the effect of stimulus and presentation on investigation duration.

4.4.5 Code and Data Availability

The Arduino and Python scripts and 3D end cap models described in the paper are freely available at https://github.com/FilipKosel/ODORS. The Python script was run using the Anaconda interpreter on a PC running Windows 10.

4.5 Results

4.5.1 Data Loss

Second session data from two males was excluded; one was euthanized due to injuries from home-cage aggression prior to session 2, and the other was tested but the video was not saved due to technical issues. No other data was excluded.

4.5.2 End Cap Designs

End cap designs resulted in distinct airflow patterns within the testing chamber (Figure 4.4). The perforated design resulted in diffuse airflow with no distinct stimulus

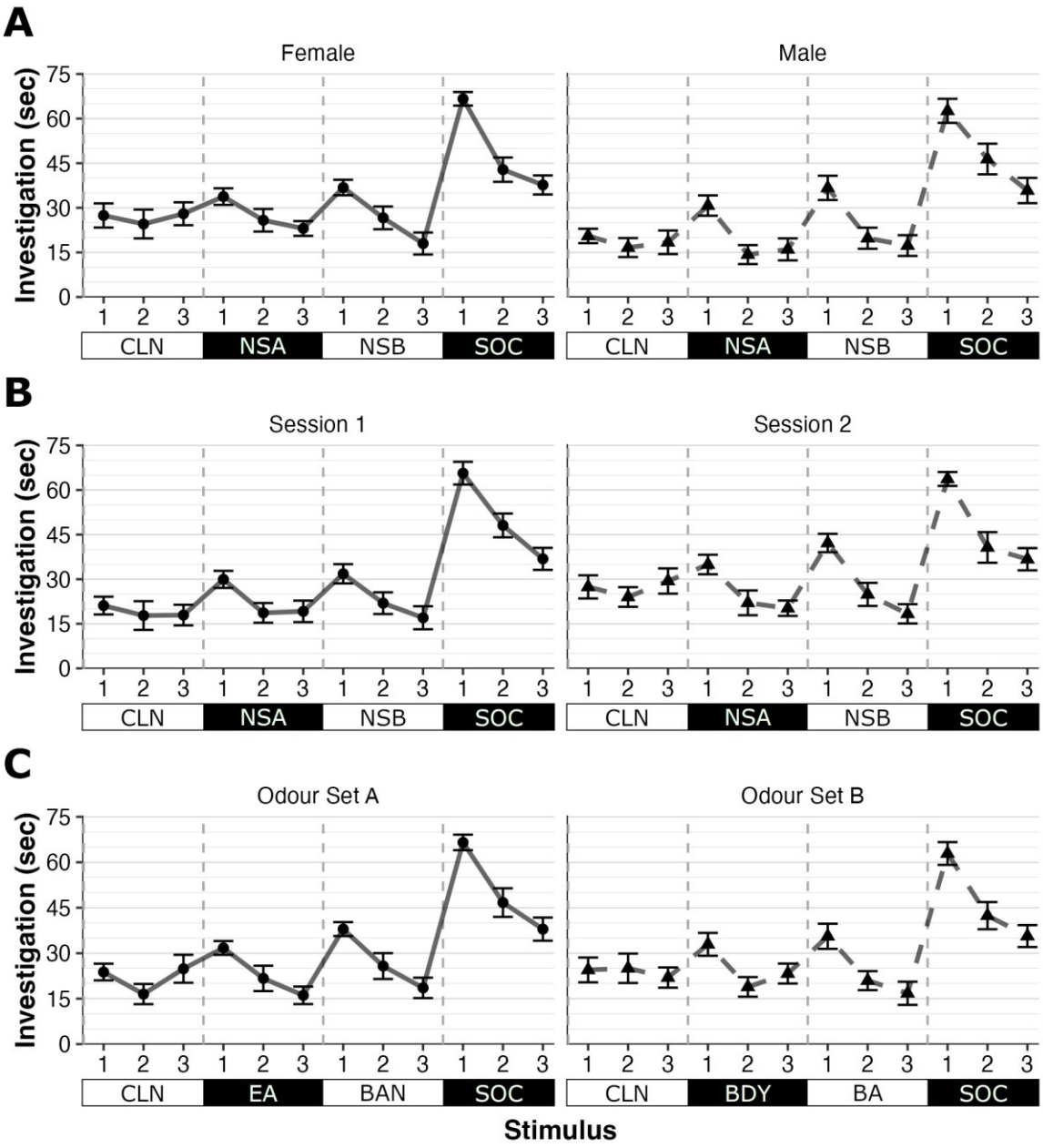

Figure 4.6: ODORS olfactory habituation/dishabituation results by sex, session, and odour set. Mean (+/- standard error) for investigation durations (in seconds) for each stimulus and trial based on ***(A)*** sex, ***(B)*** session, and ***(C)*** odour set. Vertical dashed lines represent a change from one stimulus to another. For Panel A & B, stimuli are: CLN = clean air (control) stimulus; NSA = non-social odour A; NSB = non-social odour B; SOC = social (novel, same-sex urine) stimulus; Panel C uses the same abbreviations for clean air and social stimuli. For Panel C, Odour Set A, non-social stimuli are: EA = ethyl acetate (monomolecular; non-social odour A); BAN = artificial banana (complex; non-social odour B). For Panel C, Odour Set B, non-social stimuli are: BDY = artificial brandy (complex; non-social odour A); BA = butyl acetate (monomolecular; non-social odour B).

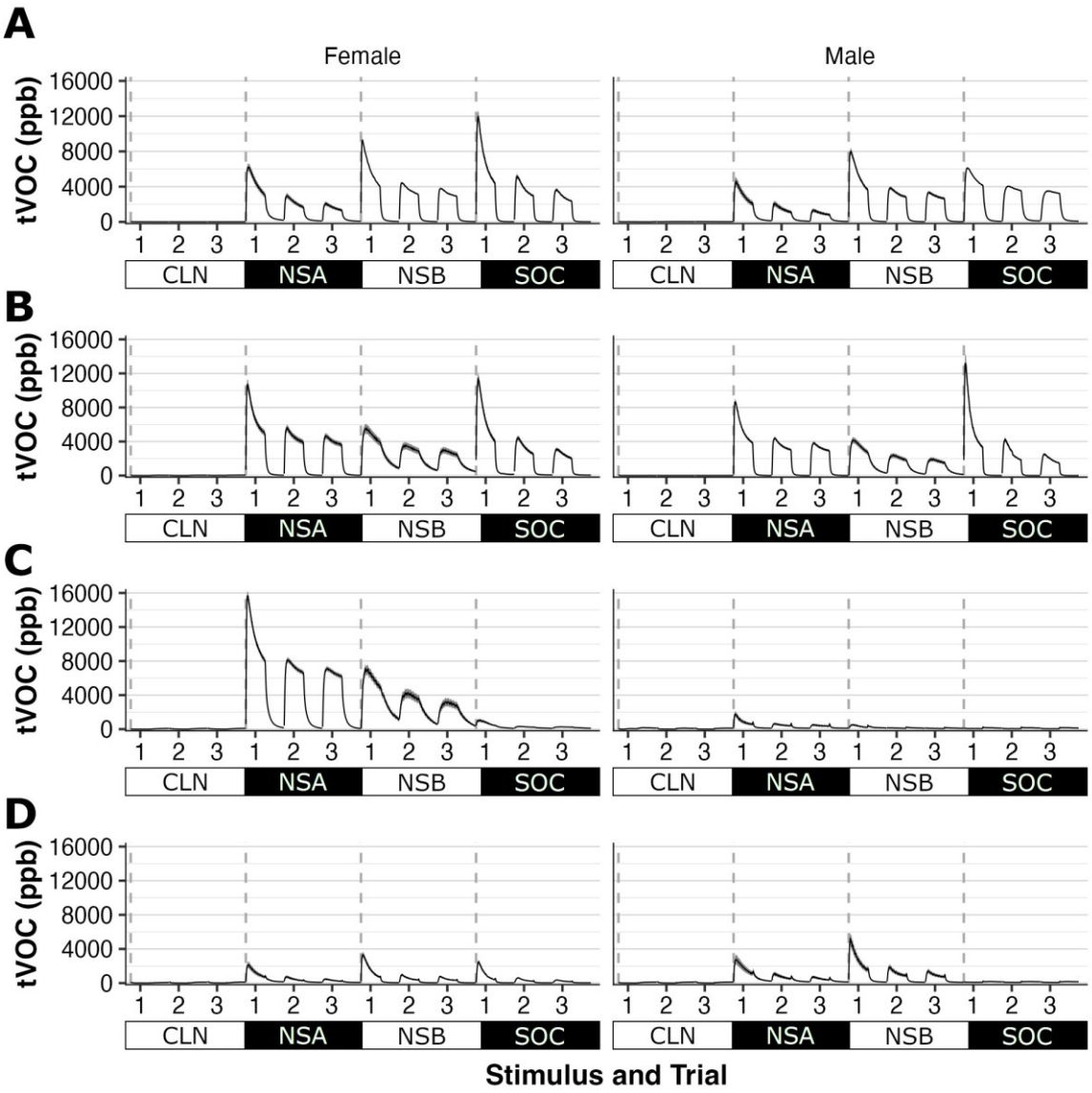

Figure 4.7: ODORS olfactory habituation/dishabituation VOC readings by cohort and session. Mean (+/- standard error) total volatile organic compound (tVOC) levels (in ppb) across sessions for each stimulus and trial for females (left) and males (right) for: ***(A)*** cohort 1, session 1; ***(B)*** cohort 2, session 1; ***(C)*** cohort 1, session 2; and ***(D)*** cohort 2, session 2. Values were calculated on a per-second basis but are presented per trial for clarity. Vertical dashed lines indicate start of each set of stimulus presentations. CLN = clean (control) odour; NSA = non-social odour A; NSB = non-social odour B; SOC = social (novel, same-sex urine) odour.

source. The odour port design resulted in a distinct stimulus source and a stream of air lengthwise into the testing chamber. The long tube design resulted in a distinct stimulus source (the odour port) with minimal airflow into the testing chamber. The short tube design resulted in a distinct stimulus source (the end of the odorant tube) and airflow localized to the primary end of the testing chamber (due to positive pressure from the secondary side). The short tube design was selected for use in the final apparatus as it allowed the odorant to enter the testing chamber from a distinct source (similar to stimuli used in the standard OHD task) while primarily localizing air to the primary end of the testing chamber (to allow location-based scoring).

4.5.3 Investigation Duration – Manual and Automatic Scoring

Both manual and automatic scoring indicate that subjects exhibit habituation (decreased investigation of a repeated odour) and dishabituation (increased investigation to a novel odour) to non-social and social stimuli, with manual scoring indicating larger habituation/dishabituation effects than automatic scoring (Figure 4.5).

For manual scoring, there was an effect of stimulus (χ^2 (3) = 178.5980, $p < .001$), presentation (χ^2 (1) = 89.8900, $p < .001$), and a stimulus by presentation interaction (χ^2 (3) = 43.2160, $p < .001$). For stimulus, overall investigation of the social odour was higher than the control ($t_{333.12} = 11.711$, $p < .001$), first non-social odour ($t_{333.12} = 10.617$, $p < .001$), and second non-social odour ($t_{333.18} = 10.036$, $p < .001$). For presentation, overall investigation was higher for the first trial than the third trial ($t_{333.16} = 9.461$, $p < .001$). For the stimulus by presentation interaction, there was habituation (decreased investigation duration from the first presentation of one stimulus to the third presentation of the same stimulus) for the first non-social odour ($t_{333.18} = 3.775$, $p = .003$), second non-

social odour ($t_{333.29} = 5.758$, $p < .001$), and the social odour ($t_{333.06} = 9.256$, $p < .001$); there was no habituation to the clean air control stimulus. There was also dishabituation (increased investigation duration from the third presentation of one stimulus to the first presentation of the subsequent stimulus) from the first to the second non-social odour ($t_{333.18} = 5.172$, $p < .001$) and from the second non-social odour to the social odour ($t_{333.29} = 14.508$, $p < .001$).

For automatic scoring, there was an overall effect of stimulus (χ^2 (3) = 46.2371, $p < .001$), presentation (χ^2 (1) = 5.6925, $p = .017$), and a stimulus by presentation interaction (χ^2 (3) = 8.1309, $p = .043$). For stimulus, overall investigation of the social odour was higher than the control ($t_{161} = 6.031$, $p < .001$), first non-social odour ($t_{161} = 4.997$, $p < .001$), and second non-social odour ($t_{161} = 5.432$, $p < .001$). For presentation, overall investigation was higher for the first trial than the third trial ($t_{161} = 2.386$, $p = .018$). For the stimulus by presentation interaction, there was habituation for the social odour ($t_{161} = 3.189$, $p = .038$). There was also dishabituation from the second non-social odour to the social odour ($t_{161} = 6.380$, $p < .001$).

Investigation duration was not related to VOC levels, as subjects exhibited high levels of investigation of social stimuli even with relatively low VOC levels (Figures 4.6 & 4.7).

4.6 Discussion

4.6.1 The ODORS OHD Paradigm is Functionally Similar to the Standard OHD Task

The ODORS OHD paradigm mimics the standard OHD task by allowing successive presentations of odour stimuli at defined intervals with an initial high odour

concentration around the stimulus source followed by gradual dissipation over the course of the trial (Figure 4.7); we achieved this by alternating odorized air with clean air, and the timing and number of trials per stimulus (three 2-minute trials) matches existing OHD paradigms. The primary end cap was the short odour tube design (Figures 4.3 & 4.4), resulting in localized stimulus presentation from a distinct source (the odour tube) and allowing location-based scoring similar to existing OHD paradigms. Finally, results from the ODORS exhibit a habituation/dishabituation pattern similar to the standard OHD task (Figure 4.5), suggesting that these two paradigms are functionally similar. Manual scoring also indicates that males and females show similar levels of investigation, that the test can be reliably performed twice in the same animals, and that investigation is largely similar across odour sets regardless of whether odorants are monomolecular or complex (Figure 4.6).

4.6.2 The ODORS Provides Additional Benefits Over the Standard OHD Task

While these two tasks are functionally similar, the use of automated stimulus delivery and VOC sensors provides a number of additional benefits. Automated stimulus delivery reduces labour requirements and variability or potential stress due to experimenter presence. Additionally, precise control of stimulus onset and offset removes any unintentional variability in trial duration or inter-trial interval that may arise with manual stimulus presentation and allows synchronization of other data with stimulus presentation. Importantly, video recordings can be synchronized with stimulus delivery on a frame-by-frame basis for fully automatic scoring. While our automatic scoring indicated lower habituation/dishabituation effects than manual scoring (Figure 4.5), it is unclear whether this is due to underestimation of investigation times by our tracking

software, overestimation by manual scoring, or some combination therein; despite this, our results (along with Oummadi *et al* (2020)) suggest that automatic scoring can be used to assess habituation/dishabituation effects to various stimuli. Additionally, the apparatus has been designed for use with tethered and untethered subjects by the inclusion of a slot along the top of the testing chamber allowing for free movement of a tether, and timing of stimulus delivery and VOC level thresholds can be used in real time to trigger electrical or optogenetic stimulation or during later analyses to identify periods of interest. Here, the sensors also identified that urine stimuli exhibited different VOC concentrations between our two sets of samples despite being undiluted, with pooled urine from Crl:CD1(ICR) mice (second session) exhibiting lower VOC concentrations than the first set (C57BL6/SJL), highlighting the potential for variability in social stimulus presentations; the addition of the VOC meter allows the researcher to assess and evaluate VOC levels that are presented to subjects. It is also important to note that VOC levels in the present study appear much lower overall during the second session; due to damage, we fabricated a replacement end cap partway through the second session testing. It is possible that this cap exhibited some outgassing that may have affected our baseline readings, further indicating the need to assess stimuli as presented to the subject as changes in the apparatus can have unexpected effects on the stimulus air. However, while the VOC levels were reduced with the use of the replacement end cap, the behavioural responses remained pronounced, indicating that this task is resistant to variability in stimulus concentrations., but again highlighting that the VOC sensors remain useful for tracking stimulus concentrations during this or other perhaps more sensitive olfactory tasks.

4.6.3 Conclusion

Herein we discuss the design, assembly, and testing of the ODORS, an economical apparatus that can efficiently and accurately test behavioural responses to odour stimuli in tethered and untethered rodents, and that can be easily modified and integrated with existing testing systems. This system can be modified to use any number of odour stimuli by adding/subtracting odorant valves and bottles, and timing of valve onset/offset can be controlled from the Arduino itself; as a result, the ODORS can be used as a stand-alone unit or be directly controlled by other software. In order to create an accessible system that could be assembled and utilized by labs with limited resources (e.g., funding, fabrication capabilities), we aimed to use inexpensive, easily accessible components that can be obtained from major suppliers or produced in-house using basic tools; the majority of the components outlined here—particularly those in contact with odorants—have either been used in previous olfactory studies (e.g., PET odorant bottles, C-Flex tubing; see Bodyak & Slotnick, 1999; Roddick et al., 2014, 2016; Slotnick & Bodyak, 2002) or are FDA-compliant for food and beverage contact (e.g., barb fittings), suggesting that they should be relatively inert and not leach chemicals into the air stream. However, it is important to note that these materials may retain odours that could result in cross-contamination of stimuli; as a result, it is key that odorant bottles, tubing, and fittings are not used with various odour stimuli, and all components should be replaced at regular intervals or if contamination is suspected. Depending on availability, alternative materials—such as glass manifolds and odorant bottles or PTFE tubing—may also reduce the likelihood of contamination.

Overall, the ODORS is a low-cost system that can be assembled from readily-

available components while allowing for modification of the design to suit the needs of a lab. Further, this system allows automation of the OHD task with similar results to the standard OHD paradigm: subjects exhibit characteristic dishabituation to a novel odour, followed by habituation to subsequent presentations of the same odour. This does not appear to be related to the concentration of odorant in the testing chamber, as subjects exhibit high levels of investigation in response to social odours even at relatively low concentrations. Due to automated stimulus delivery, the length of each trial and intertrial interval can be precisely controlled, thereby preventing unintentional over- or under-exposure to any given stimulus; additionally, experimenters no longer need to be present in the testing room to replace odour stimuli, ultimately reducing subject stress and variability due to human presence and allowing experimenters to focus on other tasks during testing. Finally, videos can be scored automatically as well as manually, further reducing the labour requirements for the task. We suggest that the ODORS will simplify and encourage olfactory research in rodent models to improve our understanding of this important modality.

Chapter 5. Aberrant Neural Activity in the mPFC of 5xFAD Mice in Response to Social and Non-social Stimuli

5.0 Preamble

This work is currently in preparation for submission to the Journal of Alzheimer's Disease. Author contributions: *Conceptualization*: F.K. and T.B.F.; *Methodology*: F.K. and T.B.F.; *Investigation*: F.K. and M.R.H.; *Formal Analysis*: F.K.; *Resources*: F.K. and T.B.F.; *Writing – Original Draft*: F.K.; *Writing – Review & Editing*: F.K. and T.B.F.; *Visualization*: F.K.; *Supervision*: T.B.F. and F.K.; *Funding Acquisition*: T.B.F.

5.1 Abstract

Evidence suggests that behavioural and psychological symptoms of dementia (BPSD)—such as apathy, anxiety, and depression—in Alzheimer's disease (AD) are linked with neuropathology. Neuroimaging studies have linked some of these symptoms with altered neural activity, although inconsistencies in operational definitions and rating scales, limited scope of assessments, and poor temporal resolution of imaging techniques have hampered human studies. However, a number of transgenic mouse models of AD exhibit BPSD-like behaviours concomitant with AD-related neuropathology, allowing examination of how neural activity may relate to BPSD-like behaviours with high temporal and spatial resolution. We have previously demonstrated age-related decreases in social investigation in transgenic 5xFAD females, and this reduced social investigation is evident in transgenic 5xFAD females and males by 6 months of age. In the present study, we examine local field potential (LFP) in the medial prefrontal cortex (mPFC) of awake, behaving 5xFAD females and males at 6 months of age during exposure to social

and non-social odour stimuli in a novel olfactometer. Our results indicate that transgenic 5xFAD mice exhibit aberrant baseline and task-dependent LFP activity in the mPFC—including higher relative delta (1-4Hz) band power and lower relative power in higher bands, and overall stronger phase-amplitude coupling—compared to wild-type controls. These results are consistent with previous human and animal studies examining emotional processing, anxiety, fear behaviours, and stress responses, and suggest that transgenic 5xFAD mice may exhibit altered arousal or anxiety. This work is the first to examine task-dependent neural activity in the mPFC of AD-model mice in response to social and non-social olfactory stimuli and further contributes to the research relating neuropathology with behavioural changes in AD.

5.2 Introduction

In addition to cognitive and motor impairments (Artero et al., 2003; Kluger et al., 1997; Lambon Ralph et al., 2003), individuals with Alzheimer's disease (AD) often exhibit behavioural and psychological symptoms of dementia (BPSD), with ~80% of community-dwelling individuals with AD exhibiting at least one BPSD since the onset of symptoms, and ~75% exhibiting at least one symptom over the course of a month (Lyketsos et al., 2002; Steffens et al., 2005). Of these, the most common are apathy (including social withdrawal), agitation and aggression, irritability, depression, and anxiety (Frisoni et al., 1999; Lyketsos et al., 2002; Steffens et al., 2005; Zhao et al., 2016). The Neuropsychiatric Inventory (NPI)—a test designed to assess the presence of BPSD, also termed neuropsychiatric symptoms (NPS)—also includes other behavioural disturbances including delusions, hallucinations, elation and euphoria, disinhibition, and aberrant motor behaviour, as well as two neurovegetative domains (sleep and night-time

disturbances, and appetite and eating disorders (Cummings, 1997; Cummings et al., 1994). While these symptoms may fluctuate over time, they tend to be recurring and worsen over the course of disease progression (Chung & Cummings, 2000). Further, BPSD can cause caregiver distress and exacerbate cognitive and functional impairments, and the presence of BPSD is a leading factor in institutionalization (Chenoweth & Spencer, 1986; Chow et al., 2002; Chung & Cummings, 2000; C. Steele et al., 1990).

