Interrogating how age and sex intersect to elicit an Alzheimer's-like phenotype in N5 TgCRND8 mice

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative brain disease characterized by memory impairment and progressive cognitive decline that leads to dementia. While a small subset (<5%) of AD cases, known as familial AD (fAD), are due to three genetic determinants (APP, PSEN-1, PSEN-2), we still do not understand the cause behind 95% of AD cases, known as sporadic AD (sAD) which are idiopathic and late-onset (> 65 years of age). Women represent two-thirds of clinically diagnosed AD cases and disproportionally experience accelerated trajectories to cognitive decline compared to men. The N5 TgCRND8 mouse model is an aggressive mouse model of Aß deposition that exhibits sexual dimorphisms in behavioural indices of cognitive decline (Chisti et al., 2001; Granger et al., 2016). In this thesis I investigated how age and sex interact with Aß load to elicit behavioural indices of ADL (i.e., nesting, sleeping, eating, drinking), BPSD (i.e., sleep-wake cycle, motoric hyperarousal, anxiety) and cognition (learning, memory, and strategy/cognitive reserve) in the N5 TgCRND8 model. First, I show that Tg females and Tg males exhibit comparable longitudinal Aß (Aß40 and Aß42) levels in the temporal cortex and hippocampus as they age from 2-12 months of age. I next characterized behavioural indices of ADL and BPSD in the N5 TgCRND8 mouse model using a battery of behavioural tests. Data presented here show that young N5 TgCRND8 mice exhibit ADL impairment and a hyperaroused phenotype that may contribute to behavioural indices of wake-dysfunction. Lastly, I investigated behavioural indices of cognition (visuospatial working memory, visuospatial learning and memory, cognitive reserve) in the N5 TgCRND8 mouse model. I show that sex-specific impairments in visuospatial working memory, visuospatial learning and memory and cognitive reserve in N5 TgCRND8 mice occurred later in life both after the onset of ADL and BPSD impairments and under identical longitudinal Aß (Aß40 and Aß42) challenge between females and

males. Taken together, this thesis provides converging evidence to indicate that age and sex interact with initial (biological) AD neuropathology to elicit early ADL and BPSD behavioural impairment in N5 TgCRND8 mice that later manifests as sex-specific cognitive decline.

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LIST OF ABBREVIATIONS

ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's Disease
AD^2	Alzheimer's disease squared
ADL	Activities of daily living
AG	Auguste Deter
AICD	APP intracellular domain
APH-1	Anterior pharynx defective 1 (APH-1)
APOE	Apolipoprotein E
APOE4	Apolipoprotein E4
APP	Amyloid precursor protein
Aβ	Amyloid beta
Aβ40	Amyloid-beta 40
Aβ42	Amyloid-beta 42
Aβ48	Amyloid-beta 48
Aβ49	Amyloid beta 49
BACE-1	Beta-secretase 1
BPSD	Behavioural and Psychological Symptoms of Dementia
CAA	Cerebral amyloid angiopathy
CDT	Clock-drawing test
CNS	Central Nervous System
CSRTT	Multiple Choice Serial Reaction Time Task
cSVD	Cerebral small vessel disease
CTFβ	C-terminal fragment B
DS	Down Syndrome
E-ADL-Test	Erlangen Test of ADLs
fAD	Familial Alzheimer's Disease
FLAIR	Fluid Attenuated Inversion Recovery
iADL	Instrumental Activities of Daily Living
MCI	Mild cognitive impairment
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment
MRI	Magnetic Resonance Imaging
MT	Microtubule
MTL	Medial Temporal Lobe
MWM	Morris Water Maze
NFT	Neurofibrillary tangles
NREM	Non Rapid Eye Movement
PAMP-DAMP	Pathogen-/Damage-Associated Molecular Pattern
PEN-2	Presenilin enhancer 2
PS	Presenilin
PS1	Presenilin-1
PS2	Presenilin-2
PT	PhenoTyper

REM	Rapid Eye Movement
RW	Running wheels
sAD	Sporadic Alzheimer's Disease
sAPPβ	Soluble amyloid beta precursor protein
SCN	Suprachiasmatic nucleus
SCN^{VIP}	Vasoactive Intestinal-Expressing Suprachiasmatic Nucleus Neurons
TBI	Traumatic Brain Injury
TREM2	Triggering Receptor Expressed on Myeloid cells 2
VIP	Vasoactive Intestinal Peptide
WHI	Women's Health Initiative
WMH	White Matter Hyperintensity
WWWhich	What-Where-Which Task
YM	Y-maze
ZT	Zeitgeber

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1. INTRODUCTION

1.1 Alzheimer's disease is a neurodegenerative brain disease characterized by memory impairment and progressive cognitive decline that leads to dementia

1.1.1. Clinical presentation of AD and related dementias

Dementia refers to a syndrome and/or group of clinical signs and symptoms associated with progressive cognitive decline that ultimately impairs ability to perform daily activities (Cunningham et al., 2015). Dementia can result from a number of neurodegenerative diseases that inflict damage and/or death to neurons in the brain, including Alzheimer's disease (AD), vascular disease and Lewy Body Dementias (Cunningham et al., 2015). Symptoms of dementia include impairments in memory, such as difficulty recalling facts and/or events, and disturbance in atleast one other cognitive domain, such as speech (i.e. difficulty finding, understanding and/or expressing words), visual-spatial skills (i.e., becoming lost in familiar places or frequently misplacing items), and/or attention and executive functions (i.e., difficulties with reasoning, judgement, planning and problem-solving skills) (Frota et al., 2011; Karantzoulis & Galvin, 2011). Persons diagnosed with dementia become progressively impaired in their capacity to think, remember, and make decisions necessary to live independently (Karantzoulis & Galvin, 2011).

Cognitive screening tests play a pivotal role in early diagnosis of dementia. The Mini-Mental State Examination (MMSE) was published by Folstein et al. in 1975 and remains a routinely used scale to evaluate cognitive function in older adults (Folstein et al., 1975). The MMSE consists of eleven questions that assess different aspects of cognition, including orientation of time and place, attention and calculation, short-term memory recall, visuospatial abilities and language skills (Folstein et al., 1975). For example, a question under the language domain may ask the patient to read and act out the sentence 'close your eyes', which assesses reading

comprehension and the ability to follow instructions (Grossman & Irwin, 2016). Importantly, the MMSE excludes questions that evaluate aspects of mental function other than cognition, such as mood and/or abnormal mental experiences that are common in neuropsychiatric disorders, hence the term "mini" (Folstein et al., 1975). MMSE scores range from 0 to 30, with scores 23 or lower indicating possible cognitive impairment (Ruchinskas & Curyto, 2003).

The clock drawing test (CDT) is another tool for assessing cognitive status and is a useful clinical addition to the MMSE (Ruchinskas & Curyto, 2003; Spenciere et al., 2017). The test takes about a minute or less to complete, whereby a person is provided with a blank piece of paper and asked to: (1) draw a clock face, (2) place the numbers on the clock face, and (3) set the clock hands to the instructed time (Hazan et al., 2018). The instructed time is a critical part of the test. While many times are used, ten after eleven is the most common due to its high sensitivity; that is, drawing this time requires correctly recoding ten minutes to the number two on the clock (Konstam & Lehmann, 2011). CDT performance engages many different cognitive domains in the brain, including executive functions (e.g., planning), attention, memory and visuospatial skills (Hazan et al., 2018). Accordingly, different kinds of errors in the clock test translate to different abnormalities in brain function (Spenciere et al., 2018). For example, drawing all twelve numbers on one side of the clock highlights deficits in spatial planning, whereas numbers drawn both inside and outside of the clock indicate visuospatial deficits (Spenciere et al., 2018). Ultimately, the CDT is a useful tool that helps clinicians and researchers screen individuals for cognitive deficits resulting from a wide range of brain disorders or injuries (i.e., stroke) (Adunsky et al., 2002; Spenciere et al., 2018).

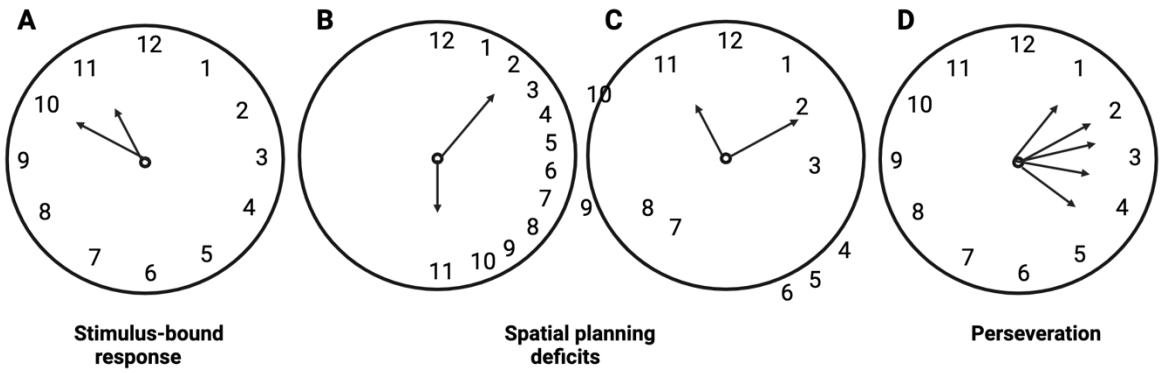

Figure 1 Representative illustrations of common errors in the clock-drawing test (CDT). Schematic depicts common CDT errors in patients diagnosed with dementia. Patients instructed to draw the time 'ten after eleven'. (A) Drawing the minute arm pointing towards the number ten indicates a stimulus-bound response error; (B) Drawing all numbers on one side of the clock face or (C) both inside and outside of the clock face indicate spatial planning deficits; (D) Drawing more than two clock arms indicates motoric perseveration. Created with BioRender.com

1.1.2. AD is defined biologically by two neuropathological hallmarks

AD is the most common cause of dementia, accounting for 60-70% of cases when including mixed AD neuropathologies (e.g., AD and vascular dementia) (Cunningham et. al., 2015; Hebert et. al., 2003). The clinical symptoms observed in AD commonly overlap with other causes of dementia, making clinical presentation alone insufficient for definitive AD diagnosis (Karantzoulis & Galvin, 2011). In fact, one study found that approximately 50% of patients with mild to moderate dementia have never received a formal diagnosis (Boustani et al., 2003). A 2023 review of criteria for diagnosis and staging of AD by the Alzheimer's Association Workgroup emphasizes the importance of defining AD as a biological disease, rather than a clinical syndrome, with persons diagnosed with biological AD prior to any detectable cognitive impairment (Alzheimer's Association Workgroup, 2023). Biological disease depends upon the biomarker assessment of two defining pathological hallmarks in the brain: deposition of extracellular amyloid-beta (Aß) plaques surrounding neurons and the formation of intracellular neurofibrilliary tangles (NFT) composed of hyperphosphorylated tau (Chong et al., 2018).

Aß peptides are the proteolytic products of amyloid precursor protein (APP) (Hampel et al., 2021; Priller et al., 2006). APP is a type I transmembrane glycoprotein abundantly expressed in the synapses of neurons (Hampel et al., 2021). APP has several isoforms that range in size from 695 to 770 amino acids; however, APP695 is the most abundant isoform in the brain (Chen et al., 2017). Amyloidogenic processing of human APP involves the sequential cleavage by ß- and γ-secretases (Fig1). The process begins when ß-secretase, predominantly through BACE-1 activity, cleaves APP into C-terminal fragment ß (CTFß or C99) and N-terminal fragment sAPPß (Chen et al., 2017). CTFß is subsequently cleaved by the γ-secretase complex, releasing extracellular Aß peptide and the APP intracellular domain (AICD) (Chen et al., 2017; Selkoe, 2001). γ-secretase is a large membrane protein complex composed of four essential subunits: nicastrin, anterior pharynx

defective 1 (APH-1), presenilin enhancer 2 (PEN-2) and presenilin (PS; including PS1 and PS2), the catalytic subunit (Iwatsubo, 2004; Kimberly et al., 2003). In AD, APP is aberrantly processed by ß- and y-secretases into neurotoxic Aß40 and Aß42 oligomers that accumulate to form extracellular plaques, subsequently leading to synaptic failure and neurodegeneration (Hampel et al., 2021).

Initial Aß-deposition occurs in brain regions important for learning and memory, including the cerebral cortex and hippocampus (Thal et al., 2002). These brain regions are target tissues of basal forebrain cholinergic neurons, which degenerate early in AD progression and correlate significantly with memory impairment in mild and moderate AD dementia (Fahnestock & Shekari, 2019). Current treatments focus on inhibiting the function of acetylcholinesterase (AChE), an enzyme responsible for the selective breakdown of the neurotransmitter acetylcholine (ACh) (Grossberg, 2003). AChE inhibitors, such as donepezil and galantamine, increase ACh availability to healthy neurons and help to slow symptoms of AD dementia, however there is no cure for AD (Grossberg, 2003).

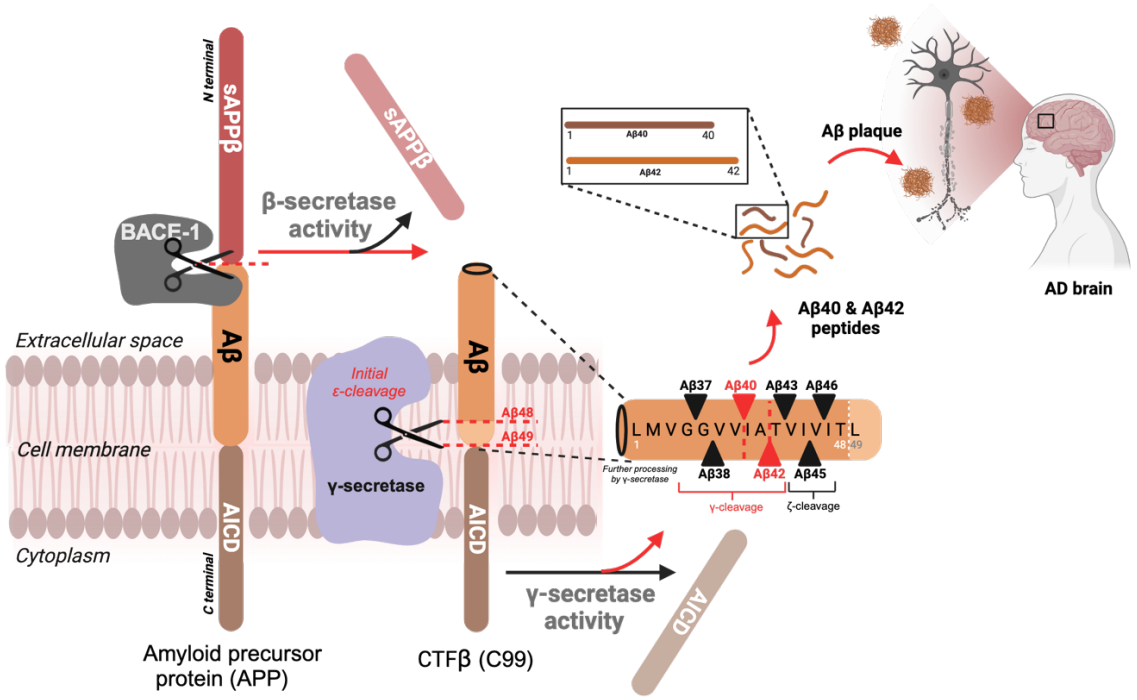

Figure 2 Amyloidogenic processing of human APP in AD. ß-secretase, primarily through BACE-1 activity, cleaves APP to produce ßCTF (C99) and sAPPß (soluble APP) is released. γ-secretase subsequently cleaves ßCTF between Aß49 or Aß48 (ε-cleavage). Further cleavages (ζ- and γ-) yield Aß40 and Aß42 that are released into the extracellular space. Aß40 and Aß42 peptides accumulate into extracellular plaques in AD brains. Figure adapted from Luo (2022). Created with BioRender.com

The second neuropathological hallmark of AD is the intracellular accumulation of neurofibrillary tangles composed of hyperphosphorylated tau protein (Chong et al., 2018). Tau is a microtubule-binding protein highly expressed in neurons (Gendron & Petrucelli, 2009). Predominantly locating to axons, tau plays many important roles in neuronal function, including stabilization of microtubules (MT) and regulation of motor protein-driven transport of cellular cargo (Barlan & Gelfand, 2017; Duan et al., 2017; Gendron & Petrucelli, 2009; Merezhko et al., 2020). Tau promotes healthy synaptic function and formation by facilitating the transport of synaptic cargo along MTs, such as neurotransmitter receptors (Schlager & Hoogenraad, 2009). Low concentrations of tau at the soma facilitate kinesin-MT binding and anterograde transport; whereas higher concentrations at the synapse promote cargo release (Dixit et al., 2008). The function of tau is highly dependent on its phosphorylation state, which is a result of the action between protein kinases and phosphatases through post-translational modifications (Mietelska-Porowska et al., 2014). In AD and a related family of neurodegenerative diseases called tauopathies, tau is abnormally hyperphosphorylated three- to four-folds higher than healthy physiological levels, leading to its disassembly from MTs and aggregation into neurofibrillary tangles (Gendron & Petrucelli, 2009; Grundke-Iqbal et al., 1986; Iqbal et al., 2010).

1.1.3. AD is a continuum that begins 10-15 years prior to the onset of clinical symptoms

AD is a continuum that begins with the silent appearance of brain neuropathological changes and slowly progresses through stages of increased neuropathological burden resulting in the onset of cognitive symptoms (Hampel et al., 2021; Alzheimer's Association Workgroup, 2023). For this reason, AD progression can be divided into three clinical stages: (1) the preclinical stage, defined by normal cognitive ability despite detectable Aß neuropathologies (i.e., Aß plaques neurofibrillary tangles); mild cognitive impairment (MCI), characterized by a decline in mental

abilities that does not interfere with daily activities; and AD dementia with functional impairment in everyday living (Hampel et al., 2021).

Aß accumulation begins 10-15 years prior to the onset of cognitive deficits, indicating that pathophysiological mechanisms involved in Aß production and clearance likely play a role early in AD progression, but remain poorly understood (Bateman et al., 2012; Hampel et al., 2021; Hardy & Selkoe, 2002; Perl, 2010). MCI represents a transition period between cognitively normal and AD dementia (Harold et al., 2009). Individuals diagnosed with MCI may experience symptoms such as forgetfulness, frequently losing train of thought, misplacing items and/or having difficulty remembering directions to familiar places (Hampel et al., 2021).While AD is the primary cause of MCI, other neurodegenerative diseases can also cause MCI (Ganguli et al., 2019). Furthermore, not all persons with MCI will progress to AD dementia (McGirr et al., 2022; Michaud et al., 2017). In fact, one-third of MCI cases due to AD progress to AD dementia within the following five years (Alzheimer's Association, 2022).

AD dementia is the final clinical stage of AD progression and ranges from mild to severe dementia. Persons with mild AD dementia may experience difficulties with planning, organizing, expressing thoughts and/or finishing tasks at work (Bird, 1993; Hampel et al., 2021). Moderate AD dementia is characterized by more pronounced cognitive changes, such as confusion about time and place, forgetfulness of personal history, and/or needing assistance dressing in appropriate clothes for the weather (Cunningham et al., 2015). Persons with moderate AD dementia also experience behavioural and/or personality changes, such as sleep pattern changes, wandering, agitation, delusions and/or compulsive repetitive behaviours such as tissue shredding (Cerejeira et al., 2012). In late-stage or severe AD dementia, individuals often lose their ability to communicate and experience a decline in physical abilities such as walking, swallowing and controlling bladder

function that significantly interferes with independent daily functioning and requires around-the-clock care (Cerejeira et al., 2012; Easterling & Robbins, 2008).

1.1.4. Preclinical (biological) AD is diagnosed in living persons by disease specific core biomarkers

At the time of Alois Alzheimer until recently, clinical diagnosis of AD was made in the living by eliminating other possible causes of dementia, termed a 'probable' AD dementia (Perl, 2010). This diagnosis was routinely confirmed postmortem through neuropathological analysis of brain tissue to identify the presence of Aß plaques and neurofibrilliary tangles (Perl, 2010). With recent advancements, preclinical AD can now be diagnosed in living persons using disease-specific core biomarkers.

The Revised Criteria for Diagnosis and Staging of AD (Jack et al., 2024) introduced the concept of Core 1 and Core 2 biomarkers. Core 1 biomarkers help to identify the presence of biological AD regardless of symptomatic status (Jack et al., 2024). Importantly, the onset of ß-amyloidosis defines the initial detectable AD stage, termed 'Initial AD', that can be visualized by amyloid positron emission tomography (PET) (Jack et al., 2024). Core 1 biomarkers are those in the A (Aß proteinopathy; e.g., Aß42) and T_1 (phosphorylated and secreted AD tau; e.g., p-tau 217, p-tau 181, p-tau 231) categories (Jack et al., 2024). Remarkably, Core 1 fluid biomarkers can help to identify the presence of biological AD regardless of symptomatic status that become abnormal with amyloid PET scans (Jack et al., 2024). In contrast, Core 2 biomarkers are more closely associated with clinical AD symptoms and become abnormal at later stages of AD progression (Jack et al., 2024). Collectively, Core 1 and Core 2 biomarkers can help to provide vital information about clinical symptoms observed and how/if they relate to biological AD and the risk of AD progression in both symptomatic and asymptomatic individuals (Jack et al., 2024).

1.1.6. The majority of AD cases are late-onset and sporadic in nature

A small subset of AD cases (<5%) are due to mutations in 3 well-known genes (Amyloid precursor protein, APP; Presenilin-1, PSEN1 and Presenilin-2, PSEN2), referred to as early-onset familial AD (fAD) (Bi et al., 2019). However, we still do not understand the cause behind 95% of cases, known as late-onset, idiopathic and sporadic AD (sAD) that affect individuals aged 65 years and older (Bird, 1993). The causal origin of sAD is not known, however, there are a variety of risk factors associated with the disease, including advancing age (i.e., >65 years), genetics (e.g., *APOE, TREM2*), sex (i.e., females), lipid metabolism (i.e., dyslipidemia) and environmental factors (i.e., traumatic brain injury) that will be explored further in the following sections (A Armstrong, 2019).

1.1.5. The amyloid cascade hypothesis is failing therapeutically

In 1906, Alois Alzheimer presented what is now known as the first Alzheimer's disease case. He described it as a "peculiar severe disease of the cerebral cortex" (Hippius & Neundörfer, 2003). The patient was Auguste Deter (known thereafter as patient A.G.), a 50-year old woman who presented with untreatable progression of sleep and memory disturbance, aggressive behaviours and confusion, and passed just four years later (Hippius & Neundörfer, 2003). Alzheimer performed a postmortem neuropathological brain examination on patient A.G., where he observed degeneration of cortical nerve cells characterized by protein fibrils and plaques described as an "extraordinary number of peculiar patches disseminated throughout the whole cortex" (Alzheimer et al., 1991). As researchers' interest in the composition of these cerebral plaques grew, a novel amyloid fibril composed of smaller, 4 kDa aggregating fragments known as Aß was identified (Allsop et al., 1983; Glenner & Wong, 2012; Masters et al., 1985). It was reported that Aß, derived from the APP gene, was neurotoxic (Yankner et al., 1989), which founded the basis of the amyloid cascade hypothesis for decades to come.

The amyloid cascade hypothesis postulates that the accumulation of Aß oligomers is the causative agent in AD that triggers a cascade of downstream pathological events, notably the formation of neurofibrillary tangles, neurodegeneration, and ultimately, dementia onset (Hardy & Selkoe, 2002). Given the complex aetiology of sAD, this simple linear disease model from Aß accumulation to AD dementia does not capture the disease etiology. Mutations in APP, PSEN-1 and PSEN-2 are indeed well-known, causal mutations of fAD (Selkoe, 2001). Moreover, there is strong genetic evidence that these mutations cause fAD through aberrant APP processing, increasing Aß42/40 ratios and promoting its aggregation (Bi et al., 2019). The development of various transgenic AD mouse models expressing these mutations recapitulate many (but not all) characteristics of AD in humans, further supporting the association between aberrant APP and AD. For example, Tg2576 mice, who overexpress APP695 with the Swedish familial AD mutation (K670N/N671L), demonstrate significant increases in Aß40 and Aß42 and show memory impairments by 6 months of age (Hsiao et al., 1996; Westerman et al., 2002). Further evidence that tightly links APP to AD is the discovery that the *APP* gene is located on chromosome 21, which has had profound clinical implications for people diagnosed with Down's Syndrome (DS) (Salehi et al., 2016). Individuals diagnosed with DS have a triple copy of APP, also known as trisomy 21, and are predicted to develop AD neuropathology by age 40 (Masters et al., 1985; Zigman et al., 2008).

While there is genetic evidence to support a causal role of APP-associated mutations in early-onset fAD, these cases represent only five percent of all AD cases (Selkoe, 2001). Furthermore, not every person with DS will progress to AD dementia despite evident plaque deposition (Salehi et al., 2016; Zigman et al., 2008). Notably, the majority of risk genes identified for sAD have no direct genetic link to APP, but rather are directly involved in lipid metabolism

and/or immune function (Schmid et al., 2002). These include the APOE ε4 allele and a variant of the *Triggering Receptor Expressed on Myeloid cells 2* (*TREM2*) gene (Stepler & Robinson, 2019).

Aberrant lipid metabolism is linked to sAD. Approximately 40% of AD patients have atleast one copy of the APOE ε4 allele (Guo et al., 2020; Harold et al., 2009, 2009; Stozická et al., 2007). The APOE gene encodes for apolipoprotein E (ApoE), an abundant lipoprotein in the CNS (Ringman et al., 2014). ApoE is highly expressed in astrocytes; its primary role is to maintain lipid brain homeostasis via phospholipid and cholesterol transport (Stepler & Robinson, 2019). Cholesterol is synthesized by astrocytes and is delivered to neurons by ApoE, which is a vital molecular process for healthy synaptic formation and function (Hatters et al., 2006). Interestingly, ApoE is also involved in Aß binding and clearance in the brain (Hatters et al., 2006). Current data affirms that the APOE genotype significantly impacts upon Aß deposition to confer differential sAD risk (Raulin et al., 2022). While the APOE ε2 allele is protective, the APOE ε4 allele is strongly associated with increased Aß deposition in the brain and cerebrovasculature, enhancing risk of sAD and related AD vascular disease called cerebral amyloid angiopathy (CAA) (Rannikmäe et al., 2014). In fact, according to Ringman et al. (2014), individuals diagnosed with severe CAA are significantly more likely to carry an APOE ε4 allele than individuals who do not have CAA (Ringman et al., 2014).

It is important to note that the biological role of ApoE4 in driving sAD neuropathology has been investigated largely in the context of its modulatory effects on Aß. ApoE4 has been reported to increase the relative levels of the more neurotoxic, oligomeric form of Aß (i.e. Aß42) and inefficiently binding to and clearing Aß from the brain, which directly contributes to its accumulation (Ringman et al., 2014; Stepler & Robinson, 2019). Importantly, these investigations rely on the assumption that Aß is at the top of the pathological chain of events, and that ApoE4

acts exclusively through Aß-dependent pathways to enhance sAD risk. However, Aß is only one target of ApoE4. ApoE4 is involved in numerous brain lipid metabolism and signalling pathways independent of Aß, which necessitates the investigation of its role in Aß-independent mechanisms (Stepler & Robinson, 2019).

Synaptic failure is an early manifestation of AD that may precede and/or drive neuronal cell death (Pelucchi et al., 2022; Selkoe, 2002). In fact, the number of synapses in the neocortex is a stronger predictor of cognitive decline compared to Aß (Colom-Cadena et al., 2020; John & Reddy, 2021). Consequently, AD can be referred to as a synaptic disorder, or synaptopathy (Pelucchi et al., 2022). Cholesterol dysregulation is one Aß-independent mechanism by which APOE ε4 may act to promote synaptic failure and facilitate enhanced sAD risk. In order to form healthy and functional synapses, neurons rely on the presence of astrocyte-derived cholesterol transported to neurons by ApoE (Burns & Duff, 2002). ApoE4 has a poorer binding affinity for lipids compared to the other isoforms, which may alter cholesterol transport to neurons and compromise synaptic function (Hatters et al., 2006; Vance, 2012).

In addition to *APOE*, *TREM2* is another important risk gene for sAD. It encodes Triggering Receptor Expressed on Myeloid cells 2 (TREM2), a transmembrane receptor selectively expressed by microglia, which are the primary resident immune cells in the brain (Schmid et al., 2002). TREM2 promotes Aß-reactive microgliosis, a process by which microglia cluster around Aß deposits in order to facilitate its removal (Wang et al., 2015). Genome-wide association studies have identified a rare mutation in TREM2, Arginine-47-Histidine (R47H), that strongly enhances sAD risk (Guerreiro et al., 2013). This finding likely indicates that microglia require TREM2 in order to mount an effective microglial response to Aß deposition (Wang et al., 2015). In fact, TREM2 deficiency in 5XFAD mice leads to apoptosis of microglia rather than their activation and

proliferation, resulting in a dysfunctional microglial response and subsequent accumulation of Aß (Wang et al., 2015). Further, a study by Wang et al. (2015) found that TREM2 acts as a lipid-sensor and mediates the microglial response to Aß deposition by recognizing and binding to a variety of damage-associated lipids. These ligands include negatively charged phospholipids, which interact with Aß in the membrane; membrane phospholipids such as phosphatidylserine, which become exposed by damaged neurons; and sphingomyelin, which is a sphingolipid released by myelin in response to damage (Wang et al., 2015). Together, these findings suggest that lipid and immune-specific genetic risk factors play an important role in enhancing sAD risk, which provides insight into alternative Aß-independent mechanisms that promote neurodegeneration in sAD.

The precise biological roles of APP and Aß in sAD have yet to be fully elucidated. Epidemiological studies demonstrate that 30% of patients who die from traumatic brain injury (TBI) have Aß plaques in their brains (Sivanandam & Thakur, 2012). Indeed, TBI is a significant epigenetic risk factor for sAD (Fleminger et al., 2003; Gottlieb, 2000; Magnoni & Brody, 2010). The term epigenetic refers to how environmental factors influence the expression of genes and associated phenotypic changes without altering DNA sequence (Dupont et al., 2009). Following TBI, APP is upregulated and serves a neuroprotective function (Corrigan et al., 2012). One study showed that APP(-/-) knockout mice who had mild TBI demonstrated more severe cognitive deficits with significant hippocampal and cortical cell loss compared to APP(+/+) mice (Corrigan et al., 2012). These findings indicate that the production of APP and Aß could be neuroprotective responses to damage rather than the upstream trigger causing neurodegeneration. In fact, neuroinflammation is a common pathological feature of both TBI and AD (Sivanandam & Thakur, 2012). Following an acute event such as TBI, microglial activation results in the upregulation APP and other inflammatory mediators, which may contribute to a vicious cycle of Aß deposition and

microglial activation that results in chronic neuropathology seen in both TBI and AD (Sivanandam & Thakur, 2012).

In addition to lipid- and immune-specific risk factors, Aß deposition also occurs in approximately one-third of cognitively healthy persons, which indicates that it may play a role in healthy aging (Chételat et al., 2013; Price & Morris, 1999; Rodrigue et al., 2012). In fact, it has been shown that Aß burden in the brain increases linearly with age (Rodrigue et al., 2012). To date, the amyloid cascade hypothesis has yet to produce effective therapies for AD (Asher & Priefer, 2022), which necessitates the investigation of other possible AD mechanistic models.

1.2 The burden of cognitive decline in AD is age- and sex-specific

1.2.1. Advancing age is the most important risk factor for AD

With the aging population growing at an exponential rate, AD is estimated to affect up to 135 million people worldwide by 2050 (Prince et al., 2013). Age is the most important risk factor for AD, with the vast majority of cases being late-onset over the age of 65 years (Guerreiro & Bras, 2015). Aging has been defined biologically as a time-dependent decline in physiological functions necessary for survival and reproduction (Gilbert, 2000). While there is no doubt that aging and AD are inextricably linked, AD is not normal aging. AD is a biological disease of aging; it is defined both clinically and biologically based on a temporal progression of neuropathological events. Initial AD can be diagnosed decades prior to cognitive symptom onset (Alzheimer's Association, 2023). Age therefore plays a major role in determining where individuals land on the AD progression spectrum. An individual's age, in combination with other risk factors such as sex and genetics, can help to identify high-risk groups and facilitate earlier interventions (Rasmussen et al., 2018).