Despite the importance of BPSD in the lives of caregivers and individuals with AD, little is known about the causes underlying these symptoms, and debate remains over the contribution of neuropathological, intrinsic (e.g., personality traits), and extrinsic (e.g., societal, environmental) factors (Cho et al., 2021; Kolanowski et al., 2017). However, Kolanowski *et al* (2017) indicate evidence of neurological involvement in the development of apathy. Evidence from mouse models of AD also suggest that apathy—specifically social withdrawal—and depressive-like behaviours worsen over the course of the disease, and increased aggression and sleep-wake disturbances are present from early stages, suggesting that neurological changes may underlie some of the BPSD observed in humans with AD (Kosel et al., 2020).

The role of the medial prefrontal cortex (mPFC) in complex behaviours like social behaviours has been well-established. Projections from the mPFC to the periaqueductal gray (PAG) are involved in mediating social investigation (T. B. Franklin et al., 2017); a sub-chronic social defeat paradigm leads to decreased social investigation and decreased functional connectivity between the regions, and these effects can be mimicked in naïve mice through inhibition of mPFC to PAG projection neurons. Projections from the mPFC to the dorsal raphe nucleus also mediate learned social avoidance, with activation of these

neurons leading to reduced social investigation in control mice, whereas inhibition of these neurons following social defeat can prevent subsequent social avoidance (Challis et al., 2014). Similarly, the mPFC is also involved in dominance and aggression; exposure to dioxins—a class of toxic organic compounds produced either by burning or as by-products of industrial processes—*in utero* and through lactation leads to decreased c-Fos expression in the mPFC and increased expression in the amygdala following a reward-based task requiring behavioural flexibility, as well as decreased competition when access to water is limited, suggesting that the change in mPFC and amygdala activity mediates dominance (Endo et al., 2012). Conversely, activation of mPFC pyramidal neurons is linked with increased dominance (as determined through the tube test) and stimulation of these neurons can lead to increased dominance whereas inhibition leads to decreased dominance (F. Wang et al., 2011; Zhou et al., 2017). Similarly, stimulation of excitatory neurons in the prelimbic cortex supresses intermale aggression, whereas inhibition of these neurons increases aggression (Takahashi et al., 2014). The prelimbic cortex is also activated following acquisition of a social interaction conditioned place preference (El Rawas et al., 2012), again suggesting that mPFC activity is recruited during social encounters.

With the importance of the mPFC in mediating social behaviours, this region has been of particular interest when examining neural mechanisms underlying social deficits in neurological disorders such as autism spectrum disorder (ASD). Shank3 knockdown mice exhibit impaired N-methyl-D-aspartate (NMDA) receptor function leading to decreased excitatory post-synaptic current in the mPFC and reduced sociability in the three-chamber sociability task (Duffney et al., 2015). Increased 4-7Hz activity together

with social deficits has also been reported in the Shank3 knock-out mouse model of ASD (Kuga et al., 2022). In addition, altered excitatory:inhibitory balance in the mPFC (due to decreased numbers of parvalbumin interneurons) has been linked with social deficits in the CNTNAP2 knock-out mouse model of ASD, and these effects can be rescued by increasing excitation of inhibitory interneurons (Selimbeyoglu et al., 2017). Further, optogenetic stimulation of parvalbumin interneurons in a theta-gamma nested pattern can rescue social deficits in the neuroligin 3 R451C knock-in mouse model of ASD (Cao et al., 2018) providing further causal evidence for the mPFC's role in mediating social interactions.

While social deficits have also been reported in a number of AD-model mice, relatively little is known about neural activity in the mPFC of these mice. Using patch-clamp electrophysiology, Bories *et al* (2012) suggested that altered background synaptic activity may be related to social deficits in 3xTg-AD mice, with transgenic males exhibiting no change in social behaviours or synaptic activity at 12 months of age, transgenic males at 18 months and transgenic females at 12 months exhibiting increased social investigation and increased synaptic activity, and transgenic females at 18 months of age exhibiting reduced synaptic activity and reduced social investigation. In the 5xFAD mouse model of AD, patch-clamp electrophysiology has also indicated reduced synaptic transmission in the mPFC by 2 months of age (Chen, Ma, et al., 2022; Chen, Wei, et al., 2022), while fibre photometry has indicated impaired cholinergic signaling from the mPFC to the supramammillary nucleus by 6 months of age (Sun et al., 2022). We have previously reported impaired social behaviours in male and female 5xFAD mice at 6 months of age (Kosel, Torres Munoz, et al., 2019; Kosel et al., 2021), and the results

identified by Bories *et al* would suggest that the altered mPFC activity reported in 5xFAD mice could contribute to these social deficits. However, the relationship between mPFC activity and social deficits in 5xFAD mice—or the relationship between mPFC activity and social behaviours in awake, behaving AD-model mice—has not yet been examined.

The 5xFAD mouse model of AD is a double-transgenic model exhibiting a human amyloid precursor protein (APP) gene with the Swedish (K670N/M671L), Florida (I716V), and London (V717I) mutations and a human presenilin-1 (PSEN1) gene with the M146L and L286V mutations (Oakley et al., 2006). Together, these mutations cause rapid accumulation of amyloid-beta (Aß) with concomitant cognitive, physiological, and behavioural impairments (Bilkei-Gorzo, 2014; Devi & Ohno, 2010; Jawhar et al., 2011, 2012; Oakley et al., 2006; M. Ohno et al., 2006, 2007; O'Leary et al., 2017, 2018; Tohda et al., 2011). In particular, transgenic 5xFAD exhibit impaired axon myelination by 1 month of age, presence of amyloid plaques and gliosis by 2 months of age, dystrophic neurites by 3 months of age, and reductions in whole brain synaptophysin by 4 months of age (Gu et al., 2018; Jawhar et al., 2012; Oakley et al., 2006). By 6 months of age, there is loss of dendritic spines on layer 5 pyramidal neurons in the prefrontal cortex, reduced excitatory synaptic density on layer 2 neurons, reduced basal synaptic transmission levels in the somatosensory cortex and hippocampus, neurodegeneration of noradrenergic and cholinergic neurons, and altered EEG power between 0.1 and ~250Hz (Crouzin et al., 2013; Crowe & Ellis-Davies, 2014; Devi & Ohno, 2010; Eimer & Vassar, 2013; Jawhar et al., 2012; H. Li, Ohta, et al., 2013; Schneider et al., 2014; Seo et al., 2021). As a result, the 5xFAD model is invaluable for exploring cognitive, physiological, and behavioural

changes—as well as their underlying mechanisms—related to AD pathology from early to late disease stages. Our previous findings demonstrated age-related deficits in social behaviours in transgenic 5xFAD females, with reduced investigation of social odours and conspecifics with age (Kosel, Torres Munoz, et al., 2019), as well as reduced social investigative behaviours in response to free-roaming conspecifics in transgenic 5xFAD females and males at 6 months of age (Kosel et al., 2021).

The present study examines whether transgenic 5xFAD mice exhibit altered local field potential (LFP) activity in the mPFC, and whether this activity changes in response to social vs non-social odour cues. Specifically, we examined power spectral density and phase-amplitude coupling (PAC) across several bands of neural oscillations (delta, theta, alpha, beta, low gamma, and high gamma) while subjects were exposed to either control (clean air), non-social (ethyl acetate; artificial banana), or social (novel, same-sex urine) odour stimuli in an olfactometer. Power spectral density has been used to identify altered power in oscillatory bands relative to wild-type controls in ASD-model mice (Cao et al., 2018; Kuga et al., 2022), and transgenic 5xFAD males at 6 months of age have previously been shown to exhibit altered relative EEG power across bands relative to wild-type controls (Schneider et al., 2014). By comparison, PAC is one form of cross-frequency coupling and has been implicated in long-range functional connectivity, working memory, attention, and other cognitive processes (Abubaker et al., 2021; Engel et al., 2013). We hypothesize that transgenic 5xFAD mice will exhibit overall differences in relative power across the oscillatory bands compared to wild-type controls, as well as altered PAC relative to wild-type controls, and that these effects will vary in response to odour stimuli.

5.3 Methods

5.3.1 Subjects

Subjects were 26 wild-type (wt) and transgenic (tg) 5xFAD females and males (*n* = 7 females/group, 6 males/group) on the C57BL6/SJL background at 6-7 months (26-30 weeks) of age. Subjects were housed in groups of 2-3 in a colony room on a 12:12 reversed light-dark cycle, with lights off from 09:30-21:30. Prior to surgery, subjects were housed in standard mouse cages (30 x 19 x 13cm; Allentown Caging Inc., Allentown, NJ, USA) containing wood-chip bedding (FreshBed; Shaw Resources, Shubenacadie, NS), a metal cage top containing *ad libitum* food (LabDiet Prolab RMH 3500; PMI Nutrition International, Arden Hills, MN) and filtered water, two (group-housed) black, opaque, polymer enrichment tubes (4cm diameter; ~8cm long), and nest material. Cages were topped with a micro-isolator filter. Following surgery, subjects were singly-housed in standard rat cages (47 x 26 x 21cm; Allentown Caging Inc.) containing wood-chip bedding (Shaw Freshbed), a black, opaque, polymer enrichment tube (4cm diameter x ~8 cm long), and a flat-bottomed ceramic bowl (~10cm diameter x 4cm tall) containing *ad libitum* filtered tap water; single housing was used to prevent wound dehiscence or other injury during recovery. Approximately 7-10 food pellets (LabDiet Prolab RMH 3500; PMI Nutrition International) were placed directly onto the bedding. Water bowls were checked for feces and debris daily and replaced as needed; fresh food was added as needed. This study was performed in accordance with CCAC and Dalhousie University guidelines and was approved by the Dalhousie University Committee on Laboratory Animals (protocol #20-113).

5.3.2 Surgery

Between 5.5 and 6.5 months (30-34 weeks) of age, subjects underwent surgery to implant four recording tetrodes (2 per side bilaterally; see Section 2.3 below for further details on the tetrode design). Surgeries were performed by using an induction chamber to anaesthetize subjects at 3.0% isoflurane (SomnoSuite; Kent Scientific Corporation, CT, USA), then transferring them to a nose cone in a stereotactic frame. Isoflurane was reduced to ~2.5% and subjects were gently held in place until breathing was stable and minor or no toe pinch reflex was evident. Subjects were then given a 5 mg/kg dose of meloxicam (INFLACAM; Chanelle Pharmaceuticals Manufacturing Ltd., Galway, Ireland) at 0.25 mg/mL subcutaneously (SQ). Subjects then had their heads shaved and fixed in place using ear bars, had their eyes coated with lubricant (Optixcare; CLC Medica, ON, Canada) to prevent formation of cataracts, and had a temperature probe inserted rectally to monitor core temp; subjects were kept on a heat pad (PhysioSuite; Kent Scientific) that was regulated based on the subject's body temperature with a target temperature of 36.5˚C and a maximum heat pad temperature of 37.0˚C (although maximal heat pad readings taken directly under the mouse were up to ~39˚C). The skin was then prepared using three consecutive cleanses using 4.0% chlorhexidine gluconate solution (Hibiclens; Mölnlycke Health Care US LLC, GA, USA), then three alternating washes of 70% ethanol and 10% povidone-iodine solution (Betadine Solution; Purdue Pharma, ON, Canada). Eye lubricant was then checked and reapplied as necessary. A slit (~1cm long) was made in the centre of one edge of a piece of 2"x2" gauze, which was then used as a drape to cover the subject's eyes to prevent damage due to the curing light.

The above preparation (including a surgical scrub) required ~30 minutes; during

this time, the subject's breathing and toe pinch reflex were monitored, and isoflurane concentration was incrementally reduced to ~1.7-2.0%. After confirming that the subject was still in surgical plane, an incision (~1cm long) was made along the midline of the skull, and lightweight vessel clips (Moira SA, Antony, France) were used to retract the skin and expose the skull surface. The fascia was gently removed from the skull surface using the scalpel blade and the head position was verified in the stereotactic frame. The head was adjusted so that the midline of the skull was parallel with the Y-axis of the stereotactic frame. The skull was levelled so that the points 1mm lateral to bregma, as well as bregma +2.25mm at the midline, were +/- 0.05mm of bregma, and all points were +/- 0.10mm of each other. A thin layer of adhesive (Adhese Universal; Ivoclar Vivadent AG, Liechtenstein) was then applied to the skull and cured with a 10s exposure to the UV light, and a 0.75mm drill bit was used to make two windows 0.75mm wide and 1.2mm long centred at bregma +2.25 and immediately adjacent to either side of the midline; these coordinates provided consistent tetrode placement in the target areas of the prelimbic and anterior cingulate cortices. Drilling across the midline was avoided due to the superior sagittal sinus, and adhesive was applied prior to drilling to prevent any adhesive from entering the holes. Two additional round holes (0.75mm diameter) were made ~2mm lateral from the midline and slightly caudal to bregma for the ground and reference screws. The surface of the skull was then flushed with sterile saline and wiped with a sterile cotton swab, and the meninges was removed to expose the surface of the brain at each hole. After removing excess adhesive and bone dust, a ground and reference screw (0.75x3.0mm; stainless steel) were placed into the two caudal holes and inserted ¼ turn into the skull to set them. A small amount of dental composite (Tetric PowerFlow

IVA; Ivoclar Vivadent) was used placed at the base of each screw and cured with a 10s exposure to the UV light to set the screws in place.

The implant was inserted into the holder and given a light rinse with sterile saline, then dabbed dry with a piece of sterile gauze. The implant was adjusted so the guide tubes were vertical, aligned with the skull, and centred in the rostral openings, then slowly lowered using the stereotactic frame until the guide tubes were resting on the surface of the brain but not applying pressure. Dental composite was then used to fix the rear portion of the implant to the skull, medial to the ground and reference screws. The holder was then removed from the implant and the upper portion of the implant was gently lowered to insert the tetrodes into the brain. Once the tetrodes were in place, dental composite was used to fix the upper and lower portions of the implant to each other, and to form the base of the head cap (around the guide tubes, rear portion of the implant, and lower part of the screws). The ground and reference wires were then each wrapped around one screw and fixed in place with dental composite, and the excess wire was cut off. Layers of dental composite were then to form the remainder of the head cap to ensure the implant was solidly fixed in place and there were no openings or hard edges. The incision was then closed up using cyanoacrylate (super glue), and front cover was placed over the implant and fixed in place using super glue. Subjects were given a 0.05 mg/kg of buprenorphine (Vetergesic Multidose; Sogeval UK Ltd., York, UK) at 0.003 mg/mL and a 0.50 mL bolus of lactated ringer's solution 5-10 minutes before the end of surgery.

Following surgery, subjects were transferred to a darkened recovery room and placed in a clean mouse cage lined with paper towel, with ½ of the cage placed on a heat pad on low heat. Subjects remained in the recovery room for ~3-4 hours following

surgery to allow them to fully recover from anaesthesia and for the buprenorphine to fully take effect. Subjects were checked regularly, and once subjects exhibited normal levels of activity, they were transferred to the colony room and placed into a post-surgery cage.

Subjects were given a booster dose of buprenorphine 6-8 hrs after the first dose, and a booster dose of meloxicam 24 hrs after the first dose, then monitored daily until testing. Subjects were checked for weight, condition of the incision, food/hydration (through the presence of feces and urine), and pain levels. Subjects exhibiting any signs of pain (e.g., squinting, hunched back, reduced activity) were given additional analgesics as needed and monitored; none of the animals required buprenorphine past the second day, and none required meloxicam past the third day.

5.3.3 Recording Implants

Implants were produced in-house from a custom design due to the use of Tucker-Davis Technologies (Jacksonville, FL) ZCA-16EIB electrode interface boards (EIB) as these EIBs use non-standard mounting. Implants were designed with four tetrodes in a rectangle spaced 0.65mm apart laterally and 0.35mm apart anterior/posterior and were pre-set for a depth of 1.5-1.6mm; due to the fixed tetrode depth and structural variability of brains across subjects, specific cortical layers were not targeted. Tetrodes were made from 12.5µm nichrome wire (RO800 wire, QH PAC/XTC coating; item #PX000028; Sandvik AB, Sweden) and connected to the EIB using gold pins. Ground and reference screws were connected with stainless steel wire (7-strand, 0.001” bare, Catalog #793200; A-M Systems, Sequim, WA, USA). Tetrodes were gold-plated to an impedance of approximately 100-200kOhm and were sterilized overnight (minimum 10 hours) in glutaraldehyde; due to the gold-plating solution being non-sterile, tetrodes could not be

tested for impedance after sterilization, although previous tests had indicated that sterilization had a minimal effect on tetrode impedance after gold-plating. Sterile implants were rinsed a minimum of 3 times in sterile saline prior to implantation. Implants were used within 48 hours of gold plating.

5.3.4 Testing Paradigm

Following the recovery period, LFP recordings were made during stimulus presentation in an olfactometer using a protocol modified from (Levy et al., 2019). In brief, the olfactometer consisted of a 2-3/4" diameter by 6" long cylindrical testing chamber with a slot along the top for the recording tether, and an inlet on one side and an outlet on the other; stimulus air was delivered to the inlet by a pump, and a series of odorant bottles and solenoid pinch valves was used to present odour stimuli one at a time. The outlet side was connected to an exhaust pump, and airflow between the two sides was adjusted to maintain positive pressure (i.e., air entered the testing chamber faster that it was removed) in order to minimize external odours from entering the chamber. Stimuli consisted of a clean air control (using clean water), ethyl acetate (monomolecular non-social stimulus), artificial banana flavouring (complex non-social stimulus) and urine from novel, same-sex conspecifics (social stimulus). Non-social stimuli were diluted to peak volatile organic compound (VOC) levels of 6000-8000 ppb above baseline as measured by a VOC sensor adjacent to the testing chamber inlet; urine stimuli were undiluted and were largely in the range of 1000-2000 ppb. Stimuli were presented in pseudo-random order in 12 blocks of 4 (a total of 48 trials), with each stimulus presented once per block, and no stimulus being presented twice in succession across blocks. Each trial consisted of a 15s stimulus presentation followed by 60s of clean air to flush the

testing chamber prior to the next trial. Testing took place over two days, with subjects being habituated to the apparatus for 30 minutes on the first day with the recording tether attached, air pumps running, and the clean air control valve being triggered 24 times in total, for a total habituation period of 30 minutes (half the length of a testing session); testing took place on the second day, starting with a 5 minute habituation period (with the recording tether attached and air pumps running, although no valve triggering) followed by a 60 minute testing period.

5.3.5 Verification of Tetrode Placement

Following testing, subjects were anaesthetized using isoflurane and lesions were made (0.9mA for 1s per channel) using the implants while subjects were in surgical plane. Following lesioning and while still in surgical plane, subjects were injected intraperitoneally with an overdose of Euthanyl, monitored to ensure surgical plane was maintained, and perfused using 0.1M phosphate-buffered saline followed by 4% wt/vol paraformaldehyde. Following perfusion, brains were removed, placed in 4% paraformaldehyde, and stored at 4ºC for a minimum of 12 hours; brains were stored for longer to allow for post-fixation if incomplete perfusion was suspected. Brains were then sliced at 70 µm and slices were imaged using a microscope to verify tetrode placement.

5.3.6 Pilot Study

To ensure functionality of the olfactometer during its development, and prior to the present study, a pilot study was undertaken using a modified version of the olfactory habituation/dishabituation task using wild-type 5xFAD mice (n = 6 females, 6 males). In brief, each trial consisted of 60s of odorized air followed by 60s of clean air (to flush the testing chamber); subjects were exposed to 3 successive trials of 3 odours: artificial

almond flavouring (first non-social odour), artificial banana flavouring (second non-social odour), and urine from novel, same-sex conspecifics (social odour). Non-social odours were diluted 1:100 in purified water; note that no clean stimulus was used, and therefore the almond stimulus served as a control for this testing. Investigation was manually scored live, with investigation considered as any period in which the subject was oriented towards the odour inlet side with its snout 2cm or less from the end. Investigation was scored as total investigation time per 2min trial.

5.3.7 Data Processing and Analyses

Data from a single channel per subject (using only tetrodes located within the anterior cingulate [$n = 21$] and prelimbic [$n = 5$] cortices, based on the Allen Mouse Brain Atlas [K. B. J. Franklin & Paxinos, 2008]; see Figure 5.S1) was imported into MATLAB (R2022b Platform Beta 9.13.0.2116060) for processing. Data was down-sampled from ~24kHz to ~500Hz and a 15s period during stimulus presentation (based on valve activation and the time VOC level started increasing) was assessed for artifact-free periods longer than 2s each (a minimum of two full cycles at 1Hz, our lowest frequency of interest); the exception to this were clean air control trials, in which trial start time was taken as valve activation time as VOC levels were expected to exhibit little variation from pretrial to trial periods. Fifteen-second periods were used as that was the overall period of active stimulus delivery. A notch filter (55-65Hz) was applied to raw data to filter out noise from the power mains and each artifact-free period was assessed for power spectral density (PSD) using the MATLAB built-in pwelch() function and phase-amplitude coupling (PAC) using the find_pac_shf() function from the Phase-Amplitude Coupling MATLAB toolbox (Onslow et al., 2011); the modulation index

('mi') setting was used for PAC analysis as results from Onslow *et al* suggested that this measure was preferable for detecting PAC across a range of frequencies under high noise conditions. The following bands were used for analysis: delta (1-4Hz), theta (4-8Hz), alpha (8-13Hz), beta (13-38Hz), low gamma (38-55Hz), and high gamma (65-150Hz). We used 1Hz as the low cut-off for the delta band as this is a value commonly used in many LFP studies; the remaining cut-offs were selected to allow comparison of our data to that obtained by Schneider *et al* (2014), as (to our knowledge) this is the only other study to examine LFP in awake, behaving 5xFAD mice. Gamma was subdivided into low (<55Hz) and high (>65Hz) bands to minimize effects of the notch filter and 60Hz noise from the power mains.

Each band was analyzed separately for raw power relative to the control stimulus (stimulus band power/control band power), percentage of band power to total power (band power/total power) for all stimuli, and PAC between bands. For PAC, the amplitude of each band was only checked against the phase of the lower bands. Outliers (mean +/- 2*SD) were removed; data from 1 wild-type female was removed as there was no control stimulus data available. No other data was removed. Data analysis was performed using mixed-effects models with subsequent estimated marginal means with the Benjamini-Hochberg correction for significant results using R (R 4.2.2 GUI 1.79 Big Sur ARM build 8160; R Foundation for Statistical Computing; Vienna, Austria) and R Studio (Version 2022.12.0+353; Posit Software PBC, Boston, MA).