1.2.2 Women represent the majority of AD cases

Women are disproportionately affected by AD and represent two-thirds of clinically diagnosed cases (Alzheimer's Association, 2013). Women also have a greater lifetime risk of developing AD (1 in 5) compared to men (1 in 10) (Lopez-Lee et al., 2024). In fact, the female sex has been named a risk factor for AD (Rahman et al., 2019). The initial explanation provided for these sex differences is the fact that women live longer than men, highlighting age as the most important risk factor for AD (Mielke et al., 2014). In fact, while women represent the majority of AD cases, several studies have shown that men have a higher risk of developing MCI (Petersen, 2011; Petersen et al., 2010). It has therefore been hypothesized that men may die earlier in life due to competing comorbidities and only the most biologically resilient live to older ages (Mielke et al., 2014). However, this explanation is insufficient to explain the fact that women exhibit accelerated trajectories to cognitive decline compared to men (Lin et al., 2015; Tschanz et al., 2011). There are many sex-specific risk and protective factors for AD that may provide insight into differences in the development and progression of AD between women and men. These include hormonal, immune and vascular related factors.

A growing body of evidence suggests that a decline in estrogen levels increases female vulnerability to AD. Estrogen is a sex steroid hormone produced by the ovaries and its primary role is to regulate menopause and maintain female reproductive health. During healthy aging, women undergo a biological cessation of the menstrual cycle, also called menopause, which can last up to 14 years (McCarthy & Raval, 2020). Importantly, women who transition to menopause or undergo ovarian surgical removal experience diminished circulating estrogen levels, which have been linked to a greater risk of AD in women (McCarthy & Raval, 2020). In addition to its reproductive role, estrogen also plays a neuroprotective role in the brain. A study conducted by

Cardinali et al. (2021) reports that lifetime estrogen exposure can modulate AD risk, such that premenopausal women experience a 0.5% decrease in AD risk with every additional month of estrogen intake (Cardinali et al., 2021). It is well-documented that postmenopausal women are far more likely to develop AD compared to age-matched men (Rahman et al., 2019). A number of studies have put forth that estrogen hormone-replacement therapy (HRT) in postmenopausal women slows and/or reduces the risk of AD dementia (Fillit et al., 1986; Kawas et al., 1997; Ohkura et al., 1994; Tang et al., 1996). However, these findings have been challenged by the Women's Health Initiative (WHI) Memory study, which concluded that estrogen HRT increases the risk of AD dementia in postmenopausal women aged 65 years and older (Shumaker et al., 2003). To date, the therapeutic potential of estrogen in mitigating sex-specific AD risk remains controversial.

Sex-specific vulnerability in AD is also linked to the innate immune response. Strikingly, women represent up to 80% of autoimmune disease patients (Jacobson et al., 1997). Autoimmune disease is a general term that refers to a group of conditions in which the body's immune system erroneously identifies its own tissues as foreign and attacks them (Jacobson et al., 1997). These include systemic lupus erythematosus, Grave's disease and Hashimoto's thyroiditis, all of which are disproportionately experienced by women (Fish, 2008; Rubtsova et al., 2015). Furthermore, the incidence and severity of infectious disease is higher in men compared to women, suggesting that women have more reactive immune systems (Fish, 2008; Klein, 2000). These findings have profound clinical implications for AD risk in women. Autoimmune disease is a significant risk factor for AD (Lin et al., 2018). A recent study conducted by Ramey et al. (2024) reported that autoimmunity enhances AD risk independently of sex, but that the prevalence of AD is higher in

women diagnosed with an autoimmune disease compared to male counterparts. These findings suggest that immunity and sex intersect to modulate AD risk.

Given the central role of neuroinflammation in AD, AD itself has been conceptualized as an innate autoimmune disease (Meier-Stephenson et al., 2022). An article published by Weaver (2022) presents a new model of AD termed "AD-squared" or "AD^2" wherein an initial pathogen-/damage-associated molecular pattern (PAMP-DAMP) immune-triggering event, such as physical trauma or infection, leads to the production and release of Aß in the brain (Weaver, 2023). Contrary to the amyloid cascade hypothesis, the AD^2 model represents Aß as the initial immunomodulatory "responder" peptide that activates downstream immunological events, notably chronic microglial activation and cytokine release, resulting in attack upon "self" neurons (Weaver, 2023). Collectively, these findings indicate that sex-specific risk in AD is, atleast in part, modulated by sexual dimorphisms in maladaptive innate immune responses.

The majority of AD cases are that of AD mixed neuropathologies (Mehta & Schneider, 2023; Schneider et al., 2007). In fact, "pure" AD neuropathology represent about a quarter of all AD cases (Strandberg et al., 2020). AD mixed neuropathologies refer to the presence of AD hallmarks with the co-occurrence of any other pathology, such as alpha synuclein characteristic of Lewy Body Dementia (Schneider et al., 2009). The most common AD mixed neuropathology is AD and vascular dementia (Attems & Jellinger, 2014). The vital role of the vasculature in healthy brain function cannot be overstated. In fact, the brain is one of the most highly perfused organs in the body (Cipolla, 2009). The brain and the cerebrovasculature are deeply interconnected by neurovascular units, which are the sites where neurons rely on healthy microvessels, as well as a family of other cells (i.e., microglia, perivascular cells, endothelial cells), to support the metabolic demands of the brain (Soto-Rojas et al., 2021). It should not come as a surprise then that vascular

co-pathologies are often intertwined in the development of AD. A highly relevant and important example is the case of cerebral small vessel disease (cSVD).

cSVD is a general term that refers to pathological processes that affect small blood vessels in the brain (i.e., arterioles, capillaries and small veins), leading to reduced and/or interrupted blood flow in affected brain regions (Q. Li et al., 2018). cSVD is a major cause of ischemic stroke and is responsible for 45% of vascular dementia cases (Cannistraro et al., 2019). White matter hyperintensities (WMH) are a neuroimaging hallmark of cSVD that appear hyperintense on fluid attenuated inversion recovery (FLAIR) magnetic resonance imaging (MRI) scans (Q. Li et al., 2018). Importantly, larger WMH volumes are associated with increased AD risk (Kandel et al., 2016). The exact cause of cSVD is not known, however, it is most strongly linked to age and vascular risk factors such as hypertension, atherosclerosis and diabetes (Q. Li et al., 2018). Paradoxically, while men are more likely to experience and die from vascular comorbidites including stroke, women represent the majority of cSVD cases (Connelly et al., 2022; de Leeuw et al., 2001; Nordström et al., 2016; Sachdev et al., 2009). Strikingly, women demonstrate significantly greater WMH volume and faster WMH progression compared to men (Fatemi et al., 2018; van den Heuvel et al., 2004). Together, these findings indicate that vascular-related co-pathologies contribute to sex-specific vulnerability in AD.

1.2.3 Age and sex intersect to elicit sex-specific cognitive decline in AD

Not only are women twice as likely to be diagnosed with AD compared to men, but they also experience faster rates of cognitive deterioration (Ardekani et al., 2016; Fatemi et al., 2018; Mielke et al., 2014; Sachdev et al., 2009). Longitudinal MRI studies have concluded that women demonstrate significantly faster hippocampal atrophy rates compared with men as they age (Ardekani et al., 2016; Hua et al., 2010). Furthermore, females with one or two copies of APOE4

demonstrate more rapid cognitive deterioration compared to APOE4-matched male counterparts (Farrer et al., 1997). These findings indicate that age and sex interact to elicit sex-specific cognitive decline in women, indicating the need for sex- and age-specific interventions.

1.3 Cognitive symptoms of AD dementia

This section provides a detailed overview of the temporal progression of clinical AD in humans and a variety of behavioural paradigms that are used to evaluate cognitive impairment in transgenic AD mouse models containing human APP mutations (Figure 3).

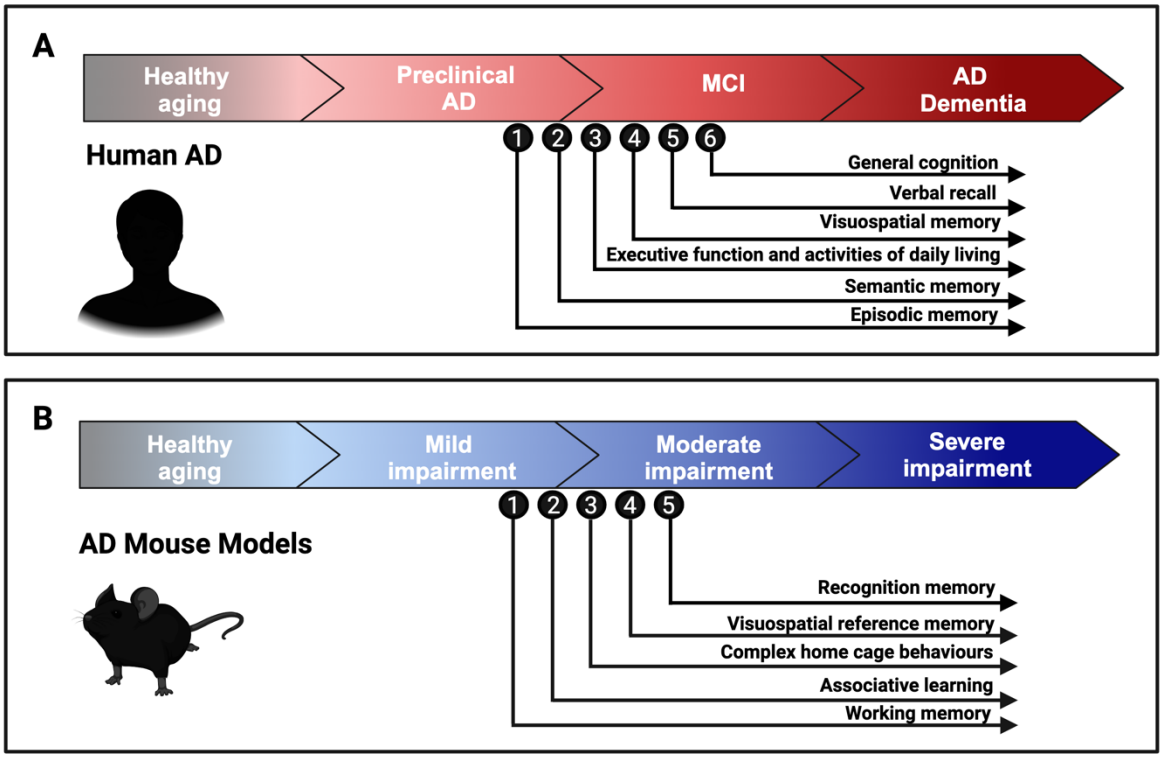

Figure 3 Summary of the temporal progression of cognitive decline in human AD and AD transgenic mouse models. (A) In human AD, episodic memory deficits in late preclinical AD are the first to occur and this is followed by semantic memory deficits. Impairment in executive function and visuospatial function develop next at the beginning of the MCI stage and this is accompanied by difficulties in performing activities of daily living (ADLs) independently. As the MCI stage progresses, deficits in verbal recall and general cognition occur. In AD dementia, all cognitive abilities mentioned are negatively affected. (B) The onset of behavioural indices of cognitive decline in APP transgenic mouse models follow a similar temporal progression. The first impairments observed are that of spatial working memory. This is generally followed by impairments in associative learning and visuospatial reference memory. Recognition memory deficits generally come later than other cognitive abilities. Figure adapted from Webster et. (2014). Created with Biorender.com

1.3.1 Episodic and semantic memory

In humans, the earliest cognitive deficits are those of medial temporal lobe-dependent (MTL) episodic memory during late preclinical AD (Bondi et al., 1999; Collie & Maruff, 2000; Schmitt et al., 2000). The hippocampus and entorhinal cortex are among the earliest brain regions affected by neuropathological changes and this is followed temporally by changes in the MTL (Webster et al., 2014). Episodic memory is a type of long-term, explicit memory that requires the conscious recall of information (Tromp et al., 2015; Tulving, 1972). Episodic memory was first defined by Tulving in 1972 as encoding, storing and retrieving memories related to temporally dated experiences of episodes and/or events (Tulving, 1972). In other words, episodic memory represents the "what, when and where" of personal experiences in a particular spatial-temporal context (Tulving, 1972). Examples of episodic memory in humans include remembering where you parked your bike this morning or what you cooked for a family dinner last month (Tromp et al., 2015). In the MMSE, questions such as orientation to time and place and the 3-word recall task are used to evaluate episodic memory in adults (Carcaillon et al., 2009). Importantly, verbal tasks are predominantly used to assess episodic memory in humans, requiring a core competency of language (Benedict et al., 1998; Coen et al., 1999). Evidently, verbal tests cannot be used to assess episodic-like memory in mice and thus, alternative spatial working memory tests are used such as forced Y-Maze (YM), Morris water maze (MWM) and the What-Where-Which Task (WWWhich) (Davis, Eacott, et al., 2013; Davis, Easton, et al., 2013; Glickman & Jensen, 1961; Morris, 1984).

As AD progresses, deficits in semantic memory follow episodic memory impairment (Storandt et al., 2006; Tuokko et al., 2005). Semantic memory is a type of long-term, explicit memory that relates to the meaning of language (Tulving, 1972). Semantic memory is a "mental thesaurus" that holds knowledge of words and their meanings as well as objects and facts (Hodges & Patterson, 1995; Tulving, 1972). Difficulties expressing language such as word finding are a

common and early manifestation in AD (Klimova et al., 2015). The picture-naming task in the Montreal Cognitive Assessment (MoCA) evaluates semantic function in older adults wherein participants are asked to name animals in the pictures provided (Mahendran et al., 2015). Increased naming errors are common in AD patients, with language impairments deteriorating as the disease progresses (Verma & Howard, 2012). In mice, associative learning is most closely associated to semantic memory in humans and is commonly measured using the active avoidance test (Vanderwolf, 1964; Webster et al., 2014).

1.3.2. Activities of daily living (ADL) and executive function

AD dementia results in a progressive decline in the ability to perform activities of daily living (ADLs) (Chatzikostopoulos et al., 2022; Foloppe et al., 2018; Palacios-Navarro et al., 2022). ADL is a general term used to describe a group of fundamental skills needed to independently care for onself and carry out daily activities (Katz, 1983). In humans, ADLs are categorized into two groups: basic ADLs and instrumental ADLs. Basic ADLs are the skills required to meet basic physical needs, including grooming (i.e., the ability to maintain proper hygiene such as bathing), dressing (i.e., the ability to successfully dress oneself in appropriate clothing), feeding (i.e., the ability to feed oneself independently), ambulation (i.e., the ability to mobilize oneself independently), continence (i.e., the ability to control bladder and bowel movements) and toileting (i.e., the ability to use the washroom appropriately and independently) (Edemekong et al., 2024). In contrast, instrumental ADLs (iADLs) are characterized by more complex behaviours of independent living, such as meal preparation, housekeeping and managing medications (Lawton & Brody, 1969). Carrying out these complex behaviours engages higher executive functions, including planning and organization (Lawton & Brody, 1969). Interestingly, impairment in executive functions in humans are observed after the onset of episodic and semantic memory

deficits, suggesting a temporal progression of neuroanatomical circuitry impairment in AD (Webster et al., 2014). The Erlangen Test of ADLs (E-ADL-Test) and the Wisconsin Sorting Task are often used to assess ADLs and executive functions in humans, respectively (Graessel et al., 2009; Grant & Berg, 2014). In mice, nest building (NB) is a goal-directed, complex homecage behaviour that is analogous to ADLs and executive functions in humans (Jirkof, 2014; Wesson & Wilson, 2011). Another commonly used test of executive functioning in mice is the multiple choice serial reaction time task (CSRTT) (Carli et al., 1983).

1.3.3. Visuospatial memory

Visuospatial memory is defined as the ability to process and retain visual information (i.e., "visuo") that allows a person to identify objects and their location in space (i.e., "spatial") (McAfoose & Baune, 2009). Visuospatial memory impairment becomes evident in AD clinical progression during the MCI stage (Webster et al., 2014). Individuals in this stage commonly demonstrate disturbances in depth perception (e.g., frequent falls, difficulty carrying out ADLs such as getting into a bathtub), increased tendency to get lost, as well as difficulty recognizing faces and locating objects (Quental et al., 2009). Accordingly, many cognitive tests evaluate visuospatial function. For example, the CDT can help to identify visuospatial impairment that commonly manifests as putting numbers exclusively on one side of the clock and/or outside of the clock (Figure 1) (Adunsky et al., 2002). Other common visuospatial tasks evaluated in humans on the MMSE is the ability of an individual to copy a complex shape, or recognize a common object (e.g., pencil) (Folstein et al., 1975). In mice, visuospatial memory is commonly measured in the MWM test, wherein mice must learn to navigate and remember the location a hidden platform using spatial cues (Morris, 1984; Webster et al., 2014).