5.3.8 Data Availability

The data supporting the findings of this study are openly available through the Federated Research Data Repository at https://doi.org/10.20383/103.0777.

5.4 Results

5.4.1 Pilot Study

Analysis of habituation/dishabituation indicated an effect of stimulus, trial, and a stimulus by trial interaction (Table 5.1; Figure 5.1). For stimulus, investigation of the social stimulus was higher than the almond stimulus. For trial, the first trial had higher investigation overall than the third trial. For the stimulus by trial interaction, the first presentations of the social and banana stimuli were higher than all other stimulus presentations. There were no main effects or interactions of sex.

5.4.2 Effect of Stimulus Delivery on Raw Band Power in Wild-type Controls

Preliminary one-sample t-tests were performed to check the mean change in raw power for all stimuli relative to the control stimulus for each band for wild-type subjects (Table 5.2; Figure 5.2). There was a significant effect for the delta (1-4Hz; $t_{11} = 2.3555$, $p = .03812$) and theta (4-8Hz; $t_{11} = 2.8277$, $p = .01644$) bands, with wild-type subjects exhibiting increased power in both bands in response to odour stimuli relative to the control trials, indicating that stimulus presentation was associated with a change in neural activity. No other bands exhibited a significant change relative to the control trial.

5.4.3 Change in Raw Power in Each Band in Response to Stimulus Presentation Relative to Control Stimulus

For the change in raw band power relative to the control trial (stimulus power/control power), there was a genotype effect for the delta band, with wild-type controls exhibiting an increase in band power relative to transgenic 5xFAD mice (χ^2 (1) = 6.5512, $p = .011$; Figure 5.3). There was also an effect of stimulus for the theta (χ^2 (2) = 8.0374, $p = .018$) and beta (χ^2 (2) = 7.9322, $p = .019$) bands, with the social stimulus

Table 5.1: Results for investigation duration during a modified olfactory habituation/dishabituation task in the olfactometer for wild-type 5xFAD females (n = 6) and males (n = 6). Significant results ($p < .05$) are bolded.

χ^2	p-value
Sex	
0.0347	.8522
Stimulus	
7.0741	**.0291**
Trial	
22.1178	**<.0001**
Sex:Stimulus	
2.6731	.2627
Sex:Trial	
1.1389	.5658
Stimulus:Trial	
10.3301	**.0352**
Sex:Stimulus:Trial	
1.2267	.8737

Note: Degrees of freedom are: 1 for sex; 2 for stimulus, trial, their separate interactions with sex; and 4 for stimulus by trial and sex by stimulus by trial interactions.

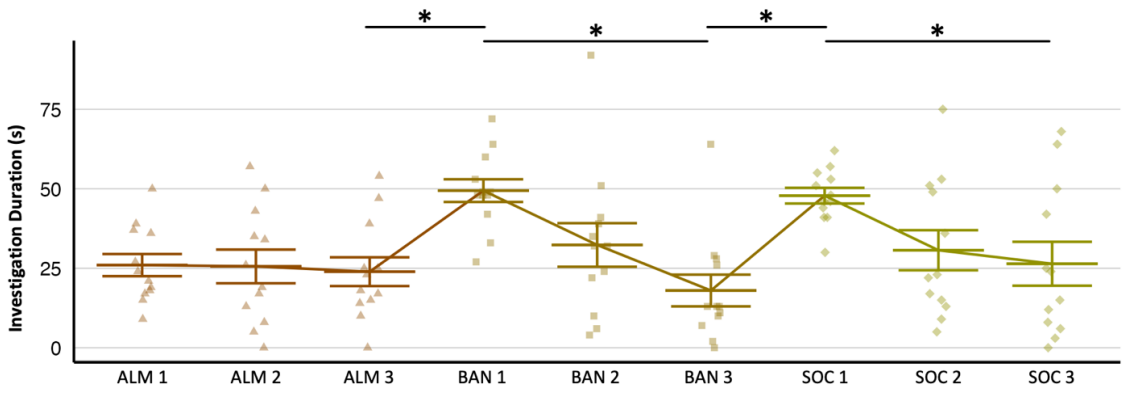

Figure 5.1: Pilot study results for investigation duration (in sec) for olfactory habituation/dishabituation for wild-type 5xFAD males and females (n = 6/group). Stimuli given as odorant (ALM = artificial almond flavouring; BAN = artificial banana flavouring; SOC = novel, same-sex urine) and presentation number (1-3). Error bars given as mean +/- SE; points are raw data. * = $p < .05$; note that only habituation/dishabituation significant effects are identified.

Table 5.2: Results for one-sample t-tests for investigation duration during a modified olfactory habituation/dishabituation task in the olfactometer for wild-type 5xFAD females (n = 6) and males (n = 6). Significant results ($p < .05$) are bolded.

Band	t-statistic	p-value
Delta	2.3555	**.0381**
Theta	2.8277	**.0164**
Alpha	2.1972	.0503
Beta	1.9439	.0779
Gamma (Low)	1.6465	.1279
Gamma (High)	1.6224	.1330

Note: Degrees of freedom are 11 for all tests.

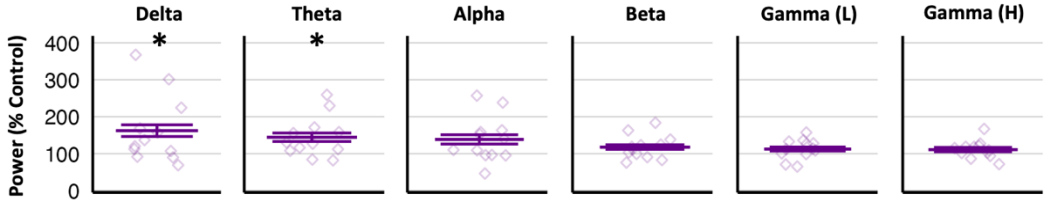

Figure 5.2: Percentage change in raw power relative to control stimulus (stimulus power/control power) for delta (1-4Hz), theta (4-8Hz), alpha (8-13Hz), beta (13-38Hz), low gamma (38-55Hz), and high gamma (65-150Hz) bands for wild-type females and males. Error bars are mean +/- SE; points represent raw data. * = $p < .05$

exhibiting a larger increase than the monomolecular non-social stimulus in both bands, and a larger increase than the complex non-social stimulus in the beta band (Figure 5.3). In addition, there was a sex by stimulus interaction for theta (χ^2 (2) = 9.7932, p = .008), alpha (χ^2 (2) = 11.9412, p = .003), and beta (χ^2 (2) = 16.8639, $p < .001$) bands; in these bands, females exhibited a larger increase in the social stimulus than the monomolecular or complex non-social stimuli, but there were no differences across stimuli in males (Figure 5.3). There was also a sex by stimulus interaction for the delta band (χ^2 (2) = 10.2388, p = .006) that was not maintained in post-hoc testing.

5.4.4 Percentage of Band Power Relative to Total Power

For percentage of band power relative to total power (band power/total power),

transgenic 5xFAD mice demonstrated significantly higher relative power in the delta band (χ^2 (1) = 32.6062, $p < .001$), and significantly lower relative power in theta (χ^2 (1) = 18.5487, $p < .001$), alpha (χ^2 (1) = 25.4754, $p < .001$), beta (χ^2 (1) = 34.0443, $p < .001$), low gamma (χ^2 (1) = 21.3100, $p < .001$), and high gamma (χ^2 (1) = 16.1424, $p < .001$) bands, compared to wild-type controls (Figure 5.4). There was also a genotype by stimulus interaction for the beta (χ^2 (3) = 16.6965, $p < .001$), low gamma (χ^2 (3) = 17.0260, $p < .001$), and high gamma (χ^2 (3) = 17.6005, $p < .001$) bands. Transgenic mice exhibited lower relative power compared to wild-type controls for all stimuli in these bands (Figure 5.4); the exception to this was the monomolecular non-social stimulus in transgenic mice and the complex non-social stimulus for wild-type mice in the high gamma band, for which there was no difference. Transgenic mice also had higher relative power for beta and low and high gamma bands for the monomolecular non-social stimulus compared to the control stimulus, and a higher relative power for the beta band for the complex non-social stimulus compared to the control stimulus. In contrast, wild-type mice had lower relative power for beta, low gamma, and high gamma bands for the complex non-social stimulus compared to control, and lower relative power for low gamma for the monomolecular non-social stimulus compared to control.

Females had higher relative power in the delta (χ^2 (1) = 4.4826, $p = .034$) band, and lower relative power in the beta (χ^2 (1) = 4.6252, $p = .032$) and low gamma (χ^2 (1) = 5.4402, $p = .020$) bands, compared to males (Figure 5.4). For low gamma, there was additionally a sex by stimulus interaction (χ^2 (3) = 11.1799, $p = .011$): males exhibited higher relative power for the monomolecular non-social stimulus compared to control and complex non-social stimulus stimuli; males also exhibited higher relative power for

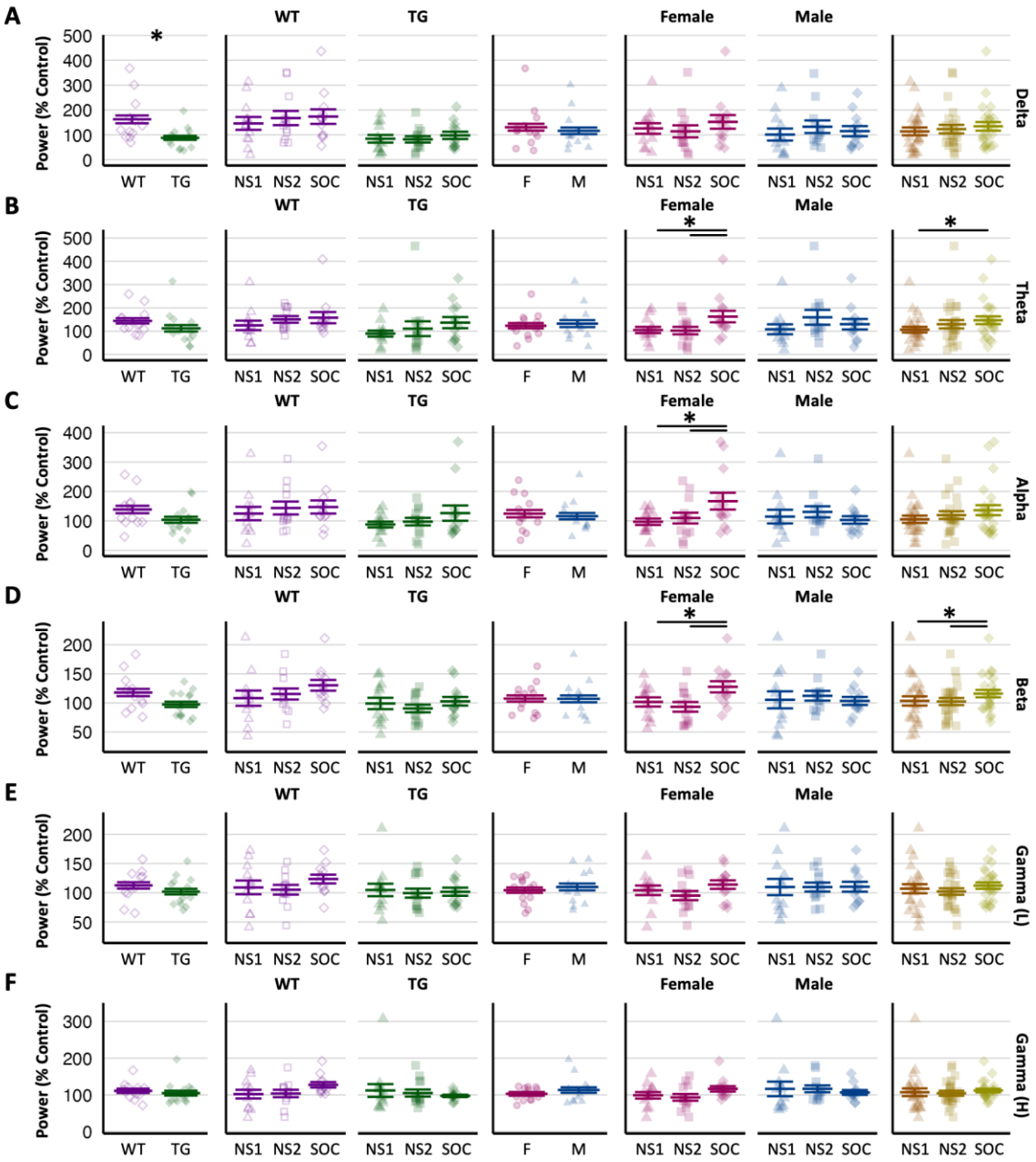

Figure 5.3: Percentage change for band power relative to control stimulus (stimulus power/control power) for ***(A)*** delta (1-4Hz), ***(B)*** theta (4-8Hz), ***(C)*** alpha (8-13Hz), ***(D)*** beta (13-38Hz), ***(E)*** low gamma (38-55Hz), and ***(F)*** high gamma (65-150Hz) bands for genotype (WT = wild-type; TG = transgenic), genotype by stimulus interaction (NS1 = non-social 1 (monomolecular); NS2 = non-social 2 (complex); SOC = social), sex (F = female; M = male), sex by stimulus interaction, and stimulus. Error bars are mean +/- SE; points represent raw data. * = $p < .05$

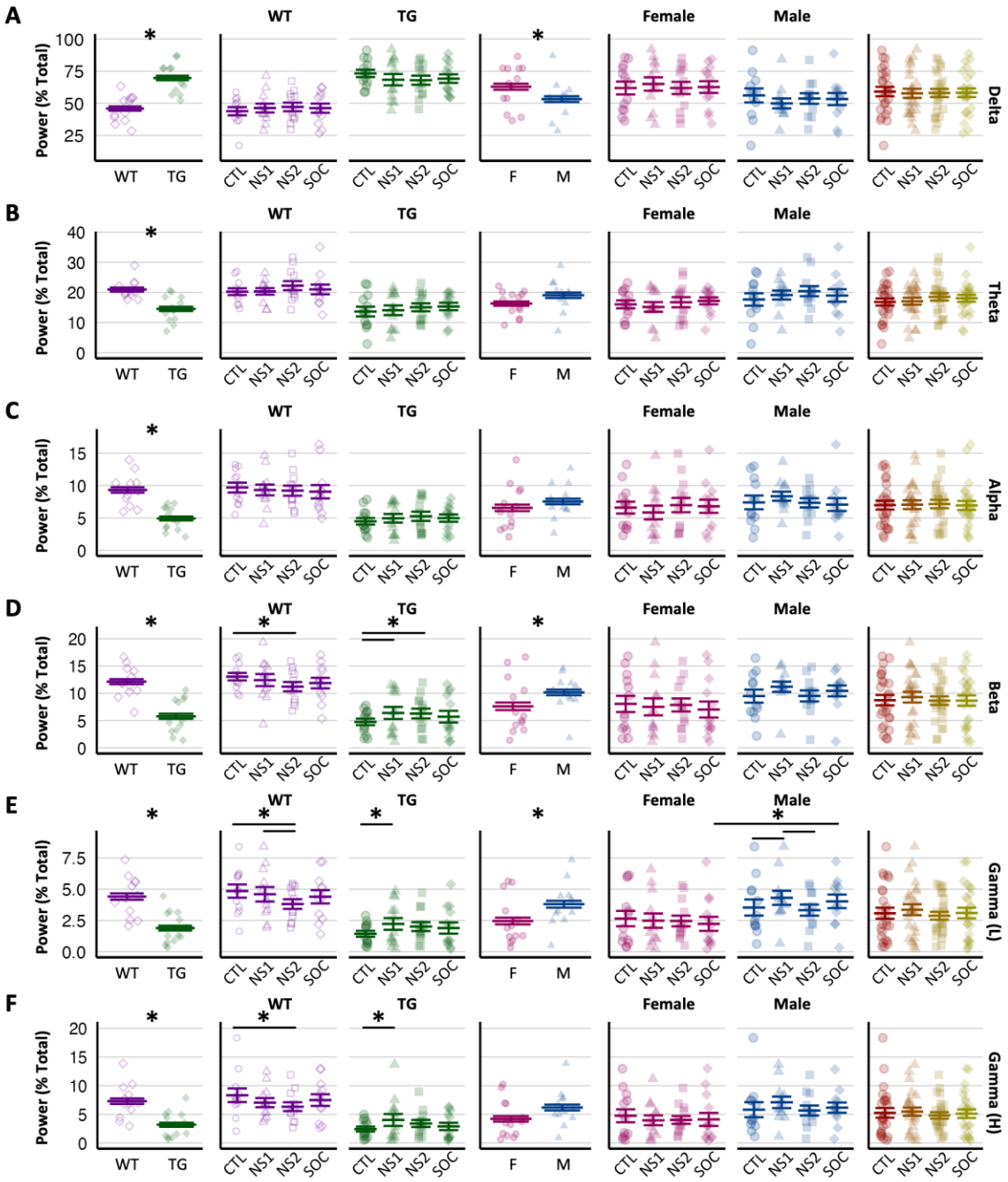

Figure 5.4: Percentage of band power relative to total power (band power/total power) for ***(A)*** delta (1-4Hz), ***(B)*** theta (4-8Hz), ***(C)*** alpha (8-13Hz), ***(D)*** beta (13-38Hz), ***(E)*** low gamma (38-55Hz), and ***(F)*** high gamma (65-150Hz) bands for genotype (WT = wild-type; TG = transgenic), genotype by stimulus interaction (CTL = clean air control; NS1 = non-social 1 (monomolecular); NS2 = non-social 2 (complex); SOC = social), sex (F = female; M = male), sex by stimulus interaction, and stimulus. Error bars are mean +/- SE; points represent raw data. * = $p < .05$

the monomolecular non-social stimulus compared to non-social and social stimuli in females (Figure 5.4). The relative power for the social stimulus was also higher for males than females. There was also a sex by stimulus interaction for the beta band (χ^2 (3) = 11.5507, p = .009) that was not maintained in post-hoc testing.

5.4.5 Phase-amplitude Coupling (PAC)

For PAC, transgenic mice exhibited higher coupling, measured by the modulation index (MI), compared to wild-type controls for delta phase on theta (χ^2 (1) = 15.9035, p < .001), alpha (χ^2 (1) = 15.0742, p < .001), beta (χ^2 (1) = 9.5284, p = .002), and low gamma (χ^2 (1) = 7.0711, p = .008) amplitude; theta phase on alpha (χ^2 (1) = 13.7073, p < .001), beta (χ^2 (1) = 7.7006, p = .006), and low gamma (χ^2 (1) = 4.7962, p = .029) amplitude; alpha phase on beta (χ^2 (1) = 9.1809, p = .002) and low gamma (χ^2 (1) = 6.2201, p = .013) amplitude; beta phase on low gamma (χ^2 (1) = 7.4633, p = .006) amplitude; and low gamma phase on high gamma (χ^2 (1) = 7.9092, p = .005) amplitude (Figure 5.5, 5.6, & 5.7). Females also demonstrated higher coupling compared to males in PAC for delta phase on theta (χ^2 (1) = 6.1207, p = .013), alpha (χ^2 (1) = 5.9205, p = .015), and beta (χ^2 (1) = 4.4255, p = .035) amplitude, and theta phase on alpha (χ^2 (1) = 4.4614, p = .035) amplitude (Figure 5.5 & 5.7).

The type of odour stimulus also influenced PAC between the phase of delta and the amplitude of alpha (χ^2 (3) = 10.1989, p = .017) and beta (χ^2 (3) = 10.7100, p = .013) with the social stimulus resulting in higher coupling compared to the complex non-social stimulus (Figure 5.5). There was also an effect of stimulus for theta phase on low gamma (χ^2 (3) = 9.2674, p = .026) amplitude, but this was not maintained in post-hoc testing (Figure 5.6). Further, there was a sex by stimulus interaction for delta phase on low

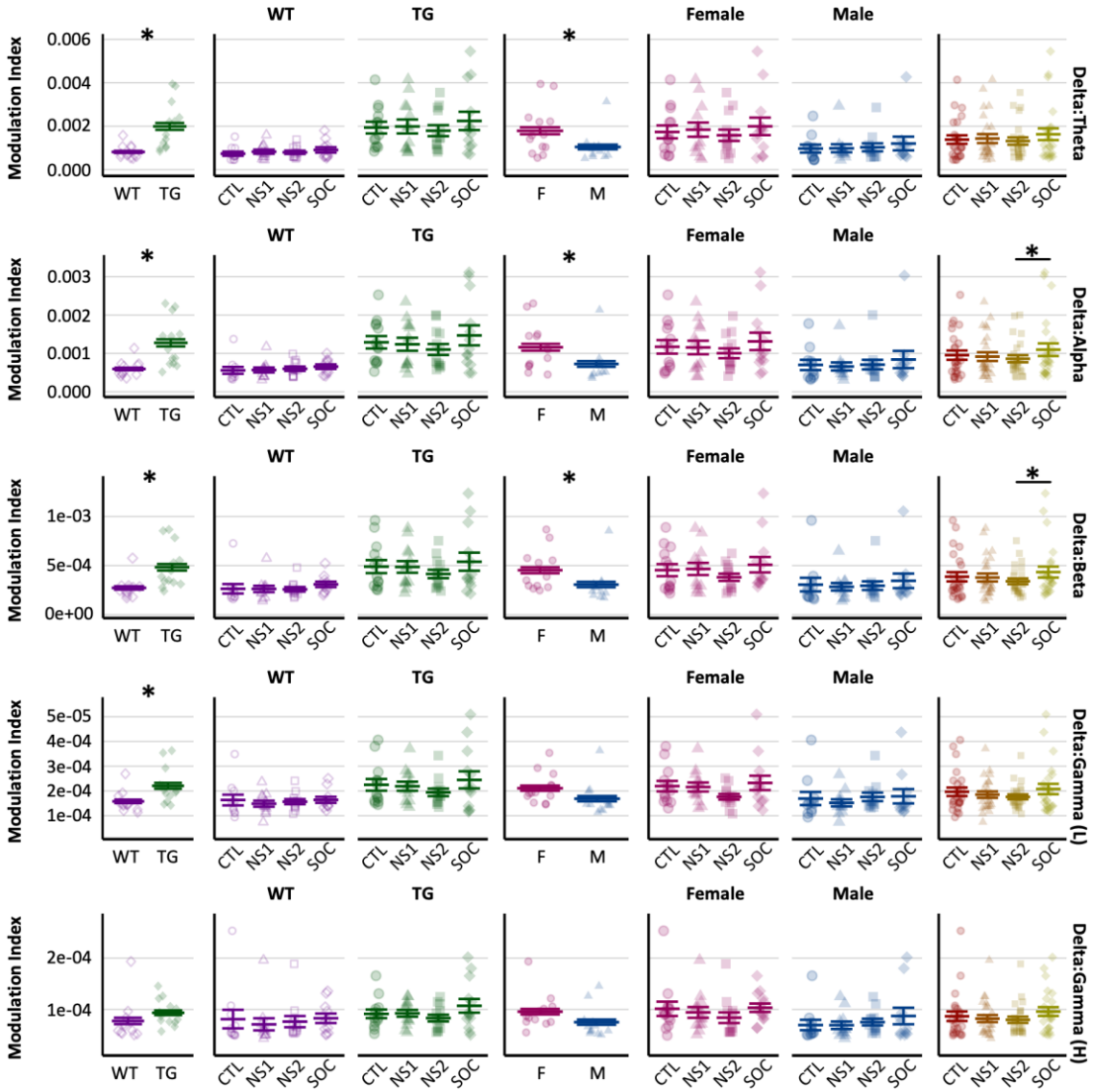

Figure 5.5: Phase-amplitude coupling modulation index based for phase of delta band (1-4Hz) on amplitude of theta (4-8Hz), alpha (8-13Hz), beta (13-38Hz), low gamma (38-55Hz), and high gamma (65-150Hz) bands for genotype (WT = wild-type; TG = transgenic), genotype by stimulus interaction (CTL = clean air control; NS1 = non-social 1 (monomolecular); NS2 = non-social 2 (complex); SOC = social), sex (F = female; M = male), sex by stimulus interaction, and stimulus. Error bars are mean +/- SE; points represent raw data. * = $p < .05$

gamma ($\chi 2$ (3) = 8.2145, p = .042) amplitude, and alpha phase on beta ($\chi 2$ (3) = 8.2368, p = .041) and low gamma ($\chi 2$ (3) = 7.8800, p = .049) amplitude, although this was not maintained in post-hoc testing (Figure 5.5 & 5.7).