1.3 Behavioural and psychological symptoms of dementia (BPSD)

Clinical AD dementia is associated with a group of non-cognitive, neuropsychiatric symptoms collectively known as behavioural and psychological symptoms of dementia (BPSD) (Cerejeira et al., 2012). BPSD is a general term used to describe highly disruptive, non-cognitive behaviours that commonly manifest in persons with dementia (Cerejeira et al., 2012). These symptoms include sleep-wake cycle disruption, appetite changes, disturbances in motoric activity and anxiety as well as repetitive, perseverative behaviours (Bayles et al., 2004; Cerejeira et al., 2012). BPSD strongly correlates with progressive cognitive decline in dementia regardless of dementia subtype and increases in severity with AD progression (Cerejeira et al., 2012; Pless et al., 2023). In fact, approximately 90% of individuals diagnosed with dementia are affected by BPSD (Cerejeira et al., 2012), which necessitates its characterization alongside cognitive assessment in preclinical AD mouse models.

1.3.1. Sleep-wake cycle disruption

Sleep disturbances are a highly prevalent and disruptive BPSD. In fact, over 40% of AD patients experience sleep disturbances (McCurry et al., 2000; Pistacchi et al., 2014). Clinical manifestations of sleep dysfunction in AD include excessive daytime napping, difficulty falling asleep, fragmented sleep, and/or early morning awakenings (Carpenter et al., 1996; Lee et al., 2007). It is well known that Aß accumulation occurs in brain regions important for memory consolidation (e.g., hippocampus) and that these neuropathological changes occur decades before cognitive symptom onset (Squire et al., 2015; Tarawneh & Holtzman, 2012). Additional evidence supports that sleep, specifically entering different sleep stages, is important for hippocampal-dependent memory (Dufort-Gervais et al., 2019). For example, a study conducted by Diekelmann

and Born (2010) found that slow wave oscillations in non-rapid eye movement (NREM) sleep are vital for the consolidation of memories in spatial learning (Diekelmann & Born, 2010). In fact, NREM sleep deprivation in rats leads to deficits in hippocampal-dependent, but not hippocampal-independent, memory systems (Chen et al., 2014). These findings suggest that sleep is important for hippocampal-dependent memory and that disturbances in sleep may worsen memory impairment in AD.

Transgenic AD mouse models that exhibit Aß accumulation in the hippocampus and cerebral cortex demonstrate increased wakefulness. For example, a study conducted by Colby-Milley et al. (2015) showed that TgCRND8 mice display increased wakefulness and reduced NREM sleep during both active and inactive periods. Increased wakefulness is characteristic of healthy aging in older adults, however, it is significantly more pronounced in the context of AD (Carrier et al., 1997; Van Erum et al., 2019). AD clinical progression severity is associated with significant reductions in NREM and REM sleep duration, suggesting that sleep disruption may contribute to memory decline in AD by affecting the consolidation of newly formed memories (Bombois et al., 2010; Moe et al., 1995).

Another important aspect of sleep disturbance in AD is sleep fragmentation, also known as interrupted sleep. Sleep fragmentation is defined as shorter and more frequent episodes of sleep and is contrary to consolidated wakefulness, which is characterized by longer and less frequent bouts of sleep (Dufort-Gervais et al., 2019). Fragmented sleep patterns, such as frequent nighttime awakenings and/or excessive daytime naps, are common manifestations in human AD and have been characterized in some preclinical AD mouse models (Carpenter et al., 1996; Moe et al., 1995). For example, 5xFAD mice, which express five human fAD mutations, demonstrate significantly reduced and shorter bouts of sleep (Sethi et al., 2015). Characterization of fragmented sleep

patterns in preclinical AD mouse models can help to elucidate the temporal relationship between Aß deposition, cognitive decline and sleep disruption in humans.

1.3.2 Changes in eating behaviour

Eating disturbance in AD can be categorized into two groups: quantitative food intake (e.g., anorexia or hyperphagia) and dietary preferences (Cerejeira et al., 2012). Clinical manifestations of eating disturbance varies with AD dementia severity (Fostinelli et al., 2020). In moderate AD dementia, loss of appetite and preferences for high-fat, sweet foods are particularly common (Mungas et al., 1990). As individuals progress from moderate to severe AD dementia, swallowing impairments become prominent and pose a significant risk for malnutrition and dehydration (Easterling & Robbins, 2008). In fact, one study shows that up to 68% of patients with severe AD dementia are malnourished and/or are at risk of malnutrition (Droogsma et al., 2013; Gillioz et al., 2009).

Nutritional status has been the primary explanation for the unintentional weight loss observed in AD patients (Gillette-Guyonnet et al., 2000). However, this explanation is insufficient to explain weight loss observed in preclinical AD when energy intake is sufficient (Jimenez et al., 2017). It is becoming increasingly recognized that AD is a metabolic disorder (Gupta et al., 2023; Kang et al., 2017). AD dementia severity has been linked to weight loss risk in AD and this phenomenon has been demonstrated in transgenic AD mouse models (Gillette-Guyonnet et al., 2000; Knight et al., 2012; Naia et al., 2023). For example, 3xTgAD mice switch to a hypermetabolic state as they age, which is accompanied by subsequent weight loss despite significantly higher food intake (Knight et al., 2012). These findings indicate that the cause of weight loss in AD is multifactorial and has yet to be fully elucidated.

1.3.3 Disturbances in motoric activity, sundown syndrome and circadian rhythm

Disturbance in motoric activity in AD consists broadly of *motoric hypoactivity* and *motoric hyperactivity*. It is important to note that disturbance in motoric activity in AD does not necessarily imply the presence of motoric abnormalities (Cerejeira et al., 2012). Rather, *motoric hypoactivity* consists of slowness of movement and/or speech and a reduction in the number of spontaneous physical movements (Cerejeira et al., 2012). Spontaneous movements are defined as movements performed in the absence of premeditation or planning (Cerejeira et al., 2012). In contrast, *motoric hyperactivity* is described behaviourally as an increase in the number of spontaneous movements and/or rapid speech (Cerejeira et al., 2012). Perseverations in speech are common in AD, which are defined as the inappropriate repetitions of a portion of a complete previous response (Bayles et al., 2004). Further, motoric hyperactivity, or restlessness, manifests in AD as increases in pacing, wandering, aggression and/or agitation (Khachiyants et al., 2011). When these disruptive, neuropsychiatric behaviours occur at a particular time of day (i.e., in the late afternoon, evening or night), they are collectively known as "sundown syndrome" or "sundowning" (Khachiyants et al., 2011).

Circadian rhythm disturbance is an important predictor of sundown syndrome (Warfield et al., 2023). Circadian rhythm is defined an endogenous 24-hour biological clock that persists in the absence of exogenous cues (e.g., light-dark cycles) (Vitaterna et al., 2001). While circadian rhythm is not driven internally by environmental or exogenous cues, if such cues exist circadian rhythms align to them through a process called entrainment (Vitaterna et al., 2001). AD patients demonstrate circadian phase delays (e.g., later bathyphases or troughs and acrophases or peaks) in biological processes such as body temperature and locomotor activity (Harper et al., 2005; Sheehan & Musiek, 2020; J. L. Wang et al., 2015). Healthy circadian rhythmicity of body temperature, for example, is critical for sleep. Body temperature declines rapidly in the evening to initiate sleep

onset and promote entry into deep sleep, while an increase in body temperature promotes wakefulness (Kräuchi et al., 1999; Murphy & Campbell, 1997).

At the neuroanatomical level, circadian rhythm is regulated by a light-dark entrained brain structure in the hypothalamus called the suprachiasmatic nucleus (SCN) (Reppert & Weaver, 2002). The SCN projects to the subparaventricular zone (SPZ) and, together, these structures regulate body temperature, locomotor activity, appetite, levels of corticosterone, and surprisingly, aggression (Lu et al., 2001; Saper, 2013; Todd et al., 2018; Watts et al., 1987; Watts & Swanson, 1987). Remarkably, while a loss of vasoactive intestinal peptide (VIP)-expressing SCN neurons (SCN^{VIP}) is found in age-related circadian changes, AD patients do not exhibit a more profound loss of SCN^{VIP} neurons (Swaab et al., 1985; J. L. Wang et al., 2015). These findings suggest that early hypothalamic dysfunction in circadian brain circuitry likely plays a role early in AD and underlies sundown syndrome (Warfield et al., 2023).

1.3.4 Alterations in anxiety

Anxiety is a common mental illness worldwide, affecting approximately 4% of the global population (World Health Organization, 2023). Anxiety is characterized by feelings of intense worry or fear in anticipation of a threat, whether it be real or perceived (Chand & Marwaha, 2024). Scientific evidence shows that anxiety may be an early neuropsychiatric manifestation of preclinical AD (Seignourel et al., 2008). Indeed, higher Aß brain burden has been linked to anxiety and depressive symptoms in cognitively healthy adults (Donovan et al., 2018). Another study found that, in preclinical AD, an increase in the severity of anxiety symptoms was associated with more rapid Aß-moderated cognitive decline (Pietrzak et al., 2015). These findings indicate that neuropsychiatric symptoms likely play a role in preclinical AD and necessitates further characterization in preclinical AD mouse models.

1.4 The N5 TgCRND8 is a validated mouse model of human Aß deposition and sex-specific cognitive decline

APP mutations are a genetic determinant of fAD, occurring in 10-15% of fAD cases (Bi et al., 2019). While only a subset of fAD patients express mutant APP, the majority of this subset show the defining neuropathological hallmarks of AD (Bi et al., 2019). The study and characterization of transgenic AD mouse models expressing human mutant APP may help to elucidate potentially common or intersecting pathomechanisms underlying AD progression in sAD cases.

1.4.1 N5 TgCRND8 female and male mice demonstrate comparable Aß burden, but exhibit sexual dimorphism in behavioural indices of cognitive decline

While there are no naturally occurring rodent forms of AD, a validated TgCRND8 mouse model of Aß deposition has been created (Chisti et al. 2001). TgCRND8 mice overexpress a double mutant form of APP695 with both Swedish (KM670/671NL) and Indiana (V717F) mutations at five-fold higher levels compared to endogenous murine APP (Chishti et al., 2001). Subsequently, TgCRND8 mice develop Aß plaques in the brain by 3 months of age and exhibit significantly higher Aß42 brain levels compared to Aß40, which is a critical marker of AD progression in humans (Chishti et al., 2001).

A novel TgCRND8 mouse model, N5 TgCRND8, was generated in Dr. Bennett's Neurolipidomics laboratory by backcrossing F1 TgCRND8 C57B1/6 x C3H/HeJ hybrid mice for 5 generations (N5) to wild-type C57B1/6 mice (Granger et al., 2016). At 6 months of age, N5 TgCRND8 mice demonstrate sexual dimorphic differences in behavioural indices of cognitive reserve despite comparable Aß burden (Granger et al., 2016). Male N5 TgCRND8 mice, but not

female N5 TgCRND8 mice, are able to overcome Aß burden and transition to spatial (productive) search strategy at 6 months of age, while females fail to make this transition (Granger et al., 2016). Rather, N5 TgCRND8 females rely on a nonproductive repetitive looping search strategy at 6 months of age which is closely related to perseveratory behaviours in humans (Granger et al., 2016). These findings indicate that the N5 TgCRND8 mouse model may help to elucidate sex-specific mechanisms in preclinical AD mouse models and more closely reflect the sexual dimorphisms observed in human AD.

1.4.2. It is unknown how age and sex interact to elicit cognitive decline in N5 TgCRND8 mice

There are many behavioural and biochemical aspects of the N5 TgCRND8 mouse model that have yet to be characterized, highlighting the importance of my thesis. To date, it is not known how behavioural indices of cognitive decline (e.g., spatial working memory, visuospatial learning and memory) change as N5 TgCRND8 mice age from birth to death (i.e., 1-12 months of age) and the subsequent relationship between cognition and Aß40/42 levels in the brain at these time points. Importantly, behavioural indices of ADLs (i.e., nesting, sleeping, eating and drinking behaviours) and BPSD (i.e., motoric activity, anxiety, perseveration) have yet to be characterized and tracked longitudinally from birth to death (i.e., 1-12 months of age) in N5 TgCRND8 mice. These behaviours are particularly important to characterize in the N5 TgCRND8 mouse model, as in human AD, many of them arise during the preclinical stage and potentially serve as indicators of early underlying neuropathologies (e.g., sleep disturbance and hypothalamic circadian dysfunction). Finally, it is unknown how age and sex interact longitudinally with Aß burden in the brain to elicit cognitive decline, impairment in ADLs and the onset of BPSD in N5 TgCRND8 mice.

1.5 Objectives and hypotheses

The overarching objective of this thesis was to elucidate how age and sex interact with Aß load to alter behavioural indices of different cognitive domains (ADL analogs, hyperarousal, anxiety, reference memory, visuospatial memory) in the N5 TgCRND8 mouse model of AD. I hypothesized that female and male mice with comparable Aß burden would exhibit a different time course of behavioural impairment that could be defined for subsequent sex-specific preclinical intervention.

To test these hypotheses, I proposed two specific aims:

(1) Longitudinally track behavioural indices of ADLs (i.e., nesting, sleeping, eating, drinking), BPSD (i.e., motoric activity, anxiety, perseveration) and cognition (i.e., spatial working memory, visuospatial learning and memory, cognitive reserve) in N5 TgCRND8 mice from birth to death (i.e., 1-12 months of age).

(2) Longitudinally quantify Aß40 and Aß42 levels in the temporal cortex and hippocampus of N5 TgCRND8 mice from birth to death (i.e., 1-12 months of age).

2 MATERIALS AND METHODS

2.1 Animal Experimentation

F1 TgCRND8 C57B1/6 x C3H/HeJ hybrid mice were backcrossed for 5 generations (N5) to wild-type C57B1/6 mice in Dr. Bennett's Neurolipidomics Laboratory. All mice were genotyped for the human AßPP transgene (See Table 1).

Table 1 Transgenic (TgCRND8) Genotyping Protocol

Gene	***Reaction Conditions***	***Cycling & Amplicon sizes***
TgCRND8	TgCRND8F: 5'-3' GGC CGC GGA GAA ATG AAG AAA CGC CAA GCG CCG TGA CT TgCRND8R: 5'-3' TGT CCA AGA TGC AGC AGA ACG GCT ACG AAA A Primer concentrations: 10 pmol/ul	94 °C 3 min 35 cycles 94°C 20 s 68°C 20 s 72°C 90 s 72°C 7 min (Tg ~1000bp)

2.2 Nest building (NB)

Nest building (NB) is a complex homecage that was assessed on female and male N5 TgCRND8 and NonTg mice longitudinally every month from 1-12 monts of age. Between 3:00-5:00 PM (prior to the beginning of the active period), standard mouse cages were collected from the animal facility. Water and food were available *ad* libitum. Individual cages were prepared by placing a single nestlet (5 cm x 5 cm) at the bottom of each cage. No additional materials were placed in the cage for the duration of the test. Prior to starting the test, two 'before' pictures of the cage were taken from two different angles (i.e., birdseye view, side view) (Fig. 4). The mice were then placed in their respective cages overnight until the following day between 8:00 and 10:00 AM. At this time, the mice were removed from the cages carefully as to not disturb the nest and were subsequently placed in a fresh cage. Two 'after' pictures were taken of the cage from the same two angles (i.e., birds eye view and side view) (Fig. 4). The after pictures were then scored by three independent scorers blinded to the genotype and sex of the mouse using a standardized scoring sheet (Table 1; Deacon, 2012). The mode of the three scores was taken as the final score for each nest. In cases where a consensus between the three scorers was not reached, a fourth scorer was included and the mode was determined after the addition of that score.

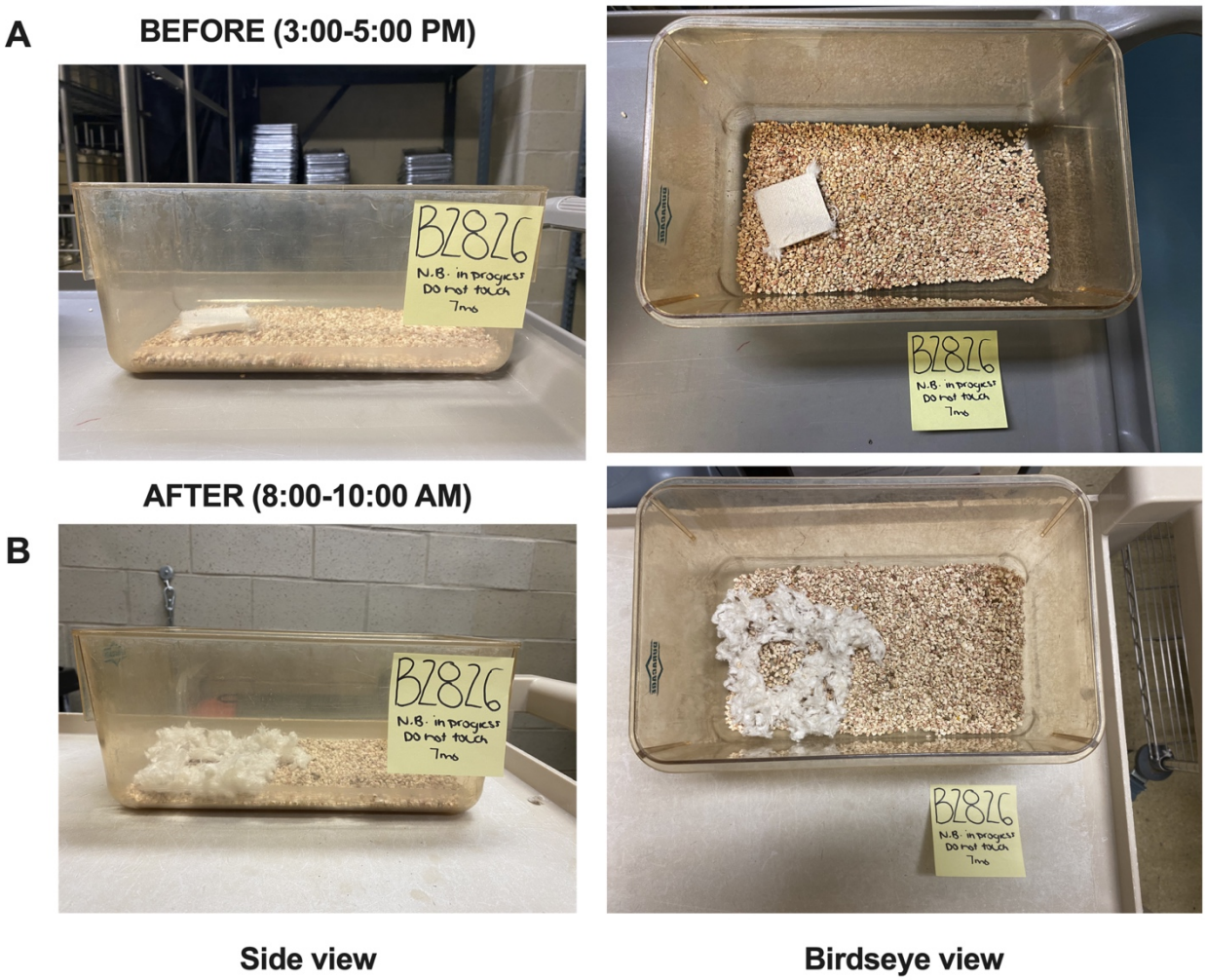

Figure 4 NB pictures taken before and after the test. (A) Between 3:00-5:00 PM prior to the start of the test, photos of the untouched nestlet are taken from the side view and birds eye view. (B) Between 8:00-10:00 AM once the test is completed, photos of the constructed nest are taken from the side view and birds eye view.

Table 2 NB Scoring system

NB Score	Scoring criteria
1	Nestlet not noticeably touched (> 90%) intact
2	Nestlet partially torn up (50-90%) intact
3	Nestlet mostly shredded; not identifiable nest site: <50% intact, <90% within a quarter of cage floor area
4	An identifiable, flat nest: >90% of nestlet is torn up, material gathered into a nest within a quarter of the cage floor area. Nest is flat. Nest walls are higher than the mouse body height (curled up on its side)
5	A (near) perfect nest: >90% of nestlet is torn up, nest is a crater with walls higher than the mouse body height on more than 50% of the circumference.

(Deacon, 2012)

2.3 PhenoTyper (PT)

Noldus PhenoTyper (PT) is an instrumented, home cage-like observation cage designed to track animal behaviours (Grieco et al., 2021). Each individual PT cage is equipped with an overhead infrared camera that was programmed to record duration and transits through three designated zones: the shelter zone, the feeding zone and the drinking zone (Fig. 5). PT was performed on N5 TgCRND8 mice and NonTg mice at 2, 4, 6, 8 and 12 months of age. At the beginning of the test, mice were placed in their respective PT cage and a maximum of eight animals were tested per run. Each test run lasted 29 hours in duration. The first five hours of the video recording is defined as the habituation period and was excluded from the analysis. Thus, mouse behaviours were analyzed over a complete sleep-wake cycle, or 24-hour Zeitgeber (ZT) period with ZT 0 defined as the lights ON cue and ZT 12 as the lights OFF cue. Homecage behaviours in PhenoTyper were evaluated over four defined ZT periods: (1) transition to the active period (ZT 11-12), (2) active period (ZT 13-22), (3) transition to the inactive period (ZT 23-0) and (4) inactive period (ZT 1-10). Data were acquired and video recordings were analyzed using Ethovision Version 17.0 software.

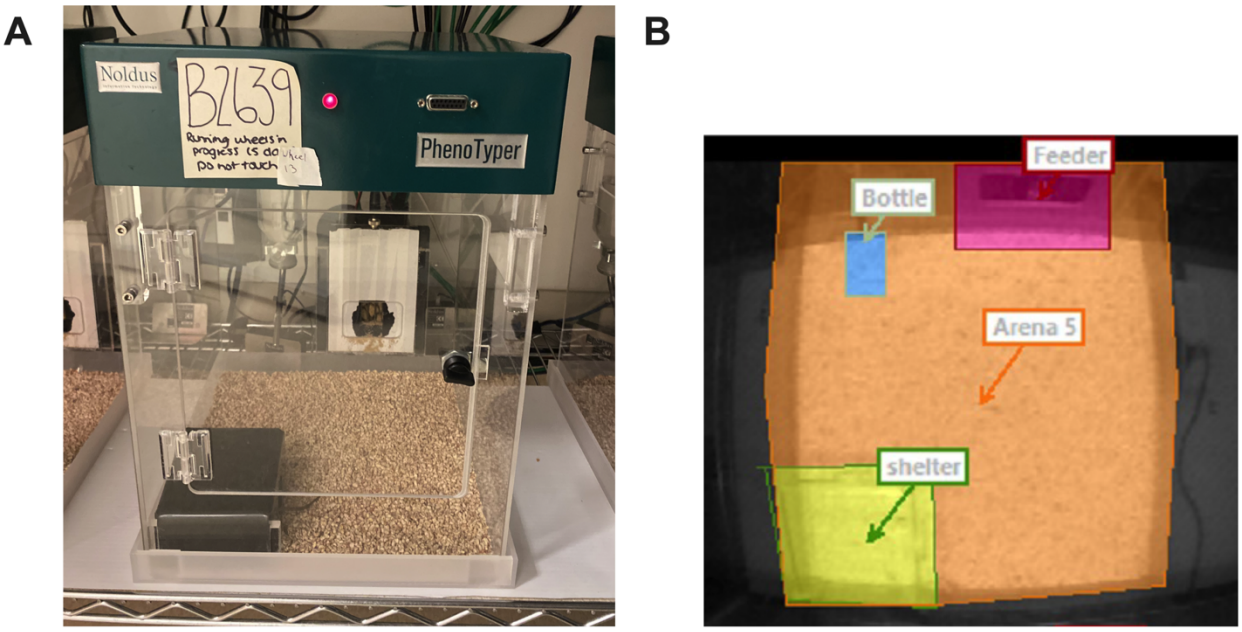

Figure 5 Experimental setup of the PT test. (A) Picture depicts experimental setup of the PT observation cage. (B) Schematic depicts overhead view of the Phenotyper observation cage. Animal behaviour was assessed in the following three zones: the shelter zone (yellow), the feeder zone (pink) and the bottle zone (blue).

2.4 Running wheels (RW)

Running wheels (RW) enable measurement of voluntary physical activity in mice. Animals were provided with a wireless running wheel (radius: 6.0198 cm, circumference: 37.82 cm) for five consecutive days (Fig. 6). Prior to the beginning of the test, clean standard mouse cages are collected. Food and water were available *ad libitum*. The distance each mouse travelled on the running wheel in kilometers was recorded for five consecutive days using Wheels Manager Software (Med associates inc.). The average distance in kilometres was calculated per hourly ZT interval and per four defined ZT periods: (1) transition to the active period (ZT 11-12), (2) active period (ZT 13-22), (3) transition to the inactive period (ZT 23-0) and (4) inactive period (ZT 1-10).

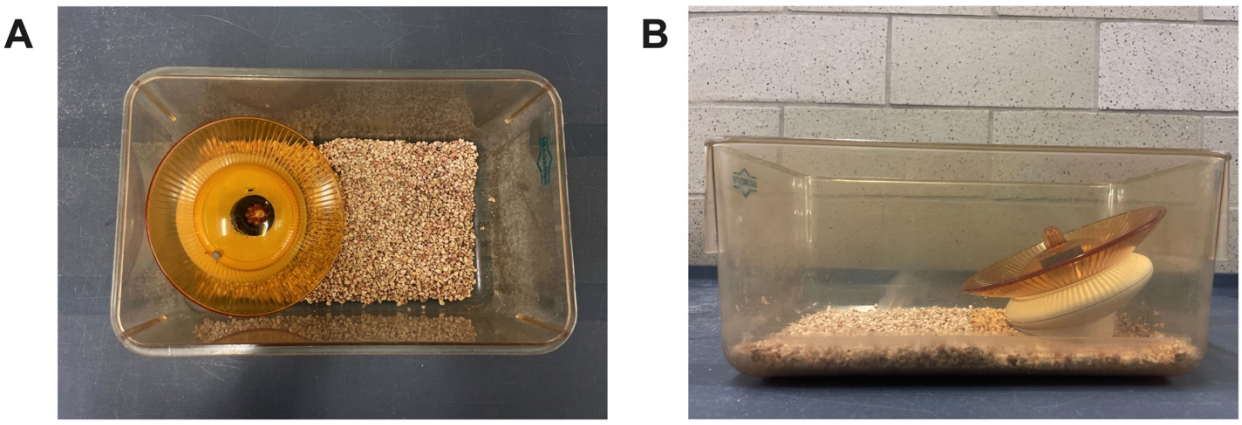

Figure 6 Experimental setup of the RW test. Schematic depicts running wheel placed in individual homecage prior to the start of the test from a birds eye view (A) and side view (B).

2.5 Forced Y-maze (YM)

Forced Y-maze (YM) is a behavioural measure of visuospatial working memory, motoric activity and can be used as a supplemental measure of anxiety in mice when combined with other tests. YM was performed on N5 TgCRND8 and NonTg mice from 2-12 months of age. In the YM paradigm, mice are tested across two, five-minute trials that are separated by a home cage period of thirty minutes. The maze is black plastic with white floor inserts (Fig. 7). It has three equal arms (38 cm long, 7.6 cm wide) that are spaced 120 degrees apart from eachother to create a Y shape (Fig. 7). Visual cues are placed both on the walls of the testing room and at the ends of arm 2 and 3 to help the mouse distinguish between the otherwise identical arms (Fig. 7). Mice were habituated in a quiet room for 30 minutes before placed in the maze. During the first trial, arm 2 or arm 3 is blocked using a black plastic insert. The blocked arm is also called the novel arm, which alternates between mice. During the first trial the mouse is placed in the entry arm (arm 1) and was allowed to freely explore the unblocked arm for five minutes. The unblocked arm is also known as the familiar arm and also alternates between mice. After five minutes, the mouse was removed from the maze and placed back in its homecage for thirty minutes. After thirty minutes, the mouse was placed back in the maze with the novel arm unblocked, where it was able to freely explore both the familiar and novel arms that are distinguished by visual vues. Behaviour was recorded and and movements tracked using Ethovision v17.0 (Noldus) software.

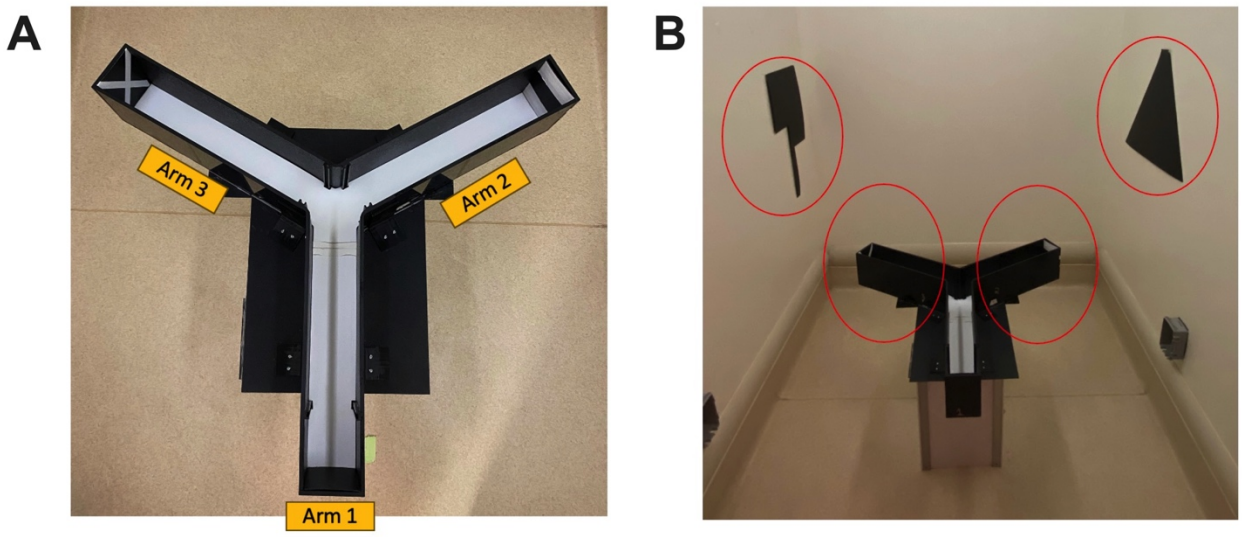

Figure 7 Experimental setup of the YM test. (A) The Y-maze has three arms equal in length and white floors. Arm 1 is the entry arm and Arms 2/3 are the novel and familiar arms that alternate between mice. (B) Arm 2 and arm 3 are distinguished by visual cues placed at the end of the arms and on the walls of the testing room.

2.6 Morris water maze (MWM)

Morris water maze (MWM) is a measure of visuospatial learning and reference memory in mice. MWM testing was performed on N5 TgCRND8 and NonTg mice from 2-12 months of age. In the MWM paradigm, the mouse learns to find a platform hidden 1 cm below the water surface using external visual cues that are placed on the walls of the testing room (Fig 8. A). Overhead white light was set to 140 lux across all testing days. Two visual cues, a black "X" and a square, were placed on the front and left walls of the testing room, respectively (Fig 8. A). The mice were habituated in the testing room for 30-60 minutes. The MWM paradigm was performed as a nine day protocol, consisting of eight training days and one probe day. The pool is 32 cm in diameter and contained tap water at 22 °C made opaque by the addition of a small quantity of white, non-toxic, tempera paint. The hidden platform (10 cm in diameter) was located 24 cm from the pool edge.

At the beginning of each of the 4 trials per day, the mouse was placed randomly in one of four starting quadrants and was given up to 60 seconds to find the hidden platform (Fig 8. B).In cases where the mouse failed to find and escape onto the hidden platform independently, the mouse was guided to the platform and ensured it remained on the platform for 3 seconds before removing the mouse from the pool. Each mouse performed four trials per day for eight days. On the ninth day, also known as the probe day, the platform was removed and the amount of time the mouse spent in the quadrant of the pool where the platform used to be is measured in order to assess spatial learning and memory.