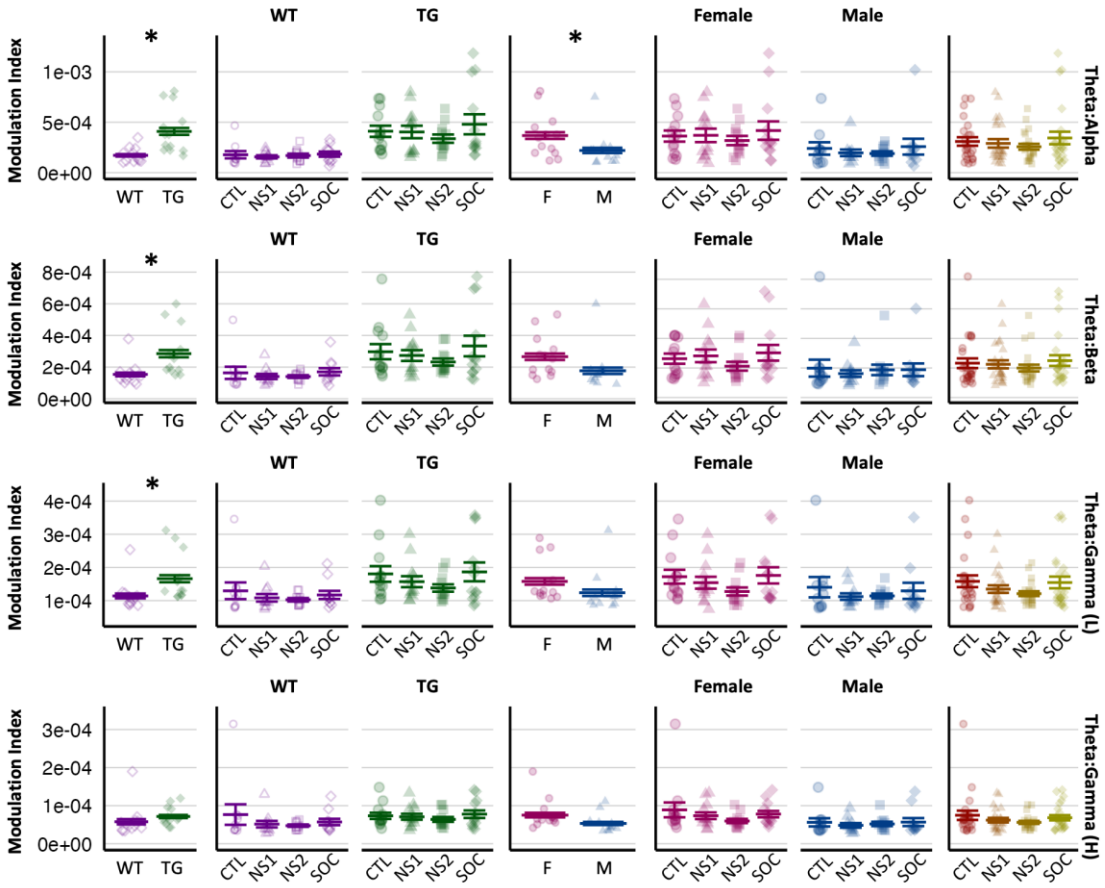

Figure 5.6: Phase-amplitude coupling modulation index based for phase of theta band (4-8Hz) on amplitude of alpha (8-13Hz), beta (13-38Hz), low gamma (38-55Hz), and high gamma (65-150Hz) bands for genotype (WT = wild-type; TG = transgenic), genotype by stimulus interaction (CTL = clean air control; NS1 = non-social 1 (monomolecular); NS2 = non-social 2 (complex); SOC = social), sex (F = female; M = male), sex by stimulus interaction, and stimulus. Error bars are mean +/- SE; points represent raw data. * = $p <$ .05

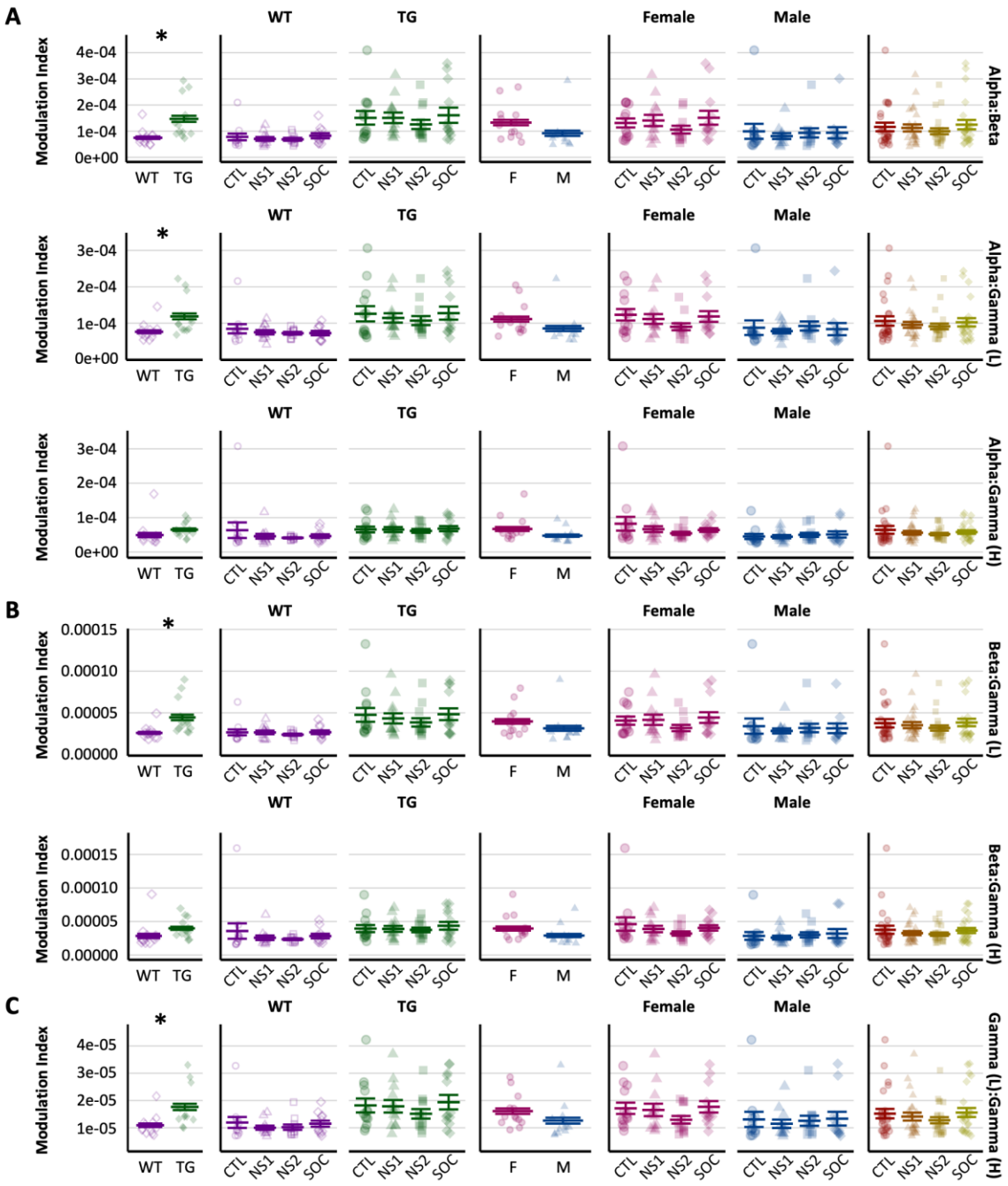

Figure 5.7: Phase-amplitude coupling modulation index based for phase of ***(A)*** alpha band (8-13Hz) on amplitude of beta (13-38Hz), low gamma (38-55Hz), and high gamma (65-150Hz) bands; ***(B)*** phase of beta on amplitude of low and high gamma; and ***(C)*** phase of low gamma on amplitude of high gamma for genotype (WT = wild-type; TG = transgenic), genotype by stimulus interaction (CTL = clean air control; NS1 = non-social 1 (monomolecular); NS2 = non-social 2 (complex); SOC = social), sex (F = female; M = male), sex by stimulus interaction, and stimulus. Error bars are mean +/- SE; points represent raw data. * = $p < .05$.

5.5 Discussion

Here, we show that transgenic 5xFAD females and males have altered neural activity in the mPFC relative to wild-type controls both in response to olfactory stimuli and under baseline conditions. In particular, the relative power of each band—given as a percentage of total power across all bands—is altered in transgenic 5xFAD mice, with the delta band (1-4Hz) accounting for a higher percentage of total power compared to wild-type controls; all other bands (theta, alpha, beta, low gamma, and high gamma) account for less of the total power in transgenic mice compared to wild-type controls. Similar effects are observed for sex, as females exhibit higher relative delta band power and lower relative beta and low gamma band power compared to males. Finally, transgenic 5xFAD mice exhibit higher PAC overall relative to wild-type controls, with stronger coupling between most bands across all odour stimuli.

Overall, these results suggest that transgenic 5xFAD mice exhibit an imbalance in LFP power relative to wild-type controls, as well as altered PAC. While we did not identify aberrant LFPs in transgenic 5xFAD mice exposed to social odour, females exhibit increased power in the theta, alpha, and beta bands in response to social stimuli relative to non-social stimuli; this is similar to results in C57BL/6J mice, which exhibit increased 20-50Hz power during social investigation (L. Liu et al., 2020). However, this effect was specific to females, as wild-type controls overall in the present study did not exhibit an increase in response to odour stimuli in this range.

The extent of neuropathology exhibited in transgenic 5xFAD mice by 6 months of age—both in the mPFC and in other cortical and subcortical regions—likely accounts for the altered neural activity found here. Transgenic 5xFAD mice exhibit a variety of

myelination abnormalities in the prelimbic cortex (as well as other regions) as early as 1 month of age, with progressive changes with age including thinner axon cross-sections by 5 months of age (Gu et al., 2018); similarly, dystrophic neurites have been reported in the cortex by 3 months of age (Jawhar et al., 2012). Amyloid deposition, along with astrogliosis and microgliosis (markers of neuroinflammation), is present in deep cortical layers by 2 months of age and progresses outwards from there, and reduced synaptophysin (relative to wild-type controls) is present by 4 months of age (Oakley et al., 2006). By 6 months of age, layer 5 pyramidal neurons in the prefrontal cortex exhibit significant loss of dendritic spines, while layer 2 neurons show reduced excitatory synaptic density (Crowe & Ellis-Davies, 2014; Seo et al., 2021); at this age, the prefrontal cortex also shows a significant increase in the number of pyknotic cells (Maiti et al., 2021).

These cellular-level changes are also accompanied by altered neural activity. Patch-clamp electrophysiology of the mPFC in brain slices from transgenic 5xFAD mice has indicated reduced intrinsic excitability and miniature excitatory post-synaptic currents, higher action potential threshold, and impaired long-term potentiation in layer 5 neurons (Chen, Ma, et al., 2022; Chen, Wei, et al., 2022). LFP recordings from cortical surface electrodes in 6-month-old transgenic 5xFAD males indicate that the sub-delta (~0.1-0.5Hz) band accounts for a higher overall proportion of total power relative to all other bands up to ~250Hz (Schneider et al., 2014). While it is impossible to confirm without raw data, their graphs suggest a similar pattern of relative band power to the one we found in the delta to high gamma range; specifically, their graphs suggest a relatively steep drop in relative power from the delta band to all higher bands in transgenic 5xFAD

males, whereas wild-type controls exhibit a more gradual reduction in relative power from delta to the higher bands, akin to the effects we observed. While our results are specific to anterior cingulate cortex, this suggests that similar and more widespread changes could be present across cortical areas. AD-related neuropathology impairs cholinergic signaling in humans and 5xFAD mice (Auld et al., 1998; Bartus, 2000; Devi & Ohno, 2010; Lehéricy et al., 1989), including reduced cholinergic input into the mPFC of transgenic 5xFAD mice (Sun et al., 2022); as stimulation of nicotinic and muscarinic acetylcholine receptors reduces low frequency (1-6Hz) power and increases high frequency (>10Hz) power (Kalmbach & Waters, 2014), the balance of low vs high frequency oscillations is modulated—at least in part—by cholinergic signaling, suggesting that the reduced cholinergic activity could result in an increase in low frequency power and a decrease in high frequency power, similar to the results indicated here. Further, our study also indicated altered PAC in transgenic 5xFAD mice relative to wild-type controls. PAC has been implicated in long-range cortical communication and mediating attention and state changes (Engel et al., 2013), suggesting that these mice may exhibit altered processing of information encoded within bands as well as altered integration of information across bands. The altered PAC reported here also supports the possibility of impaired long-range communication in transgenic 5xFAD mice as suggested in recent work with fibre-photometry examining acetylcholine release in extratelencephalic projection neurons (Sun et al., 2022), although the present study did not examine functional connectivity across brain regions.

Our findings further contribute to the literature suggesting a role for the mPFC in mediating complex behaviours. Activity in the mPFC has been closely linked with social

behaviours in general: prior evidence has demonstrated its involvement in social defeat (Challis et al., 2014; T. B. Franklin et al., 2017), dominance and aggression (Endo et al., 2012; Takahashi et al., 2014; F. Wang et al., 2011, 2014; Zhou et al., 2017), and social conditioned place preference (El Rawas et al., 2012), among other behaviours. Moreover, evidence from mouse models of autism spectrum disorder (ASD) suggests that social deficits are linked with altered mPFC activity and that modulation of mPFC activity can rescue these deficits (Cao et al., 2018; Duffney et al., 2015; Kuga et al., 2022; Selimbeyoglu et al., 2017). Differential LFP recordings have been previously associated with social investigation; increased low gamma (20-50Hz) activity is present in wild-type mice during social and non-social investigation in a three-chamber task, with a larger increase in power in response to social stimuli (L. Liu et al., 2020). This study also indicated that gamma band power bidirectionally modulates social activity, suggesting that the relatively low power we observed in the theta through high gamma ranges—including the 20-50Hz range—could contribute to the social deficits we previously reported in this model (Kosel, Torres Munoz, et al., 2019; Kosel et al., 2021). The LFP data presented here is also consistent with other studies examining mouse models with social deficits. Neuroligin 3 R451C knock-in mice (an ASD model) exhibit impaired social novelty preference as well as reduced low gamma (30-90Hz) activity (Cao et al., 2018). Finally, patch-clamp electrophysiology in the 3xTg-AD mouse model of AD has suggested that social deficits are related to altered background synaptic activity in the mPFC (Bories et al., 2012), although the relationship between social processing and mPFC activity in awake, behaving AD-model mice had not been examined until now.

While our findings provide support for the hypothesis that altered neural activity

in the mPFC could contribute to social deficits in AD, it is important to note that the mPFC is not solely involved in social behaviours, nor did we observe any genotype effects specific to the social odour stimulus. Altered mPFC activity indicated by aberrant LFPs in transgenic 5xFAD mice could also contribute to differences in anxiety or arousal, behavioural traits that have also been linked to this brain area. For example, the increased delta band power we observed is also observed in mouse models of stress and anxiety. (Lv et al., 2022) reported increased delta wave amplitude in the mPFC and hippocampus of mice exposed to a high-stress (tail suspension) but not mild stress (light exposure) situation. Similarly, increased delta band power was also reported in mice receiving intracerebroventricular (i.c.v.) injections of cholecystokinin (CCK), a peptide with reported involvement in anxiety and panic disorders (H. Li, Ohta, et al., 2013). Li *et al* reported that i.c.v. injections of CCK resulted in increased anxiety as well as increased cortical and subcortical power in the 0-30Hz range, with a particular increase in 2-4Hz (delta) band power, and these effects were ameliorated through intraperitoneal (i.p.) injection of CI-988 (a CCK_B receptor antagonist). Oscillations in the 2-6Hz range—with a specific peak at 4Hz—also mediate fear behaviour, with prefrontal 4Hz oscillations leading to synchronization of the amygdala and predicting freezing behaviour, and artificial stimulation of prefrontal cortical neurons at 4Hz leads to increased freezing behaviour (Karalis et al., 2016). Finally, recent results have indicated that male C57BL/6 mice exhibit increased relative delta band power and decreased theta and alpha band power during active investigation of the cadaver of a conspecific vs an anaesthetized conspecific, as well as increased anxiety following exposure to the cadaver (Jeon et al., 2021); despite the use of a new target (cadaver or anaesthetized) mouse for each session,

these mice also exhibit reduced investigation of the cadavers over three successive days, whereas there is no decrease in investigation of anaesthetized mice from day 1 to day 2, suggesting that the novelty of the target mouse is outweighed by an aversion to a deceased conspecific. Overall, these results suggest that low frequency oscillations, particularly those in the 2-4Hz range, are related to anxiety and fear behaviour in mice; as a result, the relatively high delta band power exhibited by transgenic 5xFAD mice in the present study suggests that these mice exhibit increased anxiety relative to wild-type controls. These findings are consistent with our previous work suggesting that reduced social investigation exhibited by transgenic 5xFAD females and males arises due to anxiety or arousal (Kosel, Torres Munoz, et al., 2019; Kosel et al., 2021). However, while we interpreted our results to be specific to social behaviours—partially due to the lack of general anxiety or apathy previously reported in this model (Kosel et al., 2020)—our three-chamber results identified a reduction in overall investigation rather than reduced social preference. In addition, the free social interaction task has been used to investigate variability in social interactions under different levels of anxiety (File & Hyde, 1978; Pentkowski et al., 2021); thus—in retrospect—we cannot rule out the possibility that reduced social investigation in our tests of sociability arise (at least in part) as a result of general anxiety or heightened arousal in transgenic 5xFAD mice.

It is of particular interest that we also observed genotype effects—particularly reduced relative power—in the beta band, and oscillations in this band have been linked with olfactory processing in rodents (Kay, 2014). In particular, rats exhibit increased beta band activity in response to odour cues during olfactory learning in a go/no-go task (Fourcaud-Trocmé et al., 2019; Hermer-Vazquez et al., 2007; Ravel et al., 2003). While

Fourcaud-Trocmé *et al* focused on brain regions specifically related to olfactory processing, they and others have indicated that beta band oscillations are highly coherent across brain regions (Fourcaud-Trocmé et al., 2019; Kay, 2014), and there is a possibility that the low relative beta band power exhibited in the mPFC of transgenic 5xFAD mice could arise due to altered olfactory processing. However, the majority of evidence suggests that transgenic 5xFAD mice exhibit no deficits in olfactory processing or learning and memory (Kosel, Torres Munoz, et al., 2019; O'Leary, Stover, et al., 2020; Roberts et al., 2020; Roddick et al., 2014, 2016), suggesting that the reduced beta band power we observed in the mPFC is not related to impaired olfaction.

Similar to our results, a number of studies have also reported altered PAC in rodent models of AD. In particular, theta-gamma PAC is reduced in the medial septum and hippocampus of J20 mice (Etter et al., 2019; Mondragón-Rodríguez et al., 2018), the entorhinal cortex of APP knock-in and rTg4510 mice (Booth et al., 2016; Nakazono et al., 2017), and the hippocampus of TgCRND8 and P301L mice and TgF344-AD rats (Ahnaou et al., 2017; Bazzigaluppi et al., 2018; Goutagny et al., 2013; Van Den Berg et al., 2023). However, APP knock-out mice also exhibit reduced theta-gamma coupling in the parietal cortex and the hippocampus (X. Zhang et al., 2016), and increased theta-gamma PAC has been reported in the mPFC of tau-injected rats (Tanninen et al., 2017) while APP/PS1 mice exhibit increased beta-gamma PAC in the striatum (Peng et al., 2021), suggesting that PAC is dependent on mechanisms other than APP and/or tau overexpression alone. Moreover, evidence from (Jacob et al., 2019) suggests that APP^{NL-G-F} mice did not exhibit significant differences in theta-gamma coupling in the mPFC relative to wild-type controls despite differences in the hippocampus and striatum,

suggesting that subcortical PAC can be dissociated from the mPFC; as most existing studies with AD-model rodents do not report mPFC data, it is unclear what the PAC relationship is between the mPFC and subcortical structures in AD-model mice in general.