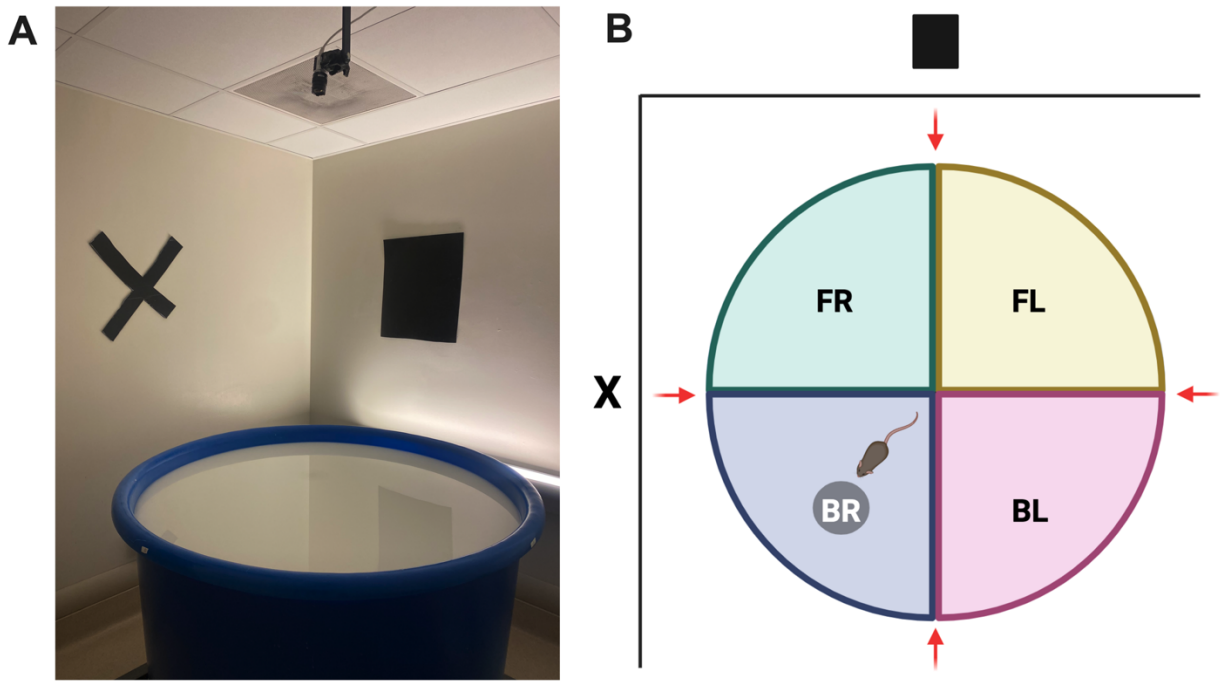

Figure 8 Experimental setup of the MWM test. In the MWM test, two visual cues are placed on the walls of the testing room. The animal's movements navigating the MWM platform are recorded using an overhead Ethovision (Noldus) camera. (B) The mouse is placed randomly in one of four starting quadrants (red arrows) and is given up to 60 seconds to find the hidden platform. Figure (B) Created with BioRender.com

2.7 Quantification of human Aß40 and Aß42 peptides

N5 TgCRND8 mice and NonTg mice were euthanized at 2, 4, 6, 8 and 12 months of age using euthanyl diluted in sterile water to a final concentration of 65 mg/ml. The right temporal cortex and right hippocampus of each animal was dissected, weighed and flash-frozen in liquid nitrogen. Tissue was prepared as per human Aß40 and human Aß42 ELISA kit instructions (human Aß40: Invitrogen #KHB3482; human Aß42: Invitrogen #KHB3442). Briefly, temporal cortex and hippocampus were homogenized together in 5 M guanidine-HCl diluted in 50 mM Tris, pH 8.0. Brain lysates were frozen and stored at -80°C for further use. Human Aß40 and human Aß42 standards were prepared as per ELISA kit instructions (human Aß40: Invitrogen #KHB3482; human Aß42: Invitrogen #KHB3442). Immediately prior to performing the ELISA, samples were pre-diluted to the optimized, age-dependent dilution with 50% Standard Diluent Buffer from the ELISA kit and 50% Delbecco's PBS, 10% BSA, 0.06% Tween and 1X protease inhibitor cocktail (see Table 2). Each sample was run in duplicate (Coefficient of variation, CV ≤ 20%). Data are reported as pg Aß peptide per mg of tissue wet weight.

Table 3 Optimized Human Aß40 and Aß42 ELISA Sample Dilutions

Age (months)	Aß40 ELISA dilution	Aß42 ELISA dilution
2	1:50	1:250
4	1:500	1:2000
6	1:8000	1:16000
8	1:16000	1:16000
12	1:32000	1:64000

2.8 Statistics

Data were analyzed using GraphPad Prism Version 10.0 software, where $p < 0.05$ were considered significant. Data were presented as the mean ± standard error of the mean (SEM). Groups of two (i.e., NonTg vs Tg collapsed across age and sex) were analyzed using an unpaired t-test. Groups of three (i.e., Tg or sex longitudinal comparisons) were analyzed using a two-way mixed ANOVA model followed by Holm-Sidak post hoc analysis as indicated in all figure captions. A repeated measures two-way ANOVA was used for escape latency and percent strategy across eight training days in MWM and distance ran across five days in RW. A one sample t and Wilcoxon test was used for percent probe during the probe day in MWM. Natural survival of different sexes and genotypes was compared using Mantel-Cox logrank tests.

3 RESULTS

3.1 N5 TgCRND8 mice exhibit age- and sex-specific behavioural impairment in the ability to perform nest building (NB)

Nest building (NB) was assessed in N5 TgCRND8 mice and NonTg mice each month from birth to death (i.e., 1-12 months of age). The first level of analysis performed sought to determine whether N5 TgCRND8 mice and NonTg mice demonstrated differences in the ability to construct nests (Fig 9. A). N5 TgCRND8 mice demonstrated significant impairment in NB score collapsed across age (Fig 9. B). Data were then disaggregated by age. N5 TgCRND8 mice demonstrated significant impairment in NB at all ages (Fig 9. C).

The final level of analysis sought to determine whether age and sex intersected to elicit NB score impairment in N5 TgCRND8 mice. Thus, NB scores were further disaggregated by sex (Fig 9. D-G). In the absence of human mutant APP, there was no sex difference in the ability to construct a nest (Fig 1. D). N5 TgCRND8 female mice demonstrated significant NB impairment at all ages tested compared to sex-matched NonTg mice (Fig 9. E). In contrast, N5 TgCRND8 male mice demonstrated initial NB impairment at early timepoints (1, 2, 4 months of age), no significant impairment between 5 and 8 months of age, with impairment re-emerging between 9 and 10 months of age compared to NonTg male mice (Fig 9. F). N5 TgCRND8 female mice demonstrated more severe NB impairment compared to N5 TgCRND8 male mice (Fig 9. G).

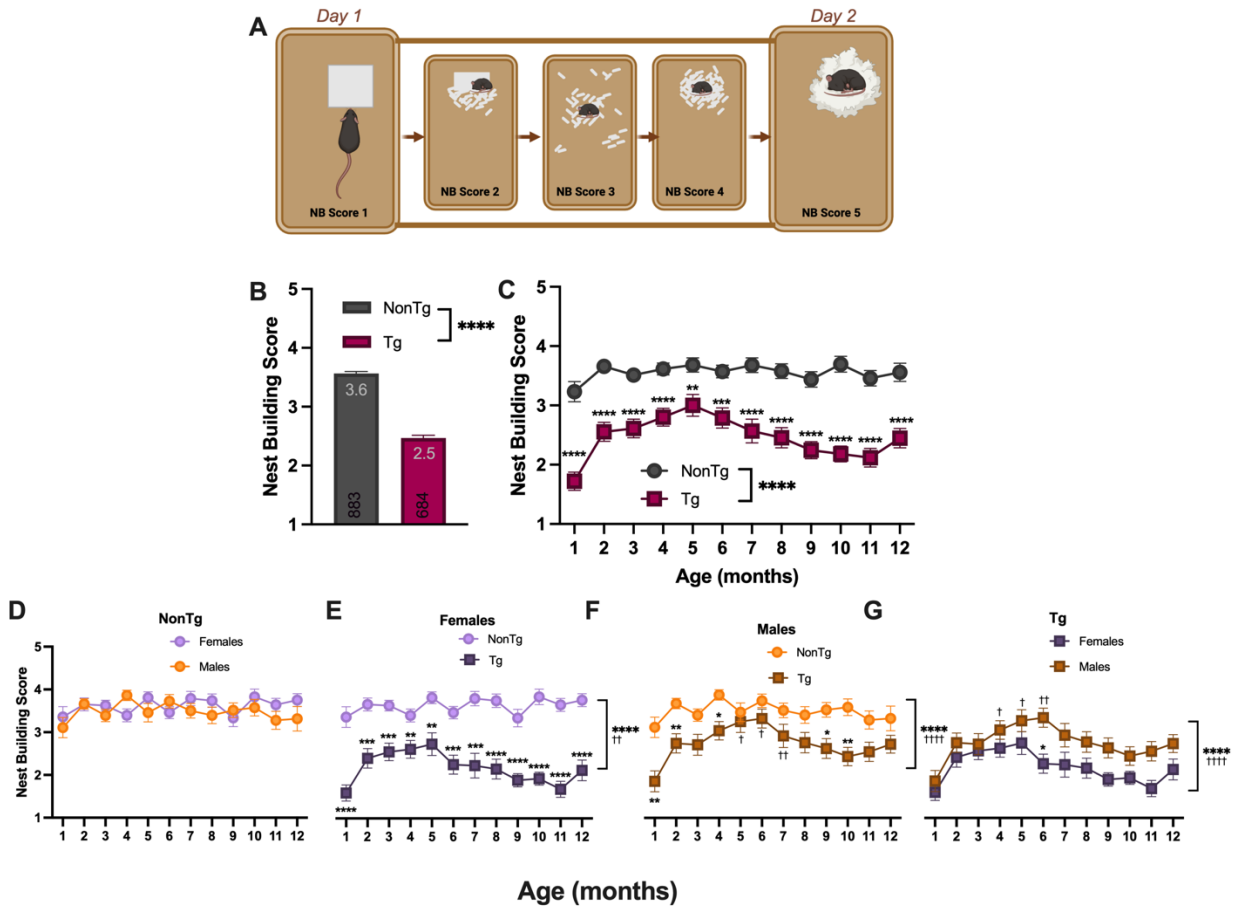

Figure 9 Male and female N5 TgCRND8 mice exhibit differences in nestbuilding (NB), a complex home cage behaviour. (A) Schematic depicts experimental assessment of nest building wherein a mouse was provided with a nestlet during their active period and nest quality was scored at the start of the inactive period. Nests were scored by three independent scorers and the mode score was taken as the final score. (B) N5 TgCRND8 mice (1-12 months of age) mice have significantly lower NB scores compared to NonTg mice. (C) N5 TgCRND8 mice demonstrate significantly lower NB scores compared to NonTg mice at all ages. Data were disaggregated by sex and age. (D) NonTg females and males show no inherent differences in NB as they age. (E) NB is significantly impaired in female Tg mice at all ages. (F) NB is impaired in young male TG mice, comparable to NonTGs between 5 and 8 months of age, then significantly declines in older mice. (G) Female Tg mice are significantly more impaired than male Tg mice. Data represent mean ± SEM of a total 1567 NB tests (NonTg Females N=468; NonTg Males N = 415; Tg Females: N = 353; Tg Males N = 331). Statistics were (B) unpaired Student's *t*-test (B) or (C-G) two-way mixed-model ANOVA with *post hoc* Šidák test comparing the main effect of sex or genotype as indicated (*p<0.05, **p<0.01,***p<0.001,****p<0.0001).

3.2 N5 TgCRND8 mice exhibit sex- and age-specific resting disturbances

Behavioural indices of sleep were characterized in NonTg and Tg mice from 2-12 months of age. Here, I refer to behavioural indices of sleep in mice as resting. Resting behaviour was defined as the duration of time the mouse spent in the shelter zone (Fig 10. A). Two resting measures were evaluated: (1) the duration of time the mouse spends in the shelter zone per hour; and (2) the number of shelter zone transits (i.e., entries and exits) the mouse performed per hour (Fig 10. A). Mice were placed in individual PT cages and resting behaviours were tracked over a complete sleep-wake cycle, or 24-hour Zeitgeber (ZT) period (Fig 10. A).

The first level of analysis performed sought to determine whether NonTg and Tg mice demonstrated differences in shelter zone duration and the number of shelter zone transits across ZT, independently of age and sex. During the active period, Tg mice demonstrated both a significant decrease in the duration of time spent in the shelter zone (ZT 13, 15-18, 20) and an increase in the number of shelter zone transits (ZT 13-18) (Fig. 2 B-C), indicative of increased restlessness during the active (but not inactive period).

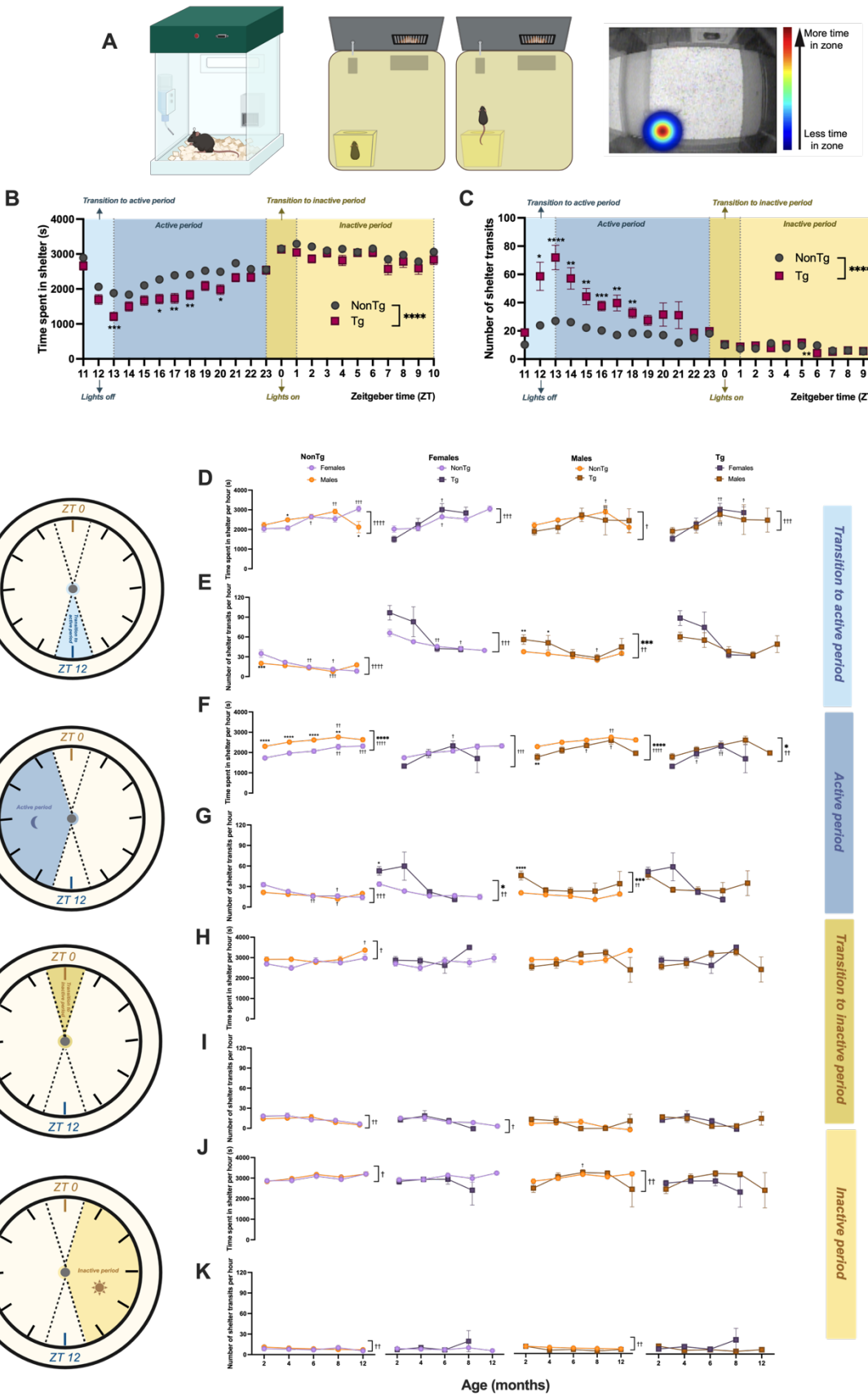
A
More time in zone
Less time in zone
B
Transition to active period
Transition to inactive period
Active period
Inactive period
Time spent in shelter (s)
NonTg
Tg
Lights off
Lights on
Zeitgeber time (ZT)
C
Number of shelter transits
D
E
F
G
H
I
J
K
NonTg
Females
Males
Tg
ZT 0
ZT 12
Active period
Inactive period
Transition to active period
Active period
Transition to inactive period
Inactive period
Age (months)

Figure 10 Females and males rest more in their active period and sleep more in their inactive period as they age; however, young Tg male and female mice are more restless in their active period than their NonTg littermates. (A) Schematics depict the experimental assessment of mice from 2-12 months of age. The duration of time mice spent in and the number of transits made to the PhenoTyper sleep shelter were quantified over 24 hours, considering the zeitgeber cues that signal transition to the active (lights off ZT 12) and inactive periods (lights on ZT 0) (left panel). A representative activity heatmap of a 4-month-old female NonTg mouse at ZT 8 is shown where the mouse is seen exclusively in the sleep shelter (right panel). (B) N5 TgCRND8 mice (age 2 to 12 months) rest less than NonTg mice during their active period (ZT 13-22) but do not show any sleep disruptions during their inactive period (ZT 1-10). (C) N5 TgCRND8 mice move in and out of the sleep shelter earlier when transitioning from the inactive to active period (ZT 11-12) and more frequently during their active period than their NonTg littermates. To interrogate when this restlessness first manifests, data were disaggregated by age and sex. (D-G) Females and males, as they age regardless of genotype, spend more time resting in the sleep shelter and less time moving in and out of the shelter zone both during transition to the active period (D,E) and during their active period (F,G). NonTg females are significantly less restless than males during the active period, spending more time in the shelter at 2,4,6, and 8-months of age (F). Young Tg males and females make significantly more shelter transits at 2-months of age during their active period compared to NonTgs (G). No difference is seen in older mice. (H-K) No genotype-related sleep disruptions were observed. Females and males as they age, regardless of genotype, spend more time sleeping and less time moving in and out of the shelter both during the transition to the inactive period and in their inactive period. Data represent mean + SEM of 201 NonTg mice (Females: n=94; Males N=107). Statistics were two-way repeated measures ANOVA with (A,B) post hoc Šidák test or (D-K) Holm-Šidák test comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.0001) or age †p<0.05, ††p<0.01, †††p<0.001, ††††p<0.0001 vs. 2 months of age). N=13-22 mice per sex (2 months of age). N=9-24 mice per sex (4 months of age). N=6-24 mice per sex (6 months of age). N=8-22 mice per sex (8 months of age). N=15 NonTg mice per sex; N=0 female Tg mice; N=3 male Tg mice at time of thesis submission (12 months of age).