Impaired hippocampal theta-gamma PAC in AD-model mice is also consistent with studies on cognitive and behavioural deficits. Angiotensin II-induced hypertension led to impaired novel object recognition memory, increased anxiety, and reduced theta-gamma PAC between the perforant pathway and the dentate gyrus (Gao et al., 2021). The *Disrupted-in-schizophrenia 1* gene similarly leads to impaired theta-gamma PAC in the dentate gyrus along with impaired memory and reduced sociability (Z. Yang et al., 2021). Social isolation housing also negatively impacts hippocampal theta-gamma coupling and leads to impaired memory and social deficits (Almeida-Santos et al., 2019; H. Wang et al., 2020). Hippocampal theta-gamma coupling has been shown to influence PAC in the mPFC in a chronic unpredictable stress model of depression (Zheng & Zhang, 2015), while altered inter-region theta-gamma PAC has been demonstrated in the mPFC and amygdala in response to both chronic and acute stress (Merino et al., 2021; Z. Wang et al., 2021). In addition to the altered gamma band power indicated above, the neuroligin 3 R451C knock-in mouse model of ASD exhibits reduced sociability and altered theta-gamma PAC, with stronger coupling between theta (4-12Hz) and high gamma (50-90Hz), whereas wild-type controls exhibit stronger coupling between theta and low gamma (30-50Hz; (Cao et al., 2018). In contrast, our study identified increased coupling between the 4-12Hz range and the 30-50Hz range—split into theta (4-8Hz), alpha (8-13Hz), beta (13-38Hz), and low gamma (38-55Hz) in our study—in transgenic 5xFAD mice compared to

wild-type controls, highlighting key mechanistic differences between models displaying similar behavioural phenotypes.

Studies in humans with AD have also indicated altered neural activity that sometimes parallels mouse models of AD and findings presented here. During mild AD, alpha oscillations slow in frequency and eventually disappear, with later stages categorized by a predominance of low frequency oscillations, and increased theta activity and decreased beta activity can be used to differentiate those with AD from cognitively healthy individuals (Czigler et al., 2008; Kanda et al., 2017; Smith, 2005); this is reflected in the present study, as transgenic 5xFAD mice similarly exhibit higher relative low frequency (1-4Hz) power and lower relative high frequency power (4-150Hz) compared to wild-type controls. Individuals with AD also exhibit reduced theta band responses and progressive reductions in delta response to oddball stimuli compared to cognitively healthy individuals, with reductions evident in those with mild cognitive impairment (MCI) and further reductions in AD (Güntekin et al., 2022). However, activity in the alpha, beta, and gamma bands is more variable, with some results indicating increased responses in individuals with AD while others indicate decreases (Başar et al., 2016; Güntekin et al., 2022). While the present study did not use an oddball paradigm, transgenic 5xFAD mice exhibited a reduced change in absolute delta band power compared to wild-type controls in response to odour stimuli. Changes in PAC have also been reported in humans with AD, with lower theta-gamma PAC in those with MCI or AD compared to cognitively healthy individuals, and both cognitively healthy and MCI individuals—but not those with AD—exhibit increased PAC in response to an olfactory oddball paradigm (Fatemi et al., 2022). However, while spatial synchronization

in the gamma band is generally reduced in individuals with AD, data from other bands is inconsistent, with studies indicating reduced (Czigler et al., 2008; Fatemi et al., 2022) or increased (Fide et al., 2023) synchronization. While these studies have largely focused on clinical measures (e.g., Mini-Mental State Exam, Clinical Dementia Rating scale, DSM-IV) to differentiate cognitively healthy individuals from those with MCI or AD, reductions in alpha band power in AD have also been linked with post-mortem density of neurofibrillary tangles (Ranasinghe et al., 2021), and reduced delta band power is correlated with reduced frontal cortical volume in mild cognitive impairment (Yener et al., 2016), indicating a relationship between neuropathology and neural activity. Although the present study also identified reduced relative alpha band power, transgenic 5xFAD mice do not exhibit tau pathology (for review, see Kosel et al., 2020); conversely, we identified a general increase in PAC and delta band power whereas AD-related pathology in humans is related to reduced PAC and delta band power, suggesting that AD-model mice exhibit some mechanistic differences compared to humans between AD-related pathology and neural activity.

In humans, patterns of neural activity have been implicated in emotional processing, anxiety, and sociability. During adolescence, typical maturation results in decreased relative power in the delta (1-3Hz) band and increased relative power in higher (e.g., alpha, beta, gamma) bands (Marek et al., 2018; Ott et al., 2021). However, in children with ASD—a condition with prominent social deficits—these age-related effects are attenuated, with less reduction in relative delta band power and less increase in relative theta, beta, and gamma band power compared to neurotypical children (Clarke et al., 2016; Vakorin et al., 2017; Yeung et al., 2016) resulting in higher relative delta band

power and lower relative theta, beta, and gamma band power, similar to the results we identified here in transgenic 5xFAD mice relative to wild-type controls. Individuals with ASD also exhibit altered fMRI activity relative to neurotypical controls across a number of regions during social tasks, including the mPFC and hippocampus (Patriquin et al., 2016). Delta band activity has been linked to emotional processing in humans, with individuals exhibiting increased delta oscillation power after viewing images designed to evoke an emotional response, with higher-arousal images leading to larger increases than low-arousal images and females exhibiting higher increases compared to males (Klados et al., 2009). Lower alpha/delta ratios—that is, lower relative power in the alpha band vs the delta band—in adolescents have also been linked to higher levels of social (but not general) anxiety (Schmidt et al., 2022), although increased alpha power has been linked with higher trait anxiety (Knyazev et al., 2004). Overall, these results—higher delta band power and/or lower power in higher bands—have been linked with impaired social behaviours and increased arousal and anxiety in humans, similar to the results reported here. Additionally, we also identified an overall increase in PAC in transgenic 5xFAD mice compared to wild-type controls, and coupling between bands in the human frontal cortex has also been implicated in social anxiety, with high socially anxious individuals exhibiting higher delta-beta coupling under baseline conditions and during a stressful (speech anticipation) condition (Miskovic et al., 2010), and harsh (as opposed to sensitive) parenting—particularly by fathers—was similarly associated with increased delta-beta coupling and increased social fear in preschoolers (Najjar & Brooker, 2017). Sriranjan *et al* (2022) also examined delta-beta coupling in children during a pre-surgical consultation and similarly reported that high frontal delta-beta coupling is associated with

higher shyness and higher state anxiety; however, children who had lower shyness (as well as lower state anxiety and delta-beta coupling) exhibited increased delta-beta coupling on the day of surgery compared to the consultation, whereas children who have high shyness did not, despite similar increases in state anxiety for the two groups. With this, Sriranjan *et al* suggest that delta-beta coupling may be linked with emotional regulation, although they highlight that they were unable to assess anxiety and cortical activity at baseline, so it's unclear whether high shyness/anxiety children exhibit increases in anxiety and delta-beta coupling relative to baseline. Still, this overall data suggests that neural activity in the frontal cortex—particularly the ratio of low to high frequencies and coupling between bands—is key for mediating behaviour in both humans and animals, and changes in neural activity arising from neurological disorders can negatively impact these behaviours.

5.5.1 Conclusions

Overall, transgenic 5xFAD mice exhibit altered LFP activity in the mPFC relative to wild-type controls, particularly in terms of altered distribution of power between 1 and 150 Hz—namely, increased relative power in the delta (1-4Hz) band and decreased power in all higher (4-150Hz) bands—as well as increased PAC across several bands. Although neuropathology was not quantified in the present study, this has been well studied in this mouse model and findings have shown that transgenic 5xFAD mice exhibit extensive neuropathology in the mPFC (and other brain regions) by 6 months of age, including altered excitability of mPFC layer 5 neurons and reduced cholinergic signalling in the mPFC, suggesting that AD-related neuropathology underlies changes in neural activity in the region (Crouzin et al., 2013; Crowe & Ellis-Davies, 2014; Devi &

Ohno, 2010; Eimer & Vassar, 2013; Gu et al., 2018; Jawhar et al., 2012; H. Li, Ohta, et al., 2013; Oakley et al., 2006; Schneider et al., 2014; Seo et al., 2021). Additionally, the role of mPFC activity in social behaviour has been well-established, and studies with mouse models of autism-spectrum disorder have linked altered neural activity in the mPFC with social deficits (Cao et al., 2018; Duffney et al., 2015; Kuga et al., 2022; Selimbeyoglu et al., 2017). However, while we have previously reported social deficits in 5xFAD mice (Kosel, Torres Munoz, et al., 2019; Kosel et al., 2021), the mPFC has also been implicated in arousal, emotional processing, and anxiety (Jeon et al., 2021; Karalis et al., 2016; Klados et al., 2009; Knyazev et al., 2004; H. Li, Ohta, et al., 2013; Lv et al., 2022; Mehak et al., 2023; Merino et al., 2021; Pentkowski et al., 2021; Z. Wang et al., 2021; Zheng & Zhang, 2015), and it is possible that these factors may also play a role in the BPSD-like behavioural phenotype we previously described in these mice. Despite this, the link between apathy (Mehak et al., 2023), anxiety (Mendez, 2021), and neuropathology in humans with AD and AD-model mice suggests that the altered neural activity we report here underlies the altered behaviours we have previously identified in this model.

It is important to note that we observed sex effects in conjunction with our genotype effects, consistent with other data suggesting sex effects on pathology in transgenic 5xFAD mice. Relative to males, transgenic 5xFAD females exhibit upregulation of a number of genes associated with neuropathology by 4 months of age, including the mutated APP and PSEN1 transgenes responsible for driving the AD-related pathology exhibited by this model (Bundy et al., 2019), consistent with increased Aß pathology observed in females compared to males at 3 and 6 months of age (Reid &

Darvesh, 2015). Transgenic 5xFAD females are also more susceptible to stress than males, with behavioural stress resulting in significant increases in ß-site APP cleaving enzyme 1 (BACE1), Aß deposition, and presence of APP C-terminal fragments in the hippocampus of females but not males (Devi et al., 2010). Similarly, no sex differences in lifespan were observed in transgenic 5xFAD mice despite C57BL6/SJL females—the background strain—living longer than males, suggesting that the rate of pathology in transgenic 5xFAD females is having a larger impact on survival than in males (Rae & Brown, 2015); however, Todorovic *et al* (2020) indicated that transgenic 5xFAD males accumulated physiological impairments associated with frailty more rapidly than females, leading to higher overall scores by 11 months of age. These sex effects are also consistent with human data; in 2012 there were approximately 85,000 females with AD in institutions in Canada, compared to ~34,000 males, and as of 2022 there were ~82,000 community-dwelling females with AD compared to ~59,000 males (Statistics Canada, 2013, 2022). While it has been suggested that the difference in number of males vs females is due to sex differences in life expectancy, there is ample evidence to indicate that both sex and gender play a role in the development of AD, and females—as a whole—tend to exhibit more severe pathology and clinical signs (Ferretti et al., 2018; Mielke et al., 2014; Oveisgharan et al., 2018; Scheyer et al., 2018; Zhu et al., 2021).

Overall, our findings provide further support that neuropathology—specifically changes in neural activity in the mPFC arising from neurodegeneration—underlies some of the BPSD observed in humans with AD. The increased relative delta power and decreased relative power in higher bands somewhat reflects the findings observed in some human AD studies (Czigler et al., 2008; Kanda et al., 2017; Smith, 2005), and

increased PAC has been reported as well (Fide et al., 2023), although this finding has been inconsistent (Czigler et al., 2008; Fatemi et al., 2022). A recent fMRI study has suggested that social dysfunction in humans with AD is related to reduced connectivity of the default mode network in the mPFC (Saris et al., 2022), consistent with our findings of altered mPFC activity, although no studies to date have reported on LFP activity relative to social deficits in humans with AD. That said, research is continually providing evidence to support the role of neuropathology in the development of BPSD—including social deficits—thereby highlighting the need for additional research in this area in both humans and animal models.

5.6 Acknowledgements

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5.7 Supplemental Materials

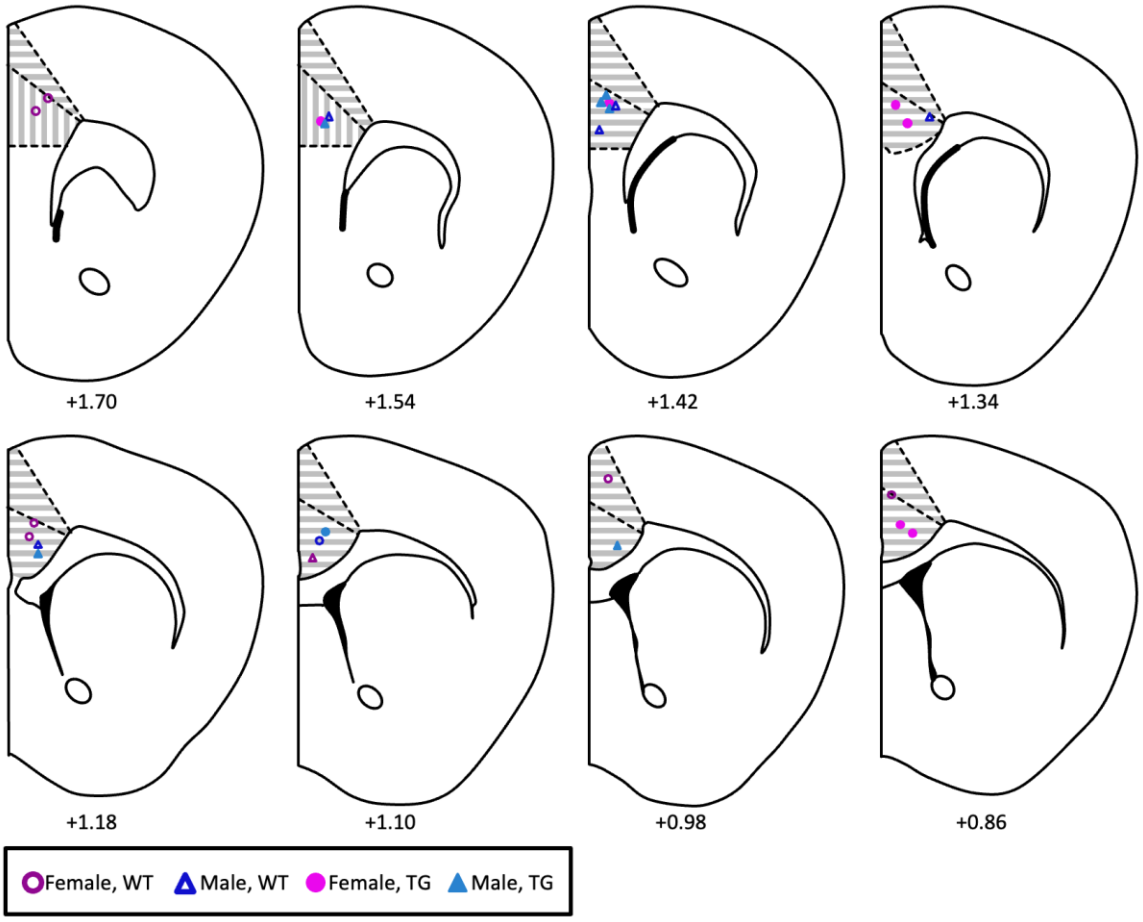

Figure 5.S1: Implant locations used for recordings for wild-type females (WT; dark pink, open circles), wild-type males (WT; dark blue, open triangles), transgenic females (TG; bright pink, filled circles), and transgenic males (TG; bright blue, filled triangles). Locations of the anterior cingulate cortex (horizontal pattern) and prelimbic cortex (vertical pattern) are indicated using dashed lines and measurements are provided from bregma based on the 3rd Edition of the Allen Mouse Brain Atlas. Implants were made bilaterally but are shown on a single hemisphere.

Chapter 6. General Discussion

6.1 Overview

The present work examined behavioural and psychological symptoms of dementia (BPSD)-like behaviours—specifically, social withdrawal and aggression—in the transgenic 5xFAD mouse model of Alzheimer's disease (AD)-related amyloid pathology, as well as the local field potential (LFP) activity in the medial prefrontal cortex (mPFC) that could contribute to abnormal social behaviours reported here. To this end, the research was performed through several stages: *1)* a review of existing literature on behavioural and psychological symptoms of dementia (BPSD)-like behaviours in mouse models of AD to identify relationships between neuropathology and these behaviours; *2)* research continuing from my MSc thesis to examine social behaviours in transgenic 5xFAD females and males at 6 months of age; *3)* development of an open-source olfactometer to perform neural recordings during delivery of social and non-social odour stimuli; and *4)* assessment of local field potential (LFP) activity in the medial prefrontal cortex (mPFC) in transgenic 5xFAD females and males in response to social and non-social stimuli. I identified that transgenic 5xFAD females and males exhibit reduced social investigation at 6 months of age in response to a free-roaming (but not contained) conspecific, and that—contrary to research in other AD-model mice—transgenic 5xFAD males do not exhibit overtly increased aggression in familiar (home-cage) or novel environments, nor does genotype of a conspecific affect social approach behaviour or aggression in novel environments. However, transgenic 5xFAD males exhibit increased injurious behaviours (i.e., behaviours directly leading to injury, such as bites that break the skin) when housed with other transgenic 5xFAD males, and these effects are not seen

in mixed-genotype or wild-type-only housing conditions suggesting that the phenotype of both the aggressor and the target plays a role in the development of abnormal home-cage aggression. To better understand the neural function that could be associated with this behavioural phenotype, I designed an open-source olfactometer and demonstrated that it could be used to perform a modified version of the olfactory habitation/dishabituation task. Finally, I identified that transgenic 5xFAD mice exhibit altered LFP activity in the mPFC compared to wild-type controls under baseline conditions and during stimulus presentation, with altered distribution of power between 1 and 150Hz as well as altered phase-amplitude coupling between bands.

6.2 The Odour Delivery Optimization Research System (ODORS) Provides an Efficient, Accurate, and Economical Method for Olfactory Testing in Tethered and Untethered Mice

To better understand the neural bases for aberrant social behaviours in transgenic 5xFAD mice, I assessed neural activity in awake behaving mice in response to relevant stimuli. I first had to identify a suitable task that would allow me to perform recordings while presenting social and non-social stimuli. Although the present work (see Chapter 3) and my previous research (Kosel, Torres Munoz, et al., 2019) identified reduced social investigation in transgenic 5xFAD mice in the free social interaction task, this task was determined to not be suitable for neural recordings due to ethical concerns, particularly the potential for serious injury due to the presence of a cortical implant and the possibility of aggressive behaviours. There were also practical considerations, primarily the unpredictability and inconsistency of social interactions given the use of a live conspecific and the subsequent difficulty with parsing neural recordings for analysis.

While the three-chamber apparatus would allow presentation of both social and non-social stimuli and reduce the likelihood of injury by preventing direct interaction of the subject and stimulus mice, the use of walls and pass-throughs posed a problem for use with a tether. As an alternative, I investigated the use of a modified Y-maze as this apparatus would separate the apparatus into three distinct zones—with the left, start, and right Y-maze arms corresponding to the left, centre, and right chambers in the three-chamber apparatus—while avoiding the use of walls and pass-throughs. Additionally, subjects would not be able to see one stimulus while in proximity to the other stimulus (e.g., seeing the conspecific while adjacent to the non-social stimulus), thereby requiring the subject to make a choice to investigate one stimulus or the other. While the Y-maze (and the similar T-maze) is typically used to assess working memory (Olton, 1979), studies have successfully used this task as an alternative to the three-chamber apparatus for conditioned place preference tasks (Baudonnat et al., 2017; Fournier et al., 2023; Negrelli et al., 2020). However, while both wild-type and transgenic subjects exhibited a preference for social over non-social interactions in the Y-maze, the investigation time in both groups was reduced for both stimuli relative to the three-chamber apparatus (Kosel, Gliklich, et al., 2019); as both the Y-maze and three-chamber apparatus are dependent on the subject choosing to investigate the stimuli, the reduced investigation time could (in some cases) result in insufficient data for analysis. However, work by Levy *et al* (2019) at this time had indicated that social and non-social olfactory stimuli were sufficient to evoke altered neural activity in the *Cntnap2* mouse model of autism spectrum disorder. Moreover, this model also exhibits altered neural activity related to impaired social behaviours (Selimbeyoglu et al., 2017), suggesting that subjects exhibiting altered neural

behaviour during the olfactory task would likely exhibit altered neural behaviour during a social interaction task with a live conspecific. While changes in neural activity may differ between the two tasks, the olfactory task provides a simplified method for a preliminary investigation of neural activity in response to social and non-social stimuli which can then be followed up with further studies. Additionally, I had previously identified reduced interest in social odour cues without overt impairments in olfaction in transgenic 5xFAD females (Kosel, Torres Munoz, et al., 2019), and olfactory studies have largely indicated no impairments in transgenic 5xFAD mice (O'Leary, Stover, et al., 2020; Roberts et al., 2020; Roddick et al., 2014, 2016), suggesting that an olfactory task could be used with this model.

To study neural activity in 5xFAD mice, my intention was to use an olfactometer that would allow the testing chamber to be flooded with odorized air (to ensure subjects were exposed to each stimulus) while allowing use of a tether (for neural recordings) and being controlled directly by our electrophysiology software (to allow accurate synchronization between neural recordings and presentation of olfactory stimuli). Unfortunately, there were no existing commercial or open-source units that would immediately meet these needs, and use of these systems would require extensive modifications to the testing chamber and control system, thereby increasing the costs and time necessary and negating any benefits of using a pre-designed system. Further, existing designs required difficult-to-source components—such as borosilicate glass manifolds and mixing chambers—and/or proprietary software, making them difficult to assemble, maintain, and integrate with existing systems, and I did not find any suitable designs for use with tethered subjects. Finally, I believe that open-source designs benefit

the entire scientific community by making tools accessible to labs with limited resources, giving researchers the freedom to integrate new features and refinements to drive continued development, and improving transparency of methodology. Thus, I sought to design my own olfactometer with the aims of making it easy to integrate with existing systems, using readily-available components, and sharing it with other researchers through open-source means.

The design and function of the olfactometer is described in full in Chapter 4; in brief, I approached the design based on the following principles: *1)* the testing chamber had to be useable with a tether and needed to be flooded with odorized air to ensure that subjects were exposed to stimuli for the entire duration of the trial; *2)* the system needed to be controlled directly from our electrophysiology suite to allow for accurate synchronization of neural activity with stimulus presentation; *3)* the design had to be cost-effective and utilize components that are easy to source; and, *4)* the design had to be modular, upgradeable, and flexible enough for long-term functionality across a variety of possible use cases. To validate the design, we selected the olfactory habituation/dishabituation task (M. Yang & Crawley, 2009); this task (and its variants) is commonly used to assess perception, discrimination, and motivation to investigate olfactory stimuli and is considered a gold standard in olfactory research. However, despite efforts to improve throughput (Oummadi et al., 2020), the task had not yet been adapted for use with an olfactometer, and addressing this limitation would allow us to validate our apparatus while simultaneously increasing our contribution to olfactory research.

Overall, the design and assembly of the apparatus met my intended goals. With

the exception of the solenoid pinch valves and (to a lesser extent) the tubing used for the supply lines, the apparatus largely consisted of generic components that could be easily sourced through online or local retailers or produced in-house. This, along with the use of open-source hardware—specifically Arduino-compatible hardware—also allows the system to be modular, upgradeable, and flexible enough to use with a variety of tasks. Additionally, the testing chamber was designed for use with a tether by incorporating a slot along the top, while the airflow into the chamber allows it to be flooded with odour stimuli during the trial, followed by being flushed out using clean air. Our electrophysiology suite includes a number of addressable digital input/output (I/O) connections that are directly controlled by our electrophysiology software, and I made use of this to allow the different stimulus valves to be triggered by the program itself. Finally, I was also able to incorporate two additional refinements into the design: first, I incorporated volatile organic compound (VOC) sensors to monitor and record odorant levels during testing sessions; and second, I developed end caps to adjust airflow within the testing chamber, allowing behaviours to be scored either manually or automatically (using tracking software) based on the location of the subject within the testing chamber.

With the overall apparatus functional, I was able to modify the olfactory habituation/dishabituation paradigm (M. Yang & Crawley, 2009) for use with the olfactometer, resulting in a similar pattern of stimulus presentation as the original task. Preliminary testing with wild-type 5xFAD mice (using artificial almond and banana flavouring as non-social stimuli, and urine as a social stimulus) resulted in a similar pattern of habituation and dishabituation to that I had previously identified in transgenic and wild-type 5xFAD females (Kosel, Torres Munoz, et al., 2019), although the lack of a

clean air control resulted in the almond stimulus serving as a control. However, subsequent testing with C57BL/6NCrl females and males—this time using a clean air control—resulted in the same characteristic habituation/dishabituation observed in the standard paradigm, indicating that the modified task in the olfactometer was functionally similar to the standard task. Additionally, this testing indicated that the paradigm could be used with both monomolecular (e.g., ethyl acetate, butyl acetate) and complex (e.g., artificial banana flavouring, artificial brandy flavouring) non-social stimuli, and that results were repeatable across testing sessions when using novel odours. Finally, automatic scoring of investigation using tracking software also indicated habituation and dishabituation, providing proof-of-concept that automatic scoring could be used with the olfactometer. While the automatic scoring suggested reduced effects compared to manual scoring, this may be partially due to limitations in our tracking software, and more recent software may provide improved tracking ability and comparable results to manual scoring.

Overall, the olfactometer that I developed provides a consistent, accurate method of delivering odour stimuli to assess behaviour or neural activity, and this is supported by behavioural testing with the modified olfactory habituation/dishabituation paradigm and the neural recordings in 5xFAD mice. The automation of the olfactory habituation/dishabituation task is of note as—to my knowledge—this task had not yet been modified for use with an olfactometer, and recent attempts to improve throughput still rely on manual presentation of odour stimuli (Oummadi et al., 2020). This may be due in part to the relatively specialized function of olfactometers, as well as the financial investment required for a commercial unit (~$15,000-$25,000 CAD in early 2020) or

difficulty in replicating designs described in existing studies, thereby leading to relatively low adoption of these systems by researchers. However, by focusing on components that are readily-available or can be easily produced in-house, the olfactometer described here can be produced at a fraction of the cost and—along with the open-source code and design information—is available to researchers even with limited resources.

6.3 Neuropathology May Underpin Altered Neural Activity in Transgenic 5xFAD Mice

Testing in the olfactometer indicated that transgenic 5xFAD mice exhibit altered LFP activity in the mPFC under baseline conditions and in response to odour stimuli. In particular, we identified that low-frequency oscillations—specifically, the 1-4Hz delta band—contribute to a larger proportion of the total LFP compared to wild-type controls, with all higher frequency bands (4-150Hz) contributing a lower relative proportion of total power. This is consistent with data from Schneider *et al* (2014), who indicate that transgenic 5xFAD males exhibit altered cortical EEG activity, with the sub-delta (0.1-0.5Hz) range contributing more to the overall power than the higher (0.5-~250Hz) ranges. While their data also suggested that the delta range contributes less to the total LFP power compared to wild-type controls, their graphs suggest that transgenic 5xFAD males exhibit a steep drop in relative power from the delta to theta bands, whereas wild-type controls exhibit a gradual decrease in relative power across bands. Although it is not possible to confirm without raw data, this distribution of relative LFP power—high delta band power with low theta band (and above) power—is consistent with the results identified here. Further, the relatively high delta band power is consistent with neuropathology observed in this model; transgenic 5xFAD mice exhibit significant

reductions in cholinergic neurons by 6 months of age (Devi & Ohno, 2010), and stimulation of nicotinic and muscarinic acetylcholine receptors leads to decreased low frequency (1-6Hz) and increased higher frequency (>10Hz) power (Kalmbach & Waters, 2014), suggesting that impairments in cholinergic signaling could lead to the shift in LFP power identified in these mice.

In addition to the shift in LFP power, I also identified altered phase-amplitude coupling (PAC) in transgenic 5xFAD mice; specifically, transgenic 5xFAD mice exhibit higher PAC relative to wild-type controls across a number of bands. PAC is a form of cross-frequency coupling in which the phase of one oscillatory band modulates the amplitude of a higher frequency band and has been implicated in working memory, decision making, and long-range communication across brain regions (Abubaker et al., 2021; M. X. Cohen, 2008; Engel et al., 2013). In particular, hippocampal coupling between the theta and gamma bands has been of interest to researchers as it has been implicated in working memory (Abubaker et al., 2021). Theta-gamma coupling appears to be related to the ability to keep information in working memory stores, and researchers have suggested that the number of "memory items" that can be maintained in working memory is related to the number of gamma oscillations that are nested within a single theta oscillation (Abubaker et al., 2021). Similarly, working memory tasks that are more demanding tend to be associated with stronger theta-gamma coupling. With the cognitive deficits—particularly memory impairments—exhibited by mouse models of AD (see Bilkei-Gorzo (2014) for a brief review on some common models), a number of researchers have examined theta-gamma PAC in rodent models of AD, and the results tend to be relatively consistent: theta-gamma PAC is reduced in the medial septum and

hippocampus of J20 mice (Etter et al., 2019; Mondragón-Rodríguez et al., 2018), the entorhinal cortex of APP knock-in and rTg4510 mice (Booth et al., 2016; Nakazono et al., 2017), and the hippocampus of TgCRND8 and P301L mice and TgF344-AD rats (Ahnaou et al., 2017; Bazzigaluppi et al., 2018; Goutagny et al., 2013; Van Den Berg et al., 2023). However, while transgenic AD-model mice bearing mutated APP exhibit an accumulation of Aß, theta-gamma PAC in the parietal cortex and hippocampus is also reduced in the absence of Aß in an APP-knock-out mouse model (X. Zhang et al., 2016) Given that APP is the key precursor for Aß production (Claeysen et al., 2012), this suggests that Aß accumulation alone is not necessary to alter hippocampal PAC. Moreover, other work has indicated increased theta-gamma PAC in the mPFC of tau-overexpressing rats (Tanninen et al., 2017) as well as increased beta-gamma PAC in the striatum of APP/PS1 mice (Peng et al., 2021), reflecting the increased PAC in transgenic 5xFAD mice I identified in the present work. Jacob *et al* (2019) have also indicated that $APP^{NL\text{-}G\text{-}F}$ mice exhibit significant differences in theta-gamma coupling in the striatum and hippocampus relative to wild-type controls, but not in the mPFC, suggesting that PAC in the mPFC can be dissociated from other brain regions. With PAC being implicated in long-range communication across brain regions (Abubaker et al., 2021; Engel et al., 2013), and the impaired cholinergic inputs to the mPFC in 5xFAD mice (Sun et al., 2022), it is possible that transgenic 5xFAD mice exhibit reduced PAC in the hippocampus (similar to other rodent models of AD), or other rodent models of AD exhibit increased PAC in the mPFC (such as the rats examined by Tanninen *et al*), but further work is necessary to determine whether our results are consistent with other models. My findings in 5xFAD mPFC newly show that this model exhibits increased

PAC (including theta-gamma PAC) in the mPFC compared to wild-type controls, similar to the results identified by Tanninen *et al* (2017). Conversely, Jacob *et al* (2019) did not identify PAC differences in the mPFC of a mouse model of amyloid pathology relative to wild-type controls. It is possible that neuropathological differences play a role in these results: as my aim was to explore the relationship between neural activity in the mPFC and BPSD-like behaviours, the 5xFAD mice used in the present study were 6 months of age, corresponding with an intermediate level of neuropathology; by comparison, both Tanninen *et al* (2017) and Jacob *et al* (2019) primarily focused on PAC during the pre-clinical stages of AD. While results for amyloid pathology, tau pathology, and neurodegeneration are somewhat inconsistent in AD-model mice (see Chapter 2 for review), disease staging in humans is more closely related to tau pathology than Aß deposition (H. Braak & Braak, 1991), and reduced alpha band power is linked with density of neurofibrillary tangles in humans (Ranasinghe et al., 2021). It is possible then that tau pathology is more strongly linked with altered mPFC neural activity at early disease stages compared to amyloid pathology, although further work is necessary to identify at what age 5xFAD mice start to exhibit altered neural activity and how this corresponds to disease staging.

The 5xFAD mouse model of AD is a useful model for studying the neuropathological and physiological changes associated with AD, and part of the reason for this is the rapid onset and progression of AD-related pathology exhibited by this model (Bilkei-Gorzo, 2014; Oblak et al., 2021). The combination of the Swedish (K670N/M671L), Florida (I716V), and London (V717I) APP mutations in conjunction with the PSEN1 M146L and L268V mutations lead to very rapid Aß production (Oakley

et al., 2006). Specifically, these mutations were selected as they exhibit additive effects, with the Swedish double mutation, the pairing of the Florida and London mutations, and the combination of the London with the PSEN1 double mutation all exhibiting more rapid Aß production than any of these mutations alone (Citron et al., 1992, 1998; Eckman et al., 1997). As a result, transgenic 5xFAD mice exhibit whole-brain levels of Aß at ~9-21 ng/mg of protein by 2 months of age, whereas Tg2576 mice do not show that level of Aß until 16 months of age with whole-brain levels at ~10 ng/mg of protein (Oakley et al., 2006). Although the resulting changes are widespread, there is extensive neuropathology in the mPFC by 6 months of age: deficits in axon myelination are present in the prelimbic cortex at 1 month of age, with amyloid deposition, astrogliosis, and microgliosis present in deep cortical layers by 2 months of age and progressing from there (Gu et al., 2018; Oakley et al., 2006). Dystrophic neurites are present in the cortex by 3 months of age, reduced synaptophysin (relative to wild-type controls) is present by 4 months of age, and thinner axon cross-sections are present by 5 months of age (Gu et al., 2018; Jawhar et al., 2012; Oakley et al., 2006). By 6 months of age, there is a loss of dendritic spines on layer 5 pyramidal neurons, reduced excitatory synaptic density on layer 2 neurons, and an increase in pyknotic cells (Crowe & Ellis-Davies, 2014; Maiti et al., 2021; Seo et al., 2021). By this age, transgenic 5xFAD mice also exhibit altered neural activity in addition to the altered mPFC LFP reported here, including: altered cortical EEG power distribution (as discussed above; Schneider *et al* (2014)), reduced intrinsic excitability and miniature excitatory post-synaptic currents (mEPSCs), higher action potential threshold, and impaired long-term potentiation in layer 5 neurons in mPFC brain slices (Chen, Ma, et al., 2022; Chen, Wei, et al., 2022), and reduced cholinergic signaling in

mPFC projection neurons (Sun et al., 2022). Overall, this suggests that altered neural activity in transgenic 5xFAD mice is underpinned by the neuropathology observed in this model.

6.4 Role of the mPFC in Social Behaviours and Beyond

Both human and animal research has demonstrated the involvement of the mPFC in decision-making, anxiety, emotional processing and arousal, and social behaviours. Some studies have implicated the mPFC in planning and assessment of reward (Hiser & Koenigs, 2018); for example, medial orbitofrontal neurons in rats responding more strongly to cues that predict a decrease in reward value than those that indicate no change or an increase (Lopatina et al., 2016), and during a gambling task humans with mPFC lesions will preferentially make choices that result in high immediate reward but long-term negative results whereas cognitively normal humans will preferentially choose options that result in modest immediate and long-term gains (Bechara et al., 1994), suggesting that the mPFC is critical in assessing value-based outcomes. A number of studies have also implicated the mPFC in emotion, anxiety, and fear in both humans and animals (Hare & Duman, 2020; Hiser & Koenigs, 2018). Viewing of images designed to elicit emotional responses results in increased delta band activity, with higher-arousal images resulting in higher delta band power (Klados et al., 2009); similar effects are observed in highly emotional individuals compared to low emotional individuals when presented with happy or angry faces (Knyazev et al., 2009). Exposure of male C57BL/6 mice to a cadaver (vs an anaesthetized conspecific) led to increased delta band power and increased anxiety following exposure (Jeon et al., 2021), and activation of locus coeruleus projections to the mPFC led to avoidance behaviour in a conditioned place

preference task and cause increased anxiety in rats (Hirschberg et al., 2017). Neural circuits involving the mPFC have also been implicated in mediating fear: exposure to a high-stress situation (tail suspension) led to increased delta band power in the mPFC and promoted mPFC-hippocampal functional connectivity (Lv et al., 2022), while prefrontal oscillations at 4Hz lead to synchronization with the amygdala and predict freezing behaviour, and artificial stimulation of mPFC neurons at this frequency can induce freezing behaviour (Karalis et al., 2016). PAC between the mPFC and the amygdala is also altered in response to both chronic and acute stress (Merino et al., 2021; Z. Wang et al., 2021). Similarly, projections to and from the mPFC are involved in generalization of fear memories (Xu & Südhof, 2013) and mPFC lesions lead to impaired extinction of fear memories (Morgan et al., 1993). The role of the mPFC in fear and anxiety is not limited to general anxiety, however; decreased relative alpha band power compared to delta band power in the frontal cortex is indicative of impaired maturation and increased social anxiety in adolescents (Schmidt et al., 2022), while delta-beta coupling is higher in high socially-anxious individuals during both baseline and stressful (speech anticipation) conditions (Miskovic et al., 2010). Similar effects of frontal delta-beta coupling have been observed in children as well, with higher delta-beta coupling under baseline conditions in children who exhibit higher social fear (in response to an unfamiliar adult male) and those who exhibit higher levels of shyness (Najjar & Brooker, 2017; Sriranjan et al., 2022). Together, these results indicate that the mPFC—both through local neuronal populations and projections to and from other brain regions—is key in decision-making, emotion, fear, and both general and social anxiety. Differences in neural activity between transgenic and wild-type 5xFAD mice could contribute to altered fear behaviours and

increased arousal and anxiety, as transgenic 5xFAD mice exhibit increased delta band power—the frequency range associated with emotional processing, arousal, anxiety, and fear behaviours—and increased delta-beta PAC (which is linked with social anxiety) compared to wild-type controls.

The mPFC also plays a role in social behaviours beyond social anxiety. Inhibition of projection neurons from the mPFC to the periaqueductal gray can recapitulate the reduced social investigation arising from a sub-chronic social defeat paradigm (T. B. Franklin et al., 2017); conversely, inhibition of mPFC projections to the dorsal raphe nucleus following social defeat prevents learned social avoidance (Challis et al., 2014). Activation of mPFC pyramidal neurons is linked with increased dominance, and artificial stimulation or inhibition of these neurons can directly modulate behaviours associated with dominance (F. Wang et al., 2011; Zhou et al., 2017); additionally, wins in the tube test of dominance arising from artificial stimulation lead to persistent increases in home-cage rank and mPFC activity. However, this increase in dominance appears to be related to motivation rather than aggressive behaviours (Zhou et al., 2017), and stimulation of excitatory neurons in the prelimbic cortex actually inhibits intermale aggression (Takahashi et al., 2014). A number of studies have also linked altered mPFC activity with social deficits in mouse models of autism spectrum disorder (ASD): neuroligin 3 R451C knock-in mice exhibit impaired social novelty preference and reduced activity in the 30-90Hz band (Cao et al., 2018), whereas Shank3 knock-out mice and mice subject to social defeat exhibit decreased social preference and increased 4-7Hz power (relative to wild-type controls) during exposure to a novel conspecific (Kuga et al., 2022). Shank3 knock-out mice also exhibit impaired N-methyl-D-aspartate (NMDA) receptor function and

reduced EPSCs (Duffney et al., 2015), while altered excitatory:inhibitory balance, social impairments, and altered mPFC neural responses to social odour stimuli have been reported in the CNTNAP2 mouse model (Levy et al., 2019; Selimbeyoglu et al., 2017). Neuroimaging studies with humans have linked mPFC activity with "Theory of Mind"—the ability to understand the mental state of others—and evidence suggests that individuals with AD exhibit impaired Theory of Mind together with social deficits (S. Braak et al., 2022; Molenberghs et al., 2016; Schurz et al., 2014). Similarly, recent fMRI work has indicated that social deficits in AD are linked with reduced mPFC connectivity, specifically the default mode network, providing support for the role of AD-related neuropathology in the development of social impairments (Saris et al., 2022). This is in-line with the work performed by Bories *et al* (2012), who indicated that transgenic 3xTG-AD mice exhibit increased miniature inhibitory post-synaptic currents (mIPSCs) in the mPFC at the same ages as they exhibit increased social investigation, suggesting that social investigative behaviours are linked with background mPFC synaptic activity in this model. Additionally, these timepoints were different in males and females, reflecting sex differences in AD-related pathology in this model, with females exhibiting more rapid neuropathology than males. Overall, these studies highlight the importance of the mPFC in social behaviours under normal and pathological conditions: studies examining functional connectivity and optogenetic stimulation/inhibition in otherwise-healthy mice have highlighted the importance of both local populations and projections in mediating behaviours such as social approach and avoidance behaviours, dominance, and aggression. Similarly, studies using mouse models of ASD have linked altered mPFC neural activity to impaired social behaviours. Overall, this evidence, as well as that on

anxiety, emotional processing, and decision-making, suggests that the mPFC does not simply modulate the behavioural output of cognition but is critical in the development and selection of these behaviours.

6.5 BPSD in Humans and Mouse Models of AD

Since the 1990's, a large number of transgenic mouse models have been developed using mutated human genes in order to express neuropathology similar to that observed in humans (for review, see Chapter 2). Many of these use mutated APP variants associated with familial (hereditary) AD such as the Swedish (M146L/L286V), Florida (I716V), London (V717I), and Indiana (V717F) mutations, as these can shift APP processing to the amyloidogenic pathway and lead to progressive accumulation of Aß with age (Claeysen et al., 2012; Eckman et al., 1997; Goate et al., 1991; Hardy, 1997; Sorrentino et al., 2014; Suzuki et al., 1994). Alternatively, a limited number of models use presenilin mutations or knockout alone (e.g., Duff et al., 1996; Saura et al., 2004) to drive AD-related neuropathology, although PSEN mutations are typically used in conjunction with mutated APP genes in order to accelerate Aß production over the use of mutated APP alone (Borchelt et al., 1997; Citron et al., 1998). While these models may recapitulate some of the amyloid pathology associated with AD, tau pathology may be minimal or non-existent (e.g., hyperphosphorylated tau in proximity to Aß deposits); as there are currently no known tau mutations associated with AD, mouse models intended to exhibit tauopathy—such as the 3xTg-AD, THY-Tau22, Tau P301S, and PLB1-triple (Oddo et al., 2003; Platt et al., 2011; Schindowski et al., 2006; Yoshiyama et al., 2007)—use tau mutations associated with frontotemporal lobar degeneration instead (for review, see Forrest *et al* (2018)). This variety of approaches has led to a gamut of transgenic

mouse models of AD exhibiting different neuropathologies, different rates of disease progression, and different clinical presentations (i.e., cognitive, physiological, and behavioural effects) and the resulting models vary in their face, construct, and predictive validity (for a review of five common models, see Bilkei-Gorzo (2014)). While this means that no single model can be used to accurately assess clinical presentation, neuropathology, and treatments together, this heterogeneity means that transgenic mouse models of AD may exhibit one or a few BPSD-like behaviours but not all of them at once, similar to what is observed in humans with AD. In humans, the Neuropsychiatric Inventory (NPI; Cummings, 1997; Cummings et al., 1994) is commonly used to assess BPSD—termed neuropsychiatric symptoms (NPS) in the test—and was designed to replace several assessments—e.g., the BEHAVE-AD, Hamilton Depression Rating Scale, Neurobehavior Rating Scale—with an all-in-one test. Specifically, it assesses symptoms across 10 behavioural domains (delusions, hallucinations, agitation/aggression, depression, anxiety, elation/euphoria, apathy/indifference, disinhibition, irritability, and aberrant motor behaviour) and 2 neurovegetative domains (sleep and nighttime behaviour disorders and appetite and eating disorders). However, despite the relative breadth of BPSD exhibited by AD patients, the most common are agitation/aggression, depression, anxiety, and apathy/indifference (including social withdrawal), as well as sleep and nighttime behaviour disorders, with each occurring in ~39-49% of individuals with AD (Chow et al., 2002; Frisoni et al., 1999; Zhao et al., 2016), and these symptoms also tend to be the ones examined in mouse models of AD. Based on my assessments, the 5xFAD mouse model exhibits reduced social investigative behaviours, suggesting social withdrawal that could be associated with apathy. However, the present study and my

previous work (Kosel, Torres Munoz, et al., 2019) suggests that this may be more closely related to anxiety or arousal than apathy, as these effects are observed in higher-anxiety situations (free social interaction) earlier than in lower-anxiety situations (three-chamber sociability task). Similarly, transgenic 5xFAD mice do not exhibit increased general apathy (see Chapter 2 for review), and data from my LFP recordings in the mPFC of 5xFAD mice are consistent with results from other studies on anxiety, arousal, and fear behaviours.