During the transition to active period (ZT 11-12), Tg mice moved in and out of the shelter zone significantly more compared to NonTg mice, yet they spent comparable amounts of time resting in the shelter zone during this transition period (Fig 10. B-C). Taken together, these data suggest that Tg mice exhibited more fragmented rest during this transition period, referring to shorter, more frequent episodes of rest despite comparable amounts of time spent resting in relation to healthy controls. Rest fragmentation was associated with ZT transition in that mice became more restless one hour prior to the lights OFF cue (i.e., ZT 12) (Fig 10. C), but spent a statistically comparable duration of time in the shelter zone during this same hour (Fig 2. B). Mice remained restless during the majority of their active period (Fig 10. B,C).

The second level of analysis performed sought to determine at what age restlessness first manifests in Tg mice and whether this behaviour is sex-specific. Thus, shelter zone duration and the number of shelter zone transits were disaggregated by age and sex (Fig 10. D-K). No inherent sex difference was detected in NonTg mice associated with aging. During all four ZT periods, NonTg female and NonTg male mice spent more time in the shelter zone and performed less shelter zone transits as they aged (Fig 10. D-K). This indicates that NonTg mice, regardless of sex, rest more in the shelter zone and transit to and from the shelter less as they age (Fig 10. D-K). However, during the active period, an inherent sex difference was observed wherein NonTg male mice spent significantly more time resting in the shelter zone compared to NonTg female mice from 2 to 8 months of age (Fig 10. F).

The next inquiry at this level of analysis was to determine whether human mutant APP intersects with age, sex and ZT that associated with restless behaviours in Tg mice. Tg female mice demonstrated significant increases in shelter zone transits compared to NonTg female mice during the active period at 2 months of age, however, this was not accompanied by impaired rest as total

time in shelter was comparable (Fig 10. F-G). Conversely, at the same ZT period and age, Tg male mice also demonstrated significant increases in the number of shelter transits; however, this was associated with a decrease in the time spent in the shelter compared to NonTg male mice (Fig 10. F-G). Taken together, these findings indicate that Tg male mice demonstrate a more restless phenotype compared to Tg female mice, as evidenced by disturbances in both the transition to active period (i.e., fragmented rest at 2 and 4 months of age) and active period (i.e., restlessness at 2 months of age). Notably, both male and female N5 TgCRND8 mice with human mutant APP did not show the age-related increase in resting behaviours observed in NonTg mice as they age (Fig 10. D-K).

3.3 N5 TgCRND8 mice exhibit enhanced food-seeking behaviour

Behavioural indices of appetite were evaluated in NonTg and Tg mice longitudinally from 2-12 months of age using two measures: (1) the duration of time the mouse spent in the feeder zone per hour (i.e., behavioural indice of time spent feeding); and (2) the number of feeder zone transits the mouse performs per hour (i.e., behavioural indice of food-seeking/foraging behaviour) (Fig 11. A).

The first level of analysis consisted of determining whether NonTg and Tg mice demonstrated differencces in the time spent feeding and the number of feeder zone transits across the ZT cycle. Intringuingly, during the transition to active period and active period, Tg mice demonstrated a significant increase in the number of feeder zone transits (i.e., foraging for food), but they did not spend significantly more time feeding during these ZT periods compared to NonTg littermates (Fig 11. B-C).

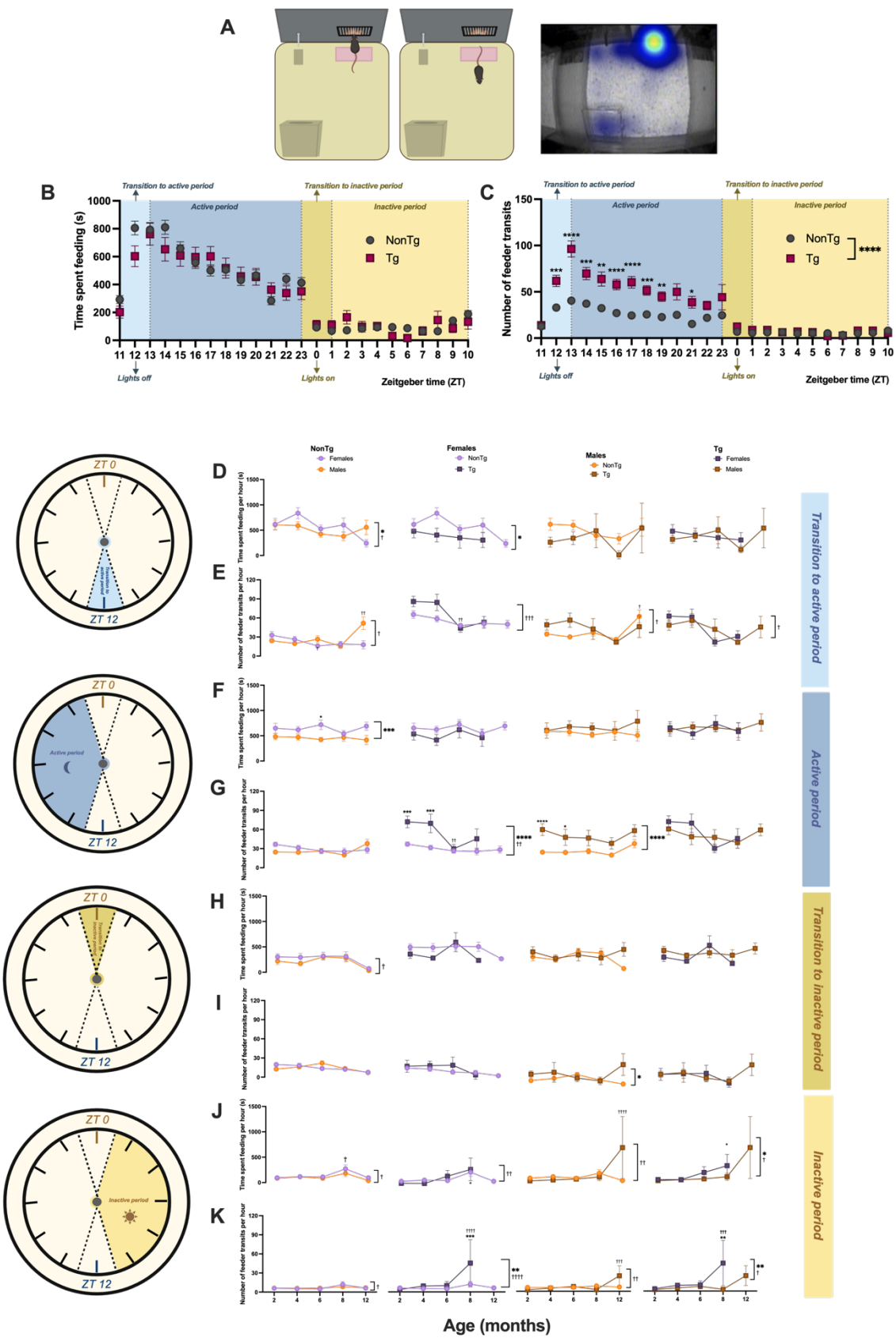
A
B
Transition to active period
Transition to inactive period
Active period
Inactive period
NonTg
Tg
Time spent feeding (s)
Lights off
Lights on
Zeitgeber time (ZT)
C
Number of feeder transits
****
D
E
F
G
H
I
J
K
NonTg
Females
Males
Females
NonTg
Tg
Males
NonTg
Tg
Tg
Females
Males
Time spent feeding per hour (s)
Number of feeder transits per hour
ZT 0
ZT 12
Active period
Transition to inactive period
Inactive period
Transition to active period
Active period
Transition to inactive period
Inactive period
Age (months)

Figure 11 Food foraging behaviours decline with age in NonTg and Tg females and NonTg males; however, Tg males eat more than NonTg males with 12 month old Tg males searching for food more frequently in their inactive period. (A) Schematics depict the experimental assessment of mice from 2-12 months of age. The duration of time mice spent in, and the number of transits made to, the PhenoTyper feeding zone were quantified over 24 hours, considering the zeitgeber cues that signal transition to the active (lights off ZT 12) and inactive periods (lights on ZT 0) (left panel). A heatmap of a 4-month-old male NonTg mouse at ZT 0 is shown where the mouse is primarily in the feeding zone with some transits to the sleep shelter and drinking zone (right panel). (B) N5 TgCNRD8 mice show no differences in feeding duration (age 2 to 12 months) yet (C) transit in and out of the feeding zone more frequently than their NonTg littermates when transit to and in their active period. When disaggregated by age and sex, (D,E) young animals initiate feeding earlier than older animals when lights turn off. (F,G) Females spend more time feeding than males with no effect of genotype on feeding duration during their active period. Younger Tg mice, regardless of sex, transit in and out of the feeding zone more often than NonTgs. (H-K) Older male Tg mice spend more time feeding and make more transits to the feeder zone during their inactive period than NonTgs. Data represent mean + SEM total of 201 NonTg mice (Females: n=94; Males N=107). Statistics were two-way repeated measures ANOVA with (A,B) post hoc Šidák test or (D-K) Holm-Šidák test comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.0001) or age †p<0.05, ††p<0.01, †††p<0.001 vs. 2 months of age). N=13-22 mice per sex (2 months of age). N=9-24 mice per sex (4 months of age). N=6-24 mice per sex (6 months of age). N=8-22 mice per sex (8 months of age). N=15 NonTg mice per sex; N=0 female Tg mice; N=3 male Tg mice at time of thesis submission (12 months of age).

The next level of analysis sought to determine at what age enhanced food-seeking behaviour first manifested in Tg mice and whether this behaviour was sex-specific. No major differences in feeding duration or food-seeking behaviour were detected between females and males, NonTg or Tg, with age during transition to active period (Fig 11. D-E). However, during the active period, NonTg females spent more time feeding than males whereas Tgs did not (Fig 11. F). Young Tg mice, regardless of sex, demonstrated significantly more feeder zone transits at 2 and 4 months of age compared to age- and sex-matched controls (Fig 11. G). This food seeking behavioural phenotype was accompanied by a ZT- and age-dependent behavioural switch wherein Tg female and Tg male mice also demonstrated an increase in the number of feeder transits at 8 months of age during the inactive period (Fig 11. K) and at 12 months of age during the transition to the inactive period (Fig 11. I), respectively.

3.4 N5 TgCRND8 mice exhibit enhanced water-seeking behaviour

Behavioural indices of thirst were evaluated in NonTg and Tg mice longitudinally from 2-12 months of age using two measures: (1) the duration of time the mouse spends in the drinking zone per hour (i.e., behavioural indice of time spent drinking); and (2) the number of drinking zone transits the mouse performs per hour (i.e., behavioural indice of water-seeking behaviour) (Fig 12. A).

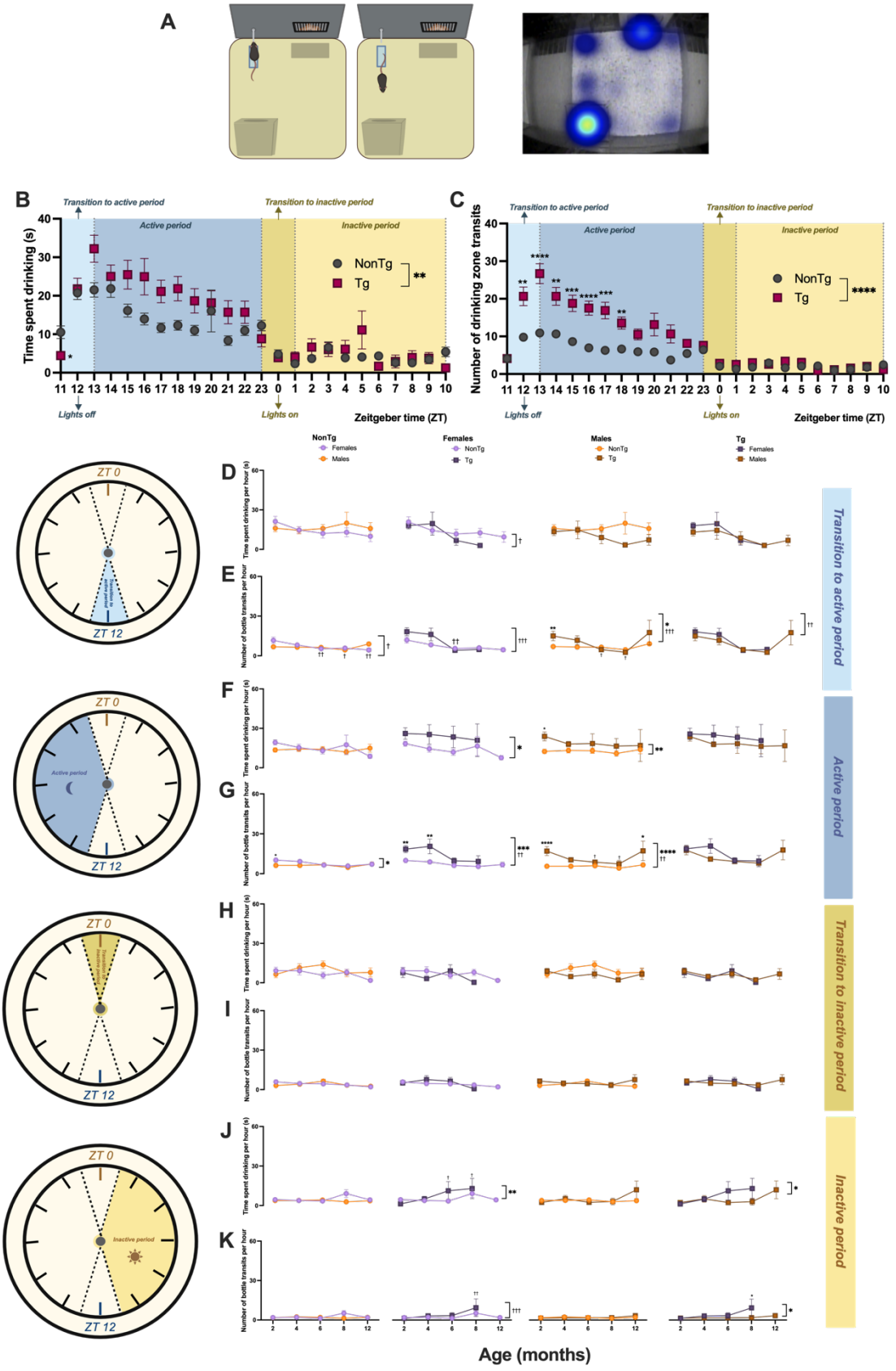
A
B
Transition to active period
Transition to inactive period
Active period
Inactive period
Time spent drinking (s)
NonTg
Tg
Lights off
Lights on
Zeitgeber time (ZT)
C
Number of drinking zone transits
D
E
F
G
H
I
J
K
NonTg
Females
Males
Females
NonTg
Tg
Males
NonTg
Tg
Tg
Females
Males
Time spent drinking per hour (s)
Number of bottle transits per hour
ZT 0
ZT 12
Transition to active period
Active period
Transition to inactive period
Inactive period
Age (months)

Figure 12 Younger Tgs, regardless of sex, seek water more often than NonTgs in their active period while older Tgs, regardless of sex, seek water more often in their inactive period. (A) Schematics depict the experimental assessment of mice from 2-12 months of age. The duration of time mice spent in, and the number of transits made to, the PhenoTyper drinking zone were quantified over 24 hours, considering the zeitgeber cues that signal transition to the active (lights off ZT 12) and inactive periods (lights on ZT 0) (left panel). A heatmap of a 4-month-old female NonTg mouse at ZT 0 is shown where the mouse primarily occupying the sleep shelter with transits to both the feeding and drinking zone (right panel). (B) N5 TgCNRD8 mice spend more time drinking and (C) move into the drinking zone more frequently in the active period than NonTgs. When disaggregated by age and sex, (D-G) younger Tgs, regardless of sex, drink for longer durations and seek water more often than NonTgs during their active period. This difference declines with age in females but not males. (H-K) Conversely, older Tgs, regardless of sex drink for longer durations and seek water more often than NonTgs during their inactive period. Data represent mean + SEM total of 201 NonTg mice (Females: n=94; Males N=107). Statistics were two-way repeated measures ANOVA with (A,B) post hoc Šidák test or (D-K) Holm-Šidák test comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.0001) or age †p<0.05, ††p<0.01, †††p<0.001 vs. 2 months of age). N=13-22 mice per sex (2 months of age). N=9-24 mice per sex (4 months of age). N=6-24 mice per sex (6 months of age). N=8-22 mice per sex (8 months of age). N=15 NonTg mice per sex; N=0 female Tg mice; N=3 male Tg mice at time of thesis submission (12 months of age).