While mouse models of AD, including the 5xFAD model, recapitulate some of the BPSD exhibited by humans, the relationship between neuropathology and BPSD-like behaviours is dependent on the specific symptom. Evidence largely suggests that depressive-like behaviours—the second most common BPSD in humans—are present at advanced stages of AD-related pathology, even if there are no genotype effects at earlier timepoints (Iascone et al., 2013; Patel et al., 2014; Romano et al., 2015; Van der Jeugd et al., 2013; Yan et al., 2013). However, these results are not always consistent; although Patel *et al* indicated increased depressive-like behaviours in transgenic 5xFAD mice at 4.5-5 months of age, Yamazaki *et al* (2015) indicated decreased depressive-like behaviours at 6 months of age, while results from the APP23 and APP/PS1 mouse models of AD suggest decreased, increased, or no difference in depressive-like behaviours relative to wild-type controls dependent on the specific task (Martín-Sánchez et al., 2021; Pfeffer et al., 2018; Vloeberghs et al., 2007; Ying et al., 2023), highlighting the importance of methodology in assessing these behaviours. Another relative common BPSD exhibited in humans is sleep/wake disturbances, and results in AD-model mice consistently indicate altered circadian rhythms and disrupted sleep patterns (Colby-

Milley et al., 2015; Duncan et al., 2012; Holth et al., 2017; Ognibene et al., 2005; Platt et al., 2011; Roh et al., 2012; Sethi et al., 2015; Sterniczuk, Dyck, et al., 2010; Wisor et al., 2005), thereby suggesting that neuropathology is involved in the development of sleep/wake disturbances in AD. Furthermore, data from Tg2576 and APP/PS1 mice indicates that Aß pathology increases proportionally with the amount of time spent awake (Kang et al., 2009; Roh et al., 2012), suggesting that the onset of sleep/wake disturbances may lead to a positive feedback loop that further drives neuropathology.

The other three major BPSD in humans—anxiety, apathy and social withdrawal, and agitation/aggression—are of particular interest with respect to the work presented here as this work touches on these three BPSD specifically. Of these symptoms, anxiety has been explored by a number of researchers, and links have been made to various neuropathological factors such as Aß accumulation in the amygdala, alterations in enzyme activity, changes in protein expression, altered dopaminergic receptor densities, and altered EEG power (Chithanathan et al., 2022; España et al., 2010; Gloria et al., 2021; Nie et al., 2017; Schneider et al., 2014). Despite this, however, there is no consistent pattern between neuropathology in AD-model rodents and anxiety, nor is there a consistent pattern within models, with conflicting results reported by various studies or even from different tasks within the same study (Kosel et al., 2020; Pentkowski et al., 2021). However, evidence is beginning to suggest that neuropathology in the medial temporal lobe and early-stage Aß accumulation are related to development of AD-related anxiety in humans, and a number of additional mechanisms—such as impaired sensorimotor gating, neuropathology in the hippocampus and amygdala, and comorbidities—have been suggested (Mendez, 2021; Pentkowski et al., 2021). Similarly,

other studies have begun to link apathy—the most common BPSD in Caucasian humans (Chow et al., 2002; Frisoni et al., 1999; Zhao et al., 2016)—with cortical Aß and neurofibrillary tangle (NFT) accumulation, particularly in the frontal and anterior cingulate cortices, and it has been suggested that frontal-subcortical pathways are likely involved in the development of apathy (Mehak et al., 2023). Interestingly, despite the relatively high prevalence of apathy in humans, only a small number of studies have examined apathy in mouse models of AD, primarily through object exploration using the novel object recognition task or a three-chamber apparatus with a novel object on one side, and the majority of these have reported no genotype effects on exploration of these objects, suggesting no apathy in these models (Bardgett et al., 2011; Bellucci et al., 2006; Braun & Feinstein, 2019; Howlett et al., 2004; Pietropaolo et al., 2012; Shruster & Offen, 2014). However, my previous results in the three-chamber apparatus with transgenic 5xFAD females (Kosel, Torres Munoz, et al., 2019) as well as the modified Y-maze (see Section 6.2 for description) have shown an overall reduction in investigation of both social and non-social stimuli. Similarly, Keszycki *et al* (2023) reported increased apathy in 6-, 12-, and 16-month-old 5xFAD mice, while Görtz *et al* (2008) reported decreased object exploration in month-old TgCRND8 females. It is important to note, however, that Keszycki *et al* identified increased apathy based on impaired nest building, marble-burying, and burrowing behaviours, and these may be susceptible to the motor deficits exhibited in this model (Fertan & Brown, 2022; Jawhar et al., 2012; O'Leary et al., 2018; O'Leary, Mantolino, et al., 2020). However, while evidence for general apathy is limited, a number of studies—including the work presented here (see Chapter 3) and my previous research (Kosel, Torres Munoz, et al., 2019)—have indicated reduced social

investigation, largely related to progressive neuropathology (Bories et al., 2012; D. Cheng et al., 2013; Filali et al., 2011a; H. Huang et al., 2016; Pietropaolo et al., 2012; Várkonyi et al., 2022; Verma et al., 2015). Despite this disconnect between social withdrawal and general apathy exhibited by AD-model mice, the evidence for a role of AD-related neuropathology in apathy is accumulating from both human and mouse studies; similarly, human studies have also provided support for the role of AD-related neuropathology in the development of anxiety despite the inconsistent results from AD-model mice.

The final BPSD commonly assessed in AD-model mice—and the third most common in humans (Zhao et al., 2016)—is agitation and aggression. While a number of studies have indicated increased aggression in AD-model mice (G. Alexander et al., 2011; Moechars et al., 1998; Pugh et al., 2007; Vloeberghs et al., 2006; Yan et al., 2013), these have largely been assessed using the resident-intruder task; this task typically involves a period of social isolation housing which causes a number of behavioural and physiological changes itself (Goldsmith et al., 1976; Koike et al., 2009; Matsumoto et al., 2005). Moreover, social isolation housing has been suggested as a method to induce behavioural abnormalities related to neuropsychiatric disorders in normal (e.g., ICR) mice (Koike et al., 2009), thereby raising the question of whether the increased aggression observed in the resident-intruder task is due to AD-related pathology or the social housing. By comparison, studies examining aggression in response to novel conspecifics in a novel environment (e.g., the work presented here in Chapter 3; (Bories et al., 2012) has not indicated increased aggression. Similarly, I did not identify any overt increases in aggression in male transgenic-only home-cages despite the increase in

injurious behaviour, suggesting that transgenic 5xFAD males do not exhibit overall increases in aggression. Results for agitation with AD-model mice are also mixed, with many studies indicating either no change or increased levels of activity relative to wild-type controls (see Chapter 2 for review). While this may be attributed to methodology—as agitation is typically measured as hyperactivity, often during tests of anxiety—there is still no clear pattern for agitation even if results from tests of anxiety are removed. Overall, these results suggest that transgenic 5xFAD mice exhibit increased aggression relative to wild-type controls, but not increased agitation. However, the caveat is that this increased aggression is primarily evident in the resident-intruder task, and it is unclear how many of these models behave under different (e.g., novel or group-housed) conditions.

Taken together, these results indicate that transgenic AD-model mice recapitulate some of the BPSD-like behaviours exhibited by humans with AD, and these symptoms—particularly sleep/wake disturbances, apathy, and depressive behaviours—are related to neuropathology. Further, apathy (or at least social withdrawal in AD-model mice) appears to be related to cortical Aß accumulation in both humans and mouse models of AD, and further research on the mechanisms underlying these behaviours in AD-model mice may point towards similar mechanisms underlying apathy in humans with AD.

6.6 Altered LFP in the mPFC of Transgenic 5xFAD Mice Suggests Increased Anxiety or Arousal

Similar to the above results on social withdrawal, the work contained herein indicates that both transgenic 5xFAD females and males exhibit reduced social investigation in the free social interaction task at 6 months of age, although there were no

genotype effects for sociability or social preference in the three-chamber apparatus. These results are consistent with my previous work indicating reduced social investigation in transgenic 5xFAD females in the free social interaction task from 9 months of age and in the three-chamber sociability task at 12 months of age (Kosel, Torres Munoz, et al., 2019), albeit at an earlier timepoint. It is possible that exposure to the tasks at 3 months of age—i.e., the prodromal stage—and/or the gradual increase in intensity of social stimuli (cotton swab with odour from dirty bedding material, urine spots, restrained conspecific, and finally free-roaming conspecific) may have reduced the genotype effects in the previous study, causing them to arise at an older age. However, social investigation in either task was not dependent on the genotype of the conspecific, indicating that the reduced social investigation exhibited by transgenic 5xFAD mice is due to intrinsic factors—such as increased anxiety or arousal—rather than the phenotype of the conspecific.

It is important to note that the social deficits previously reported in transgenic 5xFAD females (Kosel, Torres Munoz, et al., 2019) were due—in part—to an overall reduction in investigative behaviour in the three-chamber apparatus. When taken in conjunction with the reduced social investigation in the free social interaction task, and the reduced interest in social odours during the olfactory habituation/dishabituation task, this was interpreted as social deficits. However, transgenic 5xFAD females in that study did not exhibit overall changes in sociability or social novelty preference relative to wild-type controls, indicating that they were still interested in social interactions. The results from Chapter 3 indicate similar effects, with transgenic 5xFAD mice exhibiting reduced investigation of free-roaming—but not restrained—conspecifics. Overall, this suggests

that transgenic 5xFAD mice are interested in social interactions—and are likely not exhibiting apathy—although situational factors are affecting the level of interaction. This is in line with the suggested use of the free social interaction task as a method of assessing social investigation under varying levels of anxiety (File & Hyde, 1978; Pentkowski et al., 2021); cognitively normal rats will exhibit altered levels of social interaction based on the specific environmental factors relating to the task—familiar vs unfamiliar and low light vs high light environment—and the specific setup used by me—unfamiliar environment and low light—is suggested as a "moderate" anxiety situation. As a result, the reduced social investigation I report here may not be the result of social deficits per se, but rather due to overall increases in arousal or anxiety levels.

The results from Chapter 5 are consistent with these conclusions as the LFP changes reported here have been previously linked with fear behaviour, anxiety, arousal, and emotional processing in both humans and rodents. In particular, increased mPFC delta band power has been observed in mice exposed to high stress (tail suspension) but not mild stress (light exposure) conditions (Lv et al., 2022), while intracerebroventricular injections of cholecystokinin result in increased cortical 0-30Hz power—with a particular increase at 2-4Hz—along with increased anxiety (H. Li, Ohta, et al., 2013). Similarly, C57BL/6 males exhibit increased relative delta band power—along with decreased relative theta and alpha band power—when exposed to a cadaver of a conspecific vs an anaesthetized conspecific, as well as increased anxiety following exposure to the cadaver (Jeon et al., 2021). Finally, 2-6Hz oscillations (with a peak at 4Hz) in the prefrontal cortex mediate fear behaviour through synchronization with the amygdala, and stimulation of the prefrontal cortex at 4Hz leads to increased freezing behaviour (Karalis

et al., 2016). Results from human studies have been similar, highlighting the potential translational value of this research: delta band power increases with exposure to images designed to evoke emotional responses, with higher increases to high-arousal images, suggesting a role for the delta band in emotional processing (Klados et al., 2009). Similar increases in the 3-8Hz range have been reported for highly emotional individuals vs low emotional individuals when viewing happy or angry faces (Knyazev et al., 2009). Lower relative alpha band power compared to delta band power in adolescents is linked with increased social anxiety (Schmidt et al., 2022), and high delta-beta coupling (relative to controls) has been identified in children that exhibit high levels of social fear or shyness and high socially anxious individuals (Miskovic et al., 2010; Najjar & Brooker, 2017; Sriranjan et al., 2022). Overall, this evidence is consistent with the LFP findings presented here and suggests that transgenic 5xFAD mice exhibit increased anxiety or arousal that may drive the reduced investigative behaviours I previously identified.

6.7 Aggression and Injurious Behaviour in AD

My research showed that transgenic 5xFAD males do not exhibit overtly increased aggression compared to wild-type controls in either a familiar environment with a familiar conspecific (home-cage and cage-mate) or in a novel environment with a novel conspecific (free social interaction task), and the genotype of the conspecific (transgenic or wild-type) did not affect aggressive behaviours in either transgenic 5xFAD mice or wild-type controls. While aggression is a relatively common BPSD in humans with AD (Chow et al., 2002; Frisoni et al., 1999; Zhao et al., 2016) and a number of AD-model mice exhibit increased aggression (G. Alexander et al., 2011; Bergamini et al., 2022; Moechars et al., 1998; Pugh et al., 2007; Vloeberghs et al., 2006; Yan et al., 2013),

these findings have been largely based on the resident-intruder task and a period of social isolation housing. The use of social isolation housing has been shown to cause behavioural and physiological changes including increased aggression concomitant with decreased 3α,5α-tetrahydroprogesterone (THP)—a steroid hormone that regulates $GABA_A$ receptors—and both THP and $GABA_A$ receptors have been implicated in anxiety and depressive disorders (Goldsmith et al., 1976; Koike et al., 2009; Matsumoto et al., 2005). Moreover, 4 weeks of social isolation housing is sufficient to induce behavioural impairments mimicking those observed in neuropsychiatric disorders (e.g., ADHD, schizophrenia, depression) in ICR mice (a non-transgenic outbred strain) and has been suggested as a method of generating a model for these effects (Koike et al., 2009). A recent study indicated no increase in aggression during a resident-intruder task in which subjects were only isolated in their home-cage for 30 minutes prior to the test (Watt et al., 2020); similarly, I did not identify any overt increases in aggression in transgenic 5xFAD males in the home-cage (familiar environment with a familiar conspecific) or free social interaction task (novel environment with a novel conspecific; see Chapter 3), and Bories *et al* (2012) did not report any aggression in male or female 3xTg-AD mice in a free social interaction task at 12 or 18 months of age. This, then, raises the question of whether increased aggression is a characteristic of AD-model mice, or a product of the methodology commonly used to assess this aggression. While transgenic mice still exhibit higher levels of aggression in the resident-intruder task following social isolation compared to wild-type controls, it is possible that transgenic AD-model mice exhibit an increased susceptibility to the effects of social isolation rather than an intrinsic increase in aggression.

Aggression can be viewed as a negative behaviour, particularly when exhibited by humans, but it can also be viewed as a goal-oriented behaviour in mice and it falls into two categories: offensive and defensive. Offensive aggressive behaviours are usually exhibited by males and are typically territorial—intended to drive a male out of a territory—or related to dominance, can include behaviours other than overt attacks, such as chasing and mounting, and bites tend to avoid vulnerable regions (such as the head, ventral, and inguinal regions) and are rarely intended to break the skin (Brain & Parmigiani, 1990; Grant & Mackintosh, 1963; Miczek et al., 2001; Williamson et al., 2016). By comparison, defensive aggressive behaviours tend to be exhibited by lactating females against unfamiliar males and are characterized by relative uninhibition of bite location, including vulnerable regions. The study performed by Williamson *et al* illustrates the role of offensive aggression in the formation of relatively stable dominance hierarchies within groups of 12 mostly unfamiliar CD1 males (6 per group had no experience with the other mice, while the remaining 6 had previous experience with 1 other male in the group); while they do not indicate how many (if any) mice sustained injuries, it is unlikely that there were any serious injuries as all 120 subjects made it to the end of a 21-23 day observation period despite being paired at 9 weeks of age.

Despite offensive aggression in male mice being goal-oriented and not intended to cause injury, I did identify an unusually high number of home-cage injuries within transgenic-only cages in the study outlined in Chapter 3. Interestingly, while cages containing only transgenic males—as opposed to only wild-type males, or one transgenic and one wild-type—resulted in injuries necessitating separation of cage-mates, I did not observe an overt increase in home-cage aggression during the observation period, nor did

I observe an overt increase in aggressive behaviours immediately prior to visible injuries. While I did observe aggressive encounters during normal observation—e.g., after cage changes, while starting/stopping recordings, or during daily routine checks—these were not limited to transgenic-only cages, nor was the severity sufficient to warrant concern. Additionally, the appearance of injuries was very rapid; in many cases, there was no indication of increased home-cage aggression prior to the appearance of injuries. Given that cages were routinely checked on a daily basis, this indicates that subjects often progressed from normal aggression to injurious behaviour over the course of 24 hours. Similarly, transgenic 5xFAD males do not exhibit an overt increase in aggression in response to novel transgenic or wild-type conspecifics in a novel environment, suggesting that both the environment and phenotype play a role in the emergence of injurious behaviours. While I initially hypothesized that indirect genetic effects—when an individual's genotype is affected by the phenotype of a social partner (Baud et al., 2017)—would play a role in the development of these behaviours, the sudden onset and range of ages at which injuries occurred (between 8 and 23 weeks of age) suggested that this was not due to gradual physiological or developmental effects, but rather altered behaviours; specifically, the aggressor likely displays atypical aggressive behaviours towards the target, who then responds abnormally, thereby potentiating further aggression. However, it is unclear what precipitates the aggressive encounter leading to injury, what pattern of behaviours is exhibited during escalation, and what factors ultimately lead to injury (e.g., impaired bite modulation, chronic attacks on the same region, intent to harm).

Aberrant behaviours have been linked with Alzheimer's disease since Dr.

Alzheimer's original patient (Jarvik & Greenson, 1987), and behavioural disturbances such as agitation and aggression can worsen functional decline in individuals with AD and have a major impact on caregivers (Chenoweth & Spencer, 1986; Chow et al., 2002; Craig et al., 2005). Additionally, despite the benefits associated with remaining in the community, behavioural disturbances are a leading factor in institutionalization (C. A. Cohen et al., 1993; Phinney et al., 2007; C. Steele et al., 1990). Institutionalization in itself can exacerbate issues through reduction of meaningful occupations and social interactions and the potential for infantilization by care staff, and the link between behavioural disturbances and institutionalization can cause a higher prevalence of serious BPSD in long-term care facilities compared to community-dwelling individuals (C. A. Cohen et al., 1993; Craig et al., 2005; MacRae, 2011; C. Steele et al., 1990). Pharmacological interventions have also been an area of concern in long-term care facilities, where residents with dementia that exhibit aggression are more likely to receive long-term treatment with anti-psychotics, and these treatments often do not follow national guidelines, suggesting that these drugs are being used as an easy method of treating symptoms (Gustafsson et al., 2013). In particular, aggression between residents—including physical, verbal, and sexual assault—can be a major concern in long-term care facilities. Lachs *et al* (2007) indicated that 90% of police calls to long-term care facilities were due to violent episodes, with roughly half involving residents with dementia; these could include seemingly unprovoked attacks by residents with dementia on other residents, attacks against dementia patients for repetitive or disruptive behaviours, or invasion of personal space (such as a resident entering the room of another resident, in which dementia may play a role). These situations may reflect those I

identified in the present work: while I did not observe an overt increase in aggression in transgenic 5xFAD mice, I did observe increased injurious behaviours—i.e., behaviours which directly cause injury, such as bites that break the skin—in transgenic-only cages compared to wild-type-only or mixed-genotype cages, suggesting that the phenotype of the aggressor and the target play a role in mediating these behaviours; it is possible that the target is exhibiting similar instigating behaviours to those identified by Lachs *et al* (e.g., repetitive or disruptive behaviours, invasion of personal space). McMinn and Hinton (2000) indicated that allowing individuals with dementia access to an outdoor garden—rather than keeping them restricted to indoor spaces—was associated with reduced aggressive behaviours and reduced need for pharmacological interventions. While they did not measure whether aggressive behaviours were increased as a result of mandatory daytime indoor confinement, these restrictions were temporary due to maintenance on the outdoor space, and it's likely that the reductions in aggression and medication represented a return to baseline levels rather than an overall decrease. Similarly, a study by Shinoda-Tagawa *et al* (2004) examined incidents in which an injury was sustained and identified that residents living in a specialized AD ward were three times more likely to sustain injuries resulting from an altercation with another resident than non-demented residents. Again, these results are consistent with my findings with male 5xFAD mice in which cages housing only transgenic males were more likely to sustain injuries than cages containing only wild-type males or one wild-type and one transgenic male. Unfortunately, Shinoda-Tagawa *et al* suggest that attacks were either unprovoked or that the injured residents "put themselves in harm's way" and recommend that interventions focus on the behaviours of the victim. This perspective is reflective of a

larger problem wherein individuals with dementia are viewed as problematic and their behaviours as something to be managed. However, more recent research suggests a shift towards viewing aggressive behaviours as being the result of a state rather than a trait. During an observational study, Caspi (2015) identified that very few aggressive acts were unprovoked, and the majority had some sort of clear inciting event that ultimately led to aggression. Similarly, Volicer (2021) identifies the need to separate proactive aggression—premeditated behaviour with the goal of a reward or dominance—from reactive aggression, which is an aggressive response to a perceived threat or provocation. Both of these studies highlight that proactive (unprovoked) aggression is rare, with the majority of cases consisting of reactive aggression in which an event (such as touching or invasion of personal space) precipitates the attack, and that identifying risk factors and addressing them in advance can mitigate these encounters. In some cases, individuals with dementia may exhibit aggressive or disruptive behaviours arising from an unmet need—such as discomfort, hunger, thirst, or pain—which the individual has difficulty in communicating, and improving staff training on communication and addressing these needs can improve behaviour and reduce pharmacological interventions (Kolanowski, 1999). These results further support the conclusions that increased injurious behaviours in male transgenic-only cages arise due to behaviours exhibited by both the aggressor and the target, as a real or imagined provocation could lead to reactive aggression that ultimately leads to injury, possibly through a progressive escalation of aggression exhibited by both subjects.

Overall, these results suggest that individuals with AD living in a long-term care facility—particularly those in dementia wards—are more likely to be involved in

altercations, and more likely to be injured in violent altercations, than other residents. Additionally, while dementia patients occasionally exhibit seemingly unprovoked aggression, this is rare; more often, there is an antecedent event that incites the aggressive behaviours, whether exhibited by the victim, perpetrator, or both, and by identifying and addressing these events in time it may be possible to avoid an aggressive encounter. This is in-line with my findings of increased injurious behaviours in 5xFAD male transgenic-only cages. These injuries arise in familiar environments where two subjects exhibiting altered behaviours are continually in close proximity to each other, akin to the closed dementia wards used in some long-term care facilities. Similarly, the occurrence of these injuries primarily in transgenic-only cages—with reduced likelihood in wild-type-only or mixed-genotype cages—suggests that behaviour of both the aggressor and the target play a role in the development of these injuries. This may be reflective of aggressive encounters in humans with AD, as individuals with dementia may exhibit behaviours that provoke another resident, who may themselves exhibit an altered response that then intensifies the situation. Although pairing a transgenic 5xFAD male with a wild-type control led to reduced injuries in my study, a number of aggressive encounters in long-term care facilities include non-demented residents. However, it is important to note that underlying factors in non-demented residents—such as unmet needs, chronic or acute mental health concerns, or discriminatory attitudes—may similarly result in behaviours that can cause escalation of conflict. Caspi (2015) indicates that aggressive encounters were typically reactive and preceded by clear incidents that provoked the aggressor, and many took place outside of structured activities (e.g., mealtimes, in hallways, in resident rooms), recommending that changes in care strategies—such as reducing risk factors that

cause inciting events, and improved identification of and earlier intervention during these events before they escalate to aggression—could likely reduce many of these incidents. Similarly, while Volicer (2021) focuses on aggression from the perspective of care staff, their recommendations of changing nursing practices—such as improved communication and a more person-centred approach to care—are in line with the unmet needs model proposed by Kolanowski (1999) and may help with an overall reduction in aggressive or disruptive behaviours. The findings presented in this thesis suggest that early intervention—whether by addressing risk factors to minimize inciting events or early intervention during aggressive encounters to prevent escalation—is critical for reducing injuries resulting from aggression involving individuals with AD. While it is unclear what pattern of behaviours led to the increased injuries I observed in male transgenic-only cages, the reduced injuries observed in wild-type-only and mixed-genotype cages indicate that these interactions were dependent on the phenotype of both the aggressor and the target, and the presence of a wild-type male reduced the likelihood that these aggressive encounters would escalate to the point of injury. Although a large number of aggressive encounters in long-term care facilities involve non-demented individuals, it is important to note that these individuals likely exhibit one or more impairments and are therefore not equivalent to the relatively un-impaired wild-type mice in the present study. However, staff at these facilities are likely to be relatively un-impaired, and appropriate training could give them the ability to identify and intervene in volatile situations prior to escalation and injury.

6.8 Summary, Future Directions, and Final Thoughts

The work presented here was undertaken with the aim of expanding on my

previous work demonstrating an age-related reduction in social investigative behaviours in transgenic 5xFAD females from prodromic to late disease stages (Kosel, Torres Munoz, et al., 2019). The present work identified altered home-cage aggressive behaviours in 5xFAD mice that led to increased injuries, but only for transgenic males housed with other transgenic males. Counter to previous research, however, I did not identify overtly increased aggression in transgenic 5xFAD males in the home-cage or in a novel environment, nor did the genotype of a novel conspecific affect aggressive behaviours in a novel environment for either transgenic or wild-type males. I also identified reduced social investigative behaviours in response to free-roaming, but not constrained, novel conspecifics in transgenic 5xFAD males and females at 6 months of age, consistent with my previous work, and genotype of the conspecific similarly did not affect investigative behaviours. I also demonstrated that the olfactory habituation/dishabituation task (M. Yang & Crawley, 2009) could be adapted to work with an olfactometer and provided information (including design and assembly information and functional code) for an economical, modular olfactometer that can be used with both tethered and untethered rodents. Finally, I identified altered LFP activity in the mPFC of awake, behaving, transgenic 5xFAD mice under baseline conditions and in response to odour stimuli, providing a possible link between the neuropathology exhibited by this model and the reduced social investigation I previously reported.

The findings that transgenic 5xFAD mice exhibit reduced social investigative behaviours at 6 months of age is consistent with my previous research (Kosel, Torres Munoz, et al., 2019), albeit at an earlier age than I previously identified. It is likely that the study design played a role in these results, as social investigation in the present study

was done with a cross-sectional design, with subjects only tested at 6 months of age, whereas the previous study used a longitudinal design with subjects being tested at 3, 6, 9, and 12 months of age, and exposure to the tasks and novel conspecifics in the prodromal (3 month) stage may have mediated the effects at 6 months of age. Similarly, the progressive increase in "intensity" of tasks in the previous study—starting with the olfactory habituation/dishabituation task, then urine marking, then three-chamber sociability, and finally free social interaction—may have reduced the anxiety and/or arousal of transgenic mice enough to eliminate the genotype effects in the free social interaction task at 6 months of age. This would also be in line with the conclusion that reduced social investigation in transgenic 5xFAD mice arises due to increased anxiety or arousal rather than overall apathy, although recent evidence has suggested that transgenic 5xFAD mice exhibit increased apathy starting at around 6 months of age (Keszycki et al., 2023). However, evidence suggests that other transgenic AD-model mice exhibit reduced social investigation (Bories et al., 2012; D. Cheng et al., 2013; Filali et al., 2011a; H. Huang et al., 2016; Pietropaolo et al., 2012; Verma et al., 2015) in the absence of general apathy (Bardgett et al., 2011; Braun & Feinstein, 2019; Howlett et al., 2004; Pietropaolo et al., 2012; Shruster & Offen, 2014), although relatively few studies have examined sociability together with general apathy (e.g., using a novel object in the three-chamber sociability task), making it difficult to directly compare sociability with apathy.

With increased aggression reported in a number of AD-model mice (G. Alexander et al., 2011; Moechars et al., 1998; Pugh et al., 2007; Vloeberghs et al., 2006; Yan et al., 2013), and the high number of injuries I previously observed due to home-cage aggression in cages housing only transgenic 5xFAD males, the finding that transgenic

5xFAD males do not exhibit overtly increased aggression was unexpected, although some studies have reported no differences in aggressive behaviours in transgenic and wild-type males in other mouse models of AD. Bories *et al* (2012) indicated that transgenic 3xTg-AD males did not exhibit increased aggressive behaviours in a free social interaction task, while Watt *et al* (2020) indicated that the TAU58/2 mouse model of AD do not exhibit increased aggression in a resident-intruder task if isolated for only 30 minutes prior to the task, suggesting that neuropathology and environment (familiar vs novel) alone are not sufficient to cause increased aggression. While one study did report increased aggression during the resident-intruder task without social isolation housing in male PSEN conditional double-knock-out mice (Yan et al., 2013), males were housed with a female conspecific for two weeks prior to the task. Research has demonstrated links between inter-male aggression and sexual behaviours through similar neural circuits and bi-directional control of testosterone (Gleason et al., 2009; Yamaguchi, 2022), while other research has suggested increased territoriality is linked with parental behaviours (Marler & Trainor, 2020). It is possible that pair-housing of males with females used by Yan *et al* (2013) led to sexual experience, leading to increased vigilance for the "territory" (home cage) of the subject and a subsequent increase in aggression to intruders. This, of course, does not negate the increased aggression exhibited by transgenic males under these conditions; however, it suggests that AD-related pathology does not lead to increased aggression per se, but may lead to increased susceptibility to triggers that heighten aggression. In wild-type mice, these triggers (e.g., social isolation housing, sexual experience) may lead to increased likelihood that subjects will exhibit aggression towards a novel conspecific (e.g., intruder during a resident-intruder task), although this still

requires a certain level of agonistic behaviours by the target (e.g., not leaving the aggressor's territory). By comparison, these same triggers may result in a sub-threshold level for aggression in transgenic males, and lower levels of agonistic behaviours are then necessary to incite aggression against a target. This may be caused by heightened arousal in transgenic males, consistent with the results I reported here (see Chapter 3) and my previous work (Kosel, Torres Munoz, et al., 2019). This may also explain the increased injurious behaviours I identified in transgenic-only male cages: it is possible that the heightened arousal triggers one male to exhibit aggressive behaviours towards its cage-mate, which causes its cage-mate to also exhibit altered responses due to heightened arousal (e.g., fleeing, attack), leading to an escalation in aggressive encounters that ultimately leads to injury; by comparison, a wild-type male may exhibit different behaviours (e.g., upright defensive posture) that could prevent further escalation of the situation. Similarly, wild-type males may be less likely to exhibit behaviours that are considered disruptive (e.g., repetitive, stereotypic behaviours) than transgenic males, thereby reducing the likelihood of aggressive encounters in the first place. Such an encounter would only need to occur once before males were separated, so it is unclear whether these cages would continue to show increased frequency or severity of aggressive encounters over subsequent days. Moreover, these findings are consistent with data from humans with AD; specifically, individuals in AD-specific wards are more likely to be injured as a result of a violent altercation than non-demented individuals, and incidents involving individuals with dementia are primarily the result of reactive aggression to some kind of inciting event (Caspi, 2015; Lachs et al., 2007; Shinoda-Tagawa et al., 2004; Volicer, 2021), suggesting that agonistic situations involving

individuals with AD may be more likely to escalate into aggressive—and occasionally violent—encounters.

Although the exact relationship between neuropathology and BPSD-like behaviours is yet to be determined, rodent models of AD generally exhibit altered LFP in cortical and subcortical structures (Ahnaou et al., 2017; Bazzigaluppi et al., 2018; Booth et al., 2016; Bories et al., 2012; Chen, Ma, et al., 2022; Chen, Wei, et al., 2022; Etter et al., 2019; Goutagny et al., 2013; Jacob et al., 2019; Mondragón-Rodríguez et al., 2018; Nakazono et al., 2017; Peng et al., 2021; Schneider et al., 2014; Tanninen et al., 2017; Van Den Berg et al., 2023), indicating that neuropathology is playing a role in these changes. Similarly, certain BPSD-like behaviours—such as apathy, depression, aggression, and sleep/wake disturbances—are also related to neuropathology in transgenic AD-model mice (see Chapter 2); overall, this suggests a relationship between altered neural activity in cortical and subcortical structures and the BPSD-like behaviours exhibited by these models. Similarly, BPSD—particularly apathy, anxiety, agitation and aggression, depression, and sleep/wake disturbances—are prevalent in individuals with AD (Chow et al., 2002; Frisoni et al., 1999; Lyketsos et al., 2002; Steffens et al., 2005; Zhao et al., 2016), and evidence is starting to accumulate for the role of AD-related neuropathology in the development of BPSD such as apathy (Mehak et al., 2023) and anxiety (Mendez, 2021; Pentkowski et al., 2021). However, the neurological underpinnings of social impairments in AD have only just begun to be examined; to my knowledge, Saris *et al* (2022) are the only researchers to directly examine how changes in neural activity are related to altered social behaviours in AD, and their results indicated that these behaviours were related to a disconnection between the prefrontal cortex—

specifically the default mode network—and the rest of the brain. The identification of the mPFC as being involved in social deficits in AD is not surprising, as there is ample evidence linking this region to altered social behaviours in both mice and humans (Bories et al., 2012; S. Braak et al., 2022; Cao et al., 2018; Challis et al., 2014; Duffney et al., 2015; T. B. Franklin et al., 2017; Kuga et al., 2022; Levy et al., 2019; Miskovic et al., 2010; Molenberghs et al., 2016; Najjar & Brooker, 2017; Schmidt et al., 2022; Schurz et al., 2014; Selimbeyoglu et al., 2017; Sriranjan et al., 2022; Takahashi et al., 2014; F. Wang et al., 2011; Zhou et al., 2017, p. 201); however, the work by Saris *et al* relied on rating scales and resting-state fMRI data, and researchers have recently highlighted that further translational work is needed to uncover how neural activity affects these behaviours in real time (Gilmour et al., 2019; Porcelli et al., 2019).

Surprisingly, while transgenic 5xFAD mice exhibit neuropathology—including in the mPFC—concomitant with social deficits, and the mPFC has been extensively implicated in social behaviours, I did not identify any genotype effects for LFP activity in the mPFC specific to the social stimulus relative to other stimuli. Differences in LFP activity in the mPFC of transgenic 5xFAD mice are not specific to social processing, but represent overall differences compared to wild-type controls in both baseline activity and in response to odour stimuli. This is consistent with my previous conclusions that reduced social investigation in transgenic 5xFAD mice arises due to anxiety or arousal rather than social withdrawal specifically (see Chapter 3 and Kosel, Torres Munoz, *et al* [2019]). However, this does not exclude the possibility that transgenic 5xFAD mice—or other AD models—do not exhibit social deficits arising from mPFC activity. Rather, it indicates that LFP activity alone in the mPFC of 5xFAD mice does not reflect altered social

function at a behavioural level. For example, Levy *et al* (2019) identified that neuronal populations in the mPFC exhibited differential firing in response to social vs non-social odour stimuli, and this was altered in the *Cntnap2* mouse model of ASD relative to wild-type controls, providing a clear differentiation of responses to social, non-social, and control odours as well as genotype effects. Moreover, the data provided by Levy *et al* suggests that a large proportion of responsive neurons they recorded from exhibited non-specific firing in response to social vs non-social stimuli, and a smaller proportion fired only in response to either social or non-social stimuli. While they did not assess LFP data, it is possible that the overall firing of each group of neurons led to changes that could be detected at the level of single unit cell firing, but that these changes in activity cannot be differentiated at the LFP level. In addition, a number of studies either do not use a non-social stimulus or do not perform recordings during exposure to a non-social stimulus (Bories et al., 2012; Cao et al., 2018; Jeon et al., 2021; Kuga et al., 2022). The study by L. Liu *et al* (2020) did perform LFP recordings in the mPFC of C57BL/6J mice in response to social and non-social stimuli, and demonstrated that social stimuli resulted in a significant increase in gamma band power whereas the non-social stimulus resulted in a small (but non-significant) increase. That said, the lack of a non-social control in many studies highlights the need for careful study design and interpretation of results as non-specific changes in response to a stimulus—such as the non-specific firing indicated by Levy *et al*—could be misattributed social-specific effects in the absence of a non-social stimulus.

Despite these considerations, the present work has indicated that transgenic 5xFAD mice exhibit altered LFP activity compared to wild-type controls in response to

social and non-social odour stimuli. Additionally, these effects are consistent with human and animal data indicating changes in neural activity related to anxiety, arousal, and emotional processing (Hare & Duman, 2020; Hirschberg et al., 2017; Hiser & Koenigs, 2018; Jeon et al., 2021; Karalis et al., 2016; Klados et al., 2009; Knyazev et al., 2009; Lv et al., 2022; Merino et al., 2021; Miskovic et al., 2010; Najjar & Brooker, 2017; Schmidt et al., 2022; Sriranjan et al., 2022; Z. Wang et al., 2021) and further support the conclusions that reduced social investigation in transgenic 5xFAD mice is related to increased anxiety and/or arousal (see Chapter 3; Kosel, Torres Munoz, et al., 2019). However, this does not exclude the possibility that other mPFC activity—such as single-unit activity, similar to that reported in *Cntnap2* mice by Levy *et al* (2019)—is not altered in transgenic 5xFAD mice in response to social odours. Similarly, while Saris *et al* (2022) indicate that mPFC dysconnectivity (particularly the default mode network) is related to social deficits in humans with AD, this does not mean that this region is not involved in mediating other AD-related impairments as well, nor does it preclude the involvement of other brain regions or other aspects of mPFC activity—such as LFP or single-unit activity—in the development of AD-related social deficits.

It is estimated that there are ~140,000 community-dwelling Canadians currently living with AD, and there were ~115,000 additional institutionalized Canadians living with the disease as of 2012 (Statistics Canada, 2013, 2022). Roughly 80% of community-dwelling individuals with AD will exhibit at least one BPSD following the onset of cognitive symptoms (Lyketsos et al., 2002), although BPSD are a leading factor in the decision to institutionalize (Chenoweth & Spencer, 1986; C. A. Cohen et al., 1993; C. Steele et al., 1990), suggesting that prevalence of BPSD may be even higher in long-term

care facilities. However, despite the profound role of BPSD on the lives of individuals with AD, their families, and caregivers (Chenoweth & Spencer, 1986; C. A. Cohen et al., 1993; Craig et al., 2005), BPSD tend to be considered a secondary symptom to be managed with (ideally) non-pharmacological interventions or—at worst—with medication (Chung & Cummings, 2000; Corbett et al., 2012; Kolanowski, 1999; Liperoti et al., 2008; Y. Ohno et al., 2019; Tible et al., 2017). Additionally, despite building evidence to support the role of neuropathology in the development of BPSD such as apathy (Mehak et al., 2022) and anxiety (Mendez, 2021; Pentkowski et al., 2021), there is still debate about the contribution of neurological, intrinsic, and extrinsic factors in the development of BPSD (Cho et al., 2021; Kolanowski et al., 2017). Additionally, recent developments with monoclonal antibodies have led to disease-modifying drugs for AD that can effectively clear Aß deposits from the brains of individuals with AD, although this has highlighted that the majority of AD-related neuropathology is not the direct result of Aß accumulation, but rather other mechanisms that have not yet been determined (Cummings, 2023). This, then, reinforces the importance of continued exploration for the mechanisms underlying AD-related neuropathology in order to work towards a cure.

The changes in neural activity identified herein only provide a small part of the overall story, and further work is needed to determine how other factors—such as single-unit activity—are related and the mechanisms underlying these changes. Additionally, it is possible that these changes are specific to this particular mouse model under these particular circumstances; however, these findings are largely consistent with existing research with human and animal studies including both healthy controls and disease populations, including AD-related neuropathology and altered neural activity, behaviour

and neural activity in disease states and healthy controls, and altered behaviour and neuropathology in AD-model mice. Thus, this work creates a link between these three aspects of AD—neuropathology, neural activity, and behaviour—to further support the role of neuropathology in the development of BPSD. Moreover, the similarities between these results and those from mouse research on fear and anxiety are somewhat unexpected; while I have previously suggested anxiety and arousal level as factors driving the reduced social investigation observed in transgenic 5xFAD mice, the NPI (Cummings, 1997; Cummings et al., 1994)—which has become the *de facto* standard for assessing BPSD—largely includes social withdrawal under apathy ("Does the patient seem less interested in the activities and plans of others? Has the patient lost interest in friends and family members?"), with only a tangential reference to social situations under anxiety ("Does the patient avoid certain places or situations that make him/her more nervous such as riding in the car, meeting with friends, or being in crowds?"), suggesting that social withdrawal in AD arises due to apathy rather than anxiety. Similarly, the screening questions for apathy only tangentially mention social interactions ("Is he/she more difficult to engage in conversation…?"), while the only mention of social interactions in the screening questions for anxiety references separation anxiety ("Is the patient afraid to be apart from you?"). Given that the scoring questions are not asked unless the screening questions are responded to affirmatively, and questions are supposed to be asked verbatim—with the only clarifications being a restatement of the question in different words—it is possible that the prevalence of social withdrawal is not accurately assessed in humans with AD, and in some instances may be misattributed to apathy rather than anxiety. Since the original development of the NPI (Cummings, 1997; Cummings et

al., 1994) there has been a gradual shift from viewing BPSD as problematic behaviours that need to be managed (Chung & Cummings, 2000; Gustafsson et al., 2013; Liperoti et al., 2008; McMinn & Hinton, 2000; Shinoda-Tagawa et al., 2004) to a more person-centric approach aimed at understanding and addressing the proximal factors leading to these behaviours (S. Braak et al., 2022; Caspi, 2015; Corbett et al., 2012; Mehak et al., 2023; Mendez, 2021; Tible et al., 2017; Volicer, 2021), although these approaches have been advocated in the past (Kolanowski, 1999). With this shift comes an acknowledgement of the nuances underlying BPSD, including neurological, psychological, social, and environmental factors (Kolanowski et al., 2017), and Volicer (2021) has suggested that the NPI in its present state is not sufficient to capture these subtleties, at least for aggression and agitation. Similarly, a number of hypotheses of AD etiology have accepted the relative heterogeneity of the disease and have indicated that multiple mechanisms—whether upstream or downstream of Aß and NFT accumulation—may be involved in neuropathology (Nunomura et al., 2006; Small & Duff, 2008; Sorrentino et al., 2014); these hypotheses have been further supported by recent monoclonal antibody treatments of AD which indicate continued neuropathology despite reductions in Aß to below levels identifiable by scans (Cummings, 2023), suggesting that Aß alone is not driving AD-related pathology. Similarly, the brain regions affected by AD-related neuropathology—such as the mPFC—are implicated in numerous cognitive processes through local neuron populations and projections to other brain regions; thus, it's possible that subtle changes in one area can lead to different symptoms, whereas gross changes in discrete areas could lead to similar symptoms. As a result, while there is human evidence to support the role of AD-related neuropathology in the development of

apathy and anxiety (Mehak et al., 2023; Mendez, 2021), efforts to correlate neuropathology with behaviour may be hindered by the relatively narrow classifications of BPSD in the NPI, and this is supported by the poor correlation of pathology and anxiety in rodent models of AD (Kosel et al., 2020; Pentkowski et al., 2021). Similarly, transgenic AD-model mice (including 5xFAD mice) exhibit social withdrawal—which is classified as apathy in the NPI—with minimal evidence of general apathy; however, I have previously suggested that the reduced social investigation in transgenic 5xFAD mice arises from increased anxiety and/or arousal—which is not consistent with the NPI—and the present LFP results support those conclusions. Ultimately, it is clear that neuropathology is related to the development of BPSD, and the present work contributes to this body of knowledge while simultaneously highlighting the need for reappraisal of the classifications and relationships between BPSD—such as the role of apathy and anxiety in social withdrawal, as well as the mediating effects of arousal on anxiety, aggression, and social withdrawal—in order to better resolve the nuances between neuropathology and altered behaviours.

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