The first level of analysis sought to determine whether NonTg and Tg mice demonstrated differences in the time spent drinking and the number of drinking zone transits across ZT, independently of age and sex. Tg mice spent more time drinking overall than NonTg mice (Fig 12. B). During both the transition to active period and active period, Tg mice also demonstrated a significant increase in the number of drinking zone transits (ZT 12-18; Fig 12. C).

The next level of analysis sought to determine at what age enhanced water-seeking behaviour manifested in Tg mice and if this behaviour was sex-specific. Time spent in the drinking zone and the number of drinking zone transits were therefore disaggregated by age and sex (Fig 12. D-K). Young Tg mice demonstrated enhanced water-seeking behaviours that are both sex and ZT period-specific. Tg female mice demonstrated a significant increase in the number of drinking zone transits during the active period at 2 and 4 months of age compared to NonTg female mice (Fig 12. G). Likewise, Tg male mice demonstrated a significant increase in both the time spent in the drinking zone and the number of drinking zone transits during both the transition to active and active period at 2 months of age (Fig 12. E-G).

3.5 N5 TgCRND8 demonstrate a hyperaroused phenotype that intersects with sex, age and environmental factors

Spontaneous activity in the PT test was assessed longitudinally in N5 TgCRND8 mice from 2-12 months of age. Here, we defined spontaneous activity as the total number of zone transits between shelter, feeding and drinking zones (Fig 13. A). The first level of analysis sought to determine whether N5 TgCRND8 mice demonstrated differences in spontaneous activity across the ZT cycle. During both the transition to active period and active period, N5 TgCRND8 mice demonstrate significant increases in the total number of PT zone transits (Fig 13. B). This indicates that, regardless of sex and age, N5 TgCRND8 mice associated with hyperarousal (Fig 5. B).

The next level of analysis sought to determine at what age this hyperarousal phenotype first manifested and whether this behaviour was sex-specific. Data were therefore disaggregated by age and sex (Fig 13. C-F). We found that spontaneous activity declined significantly with age both during the transition to active period and the active period in all groups (Fig 13. C-F). Young N5 TgCRND8 mice, regardless of sex, showed more spontaneous activity during the transition to active period and active period at 2 and 4 months of age (Fig 13. D). No differences were observed during transition to the inactive period; however, older N5 TgCRND8 female mice demonstrate increased spontaneous activity during the inactive period compared to both NonTg littermates and N5 TgCRND8 male mice (Fig 13. F).

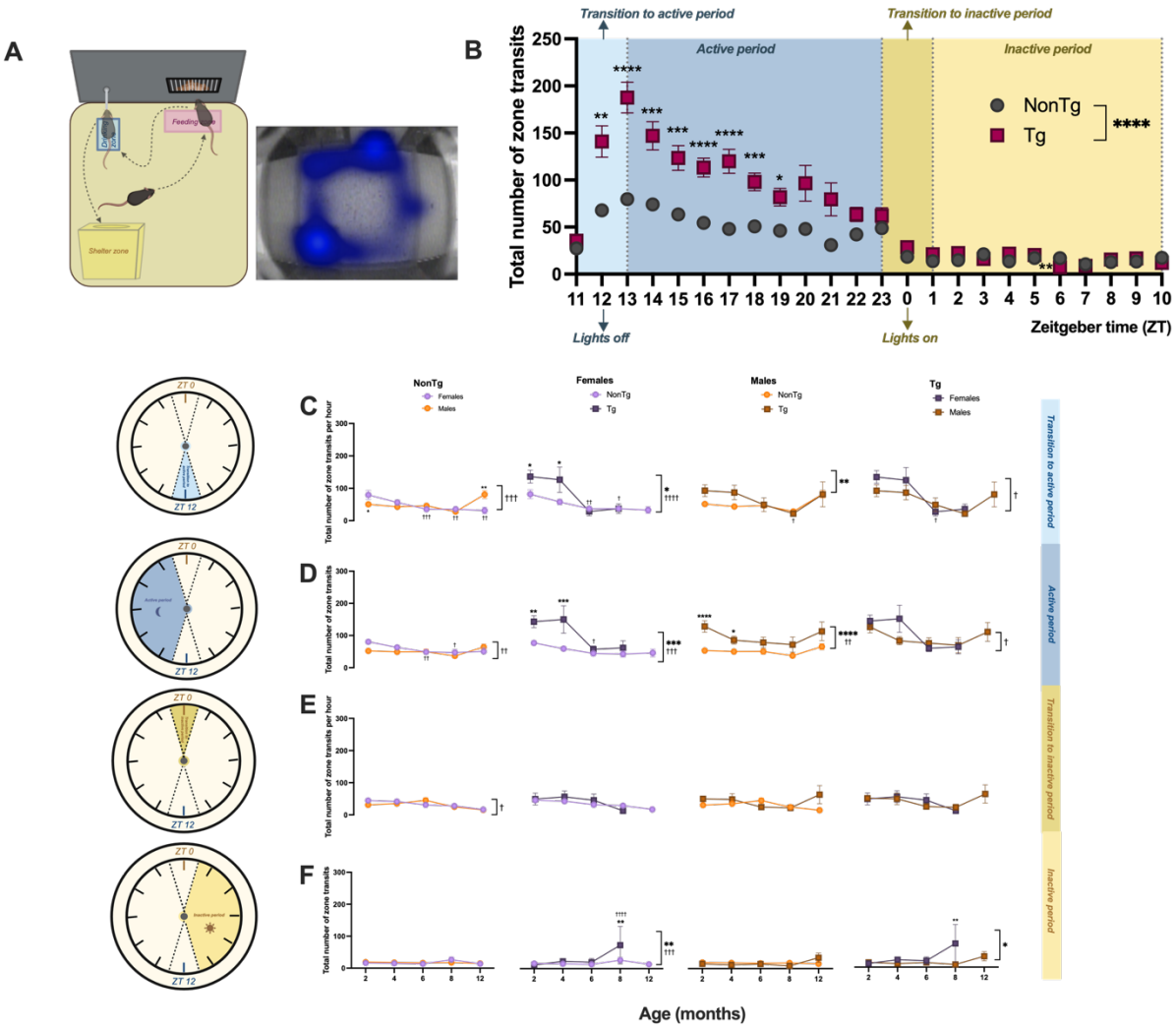

Figure 13 Spontaneous activity declines with age regardless of sex in NonTgs; however, younger Tg females and males are more active than NonTgs during the active period while older Tg females but not male are more active during their inactive period than NonTgs. (A) Schematics depict the experimental assessment of mice from 2-12 months of age. The total number of transits per hour made between the PhenoTyper drinking, feeding, and shelter zones were quantified, considering the zeitgeber cues that signal transition to the active (lights off ZT 12) and inactive periods (lights on ZT 0) (left panel). A heatmap of a 4-month-old female Tg mouse at ZT 20 is shown where the mouse is traversing all zones (right panel). (B) N5 TgCNRD8 mice become significantly more active earlier (at ZT 12) and remain more active during their active period than NonTgs but do not show increased activity during their inactive period. When disaggregated by age and sex, (C-E) activity declines with age regardless of sex in NonTgs during the transitions to the active and inactive periods and during the active period. (F) Age does not significantly alter activity during the inactive period. (C-D) Younger Tg females and males are more active during their active period than NonTgs. (E) Older Tg females but not males are more active during their inactive period than NonTgs.. Data represent mean ± SEM total of 201 NonTg mice (Females: n=94; Males N=107). Statistics were two-way repeated measures ANOVA with (A,B) *post hoc* Šidák test or (D-K) Holm-Šidák test comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.0001) or age †p<0.05, ††p<0.01, †††p<0.001 vs. 2 months of age). N=13-22 mice per sex (2 months of age). N=9-24 mice per sex (4 months of age). N=6-24 mice per sex (6 months of age). N=8-22 mice per sex (8 months of age). N=15 NonTg mice per sex; N=0 female Tg mice; N=3 male Tg mice at time of thesis submission (12 months of age).

3.6 N5 TgCRND8 exhibit increased voluntary running wheel activity

Voluntary running wheel activity was assessed longitudinally in N5 TgCRND8 mice and NonTg mice from 2-12 months of age over five consecutive ZT cycles (Fig 14. A). The first level of analysis sought to determine whether N5 TgCRND8 mice exhibited differences in voluntary running wheel activity compared to NonTg mice. Across all time periods of the ZT cycle (i.e., transition to active period, active period, transition to inactive period and part of the inactive period at ZT 1-3). N5 TgCRND8 mice ran significantly greater distances than NonTg mice (Fig 6. B).

The next level of analysis sought to determine at what age N5 TgCRND8 mice demonstrated increased voluntary running wheel activity and whether this behaviour was sex-specific. Thus, data were disaggregated by age and sex (Fig 14. C-F). An inherent sex difference in running wheel activity was observed wherein NonTg female mice ran significantly greater distances on the running wheel compared to NonTg male mice during the transition to active period and active period from 2-8 months of age (Fig 14. C-D). Voluntary running activity declined in NonTg females and males as they aged manifested during the transition to active period and the active period (Fig 14. C-D). Age did not impact on Tg running wheel activity. Running did not decline with age in N5 TgCRND8 female or male mice although Tg females, like their NonTg counterparts, ran more than Tg males in the active period (Fig 14. C-D). Further, N5 TgCRND8 mice exhibited significantly more voluntary running behaviour during both the transition to the and the inactive period compared to NonTg sex-matched counterparts (Fig 14. E-F).

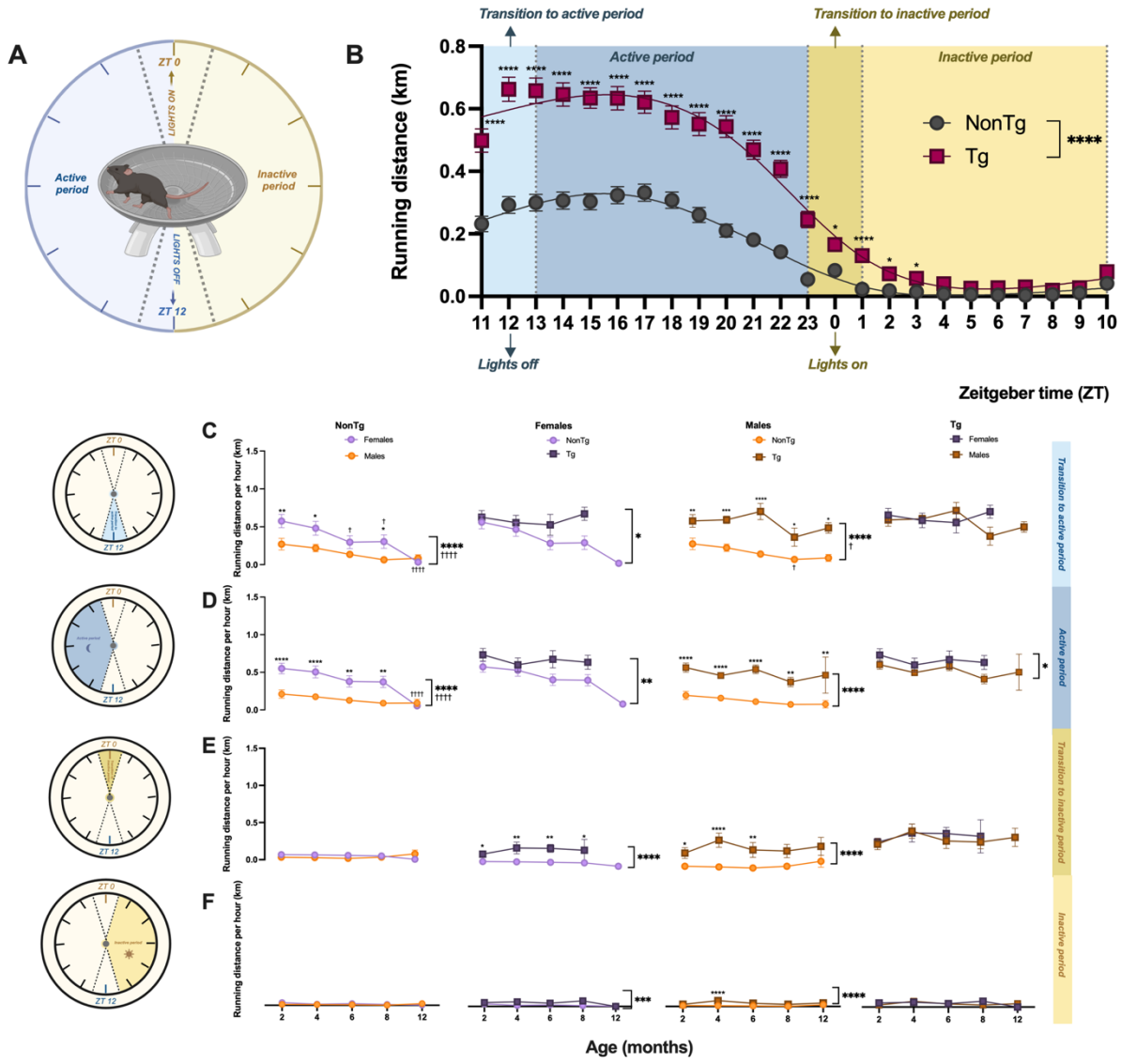

Figure 14 Tg mice, regardless of sex, run more than NonTg mice across the active-inactive periods and voluntary running does not decline with age in Tg as it does in NonTg mice. (A) Schematic depicts the experimental assessment of mice from 2-12 months of age. Mice were provided with running wheels for 5 days at 2, 4, 6, 8, and 12 months of age. (B) Tg mice run more than NonTg mice during transition to the active period, their active period, transition to the inactive period, and sustain running longer during their inactive period. When disaggregated by age and sex, (C,D) NonTg females run more than NonTg males until 12 months of age during their active period but not (E,F) their inactive period. (C-D) Voluntary running activity declines with age regardless of sex in NonTgs. (C-F) Tgs, regardless of sex, run more than NonTgs across all active-inactive periods with (D) Tg females more active than Tg males during the active period. Statistics were two-way repeated measures ANOVA with (B) *post hoc* Šidák test or (C-F) Holm-Šidák tests comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.0001) or age †p<0.05, ††p<0.01, †††p<0.001 vs. 2 months of age). N=13-22 mice per sex (2 months of age). N=9-24 mice per sex (4 months of age). N=6-24 mice per sex (6 months of age). N=8-22 mice per sex (8 months of age). N=15 NonTg mice per sex; N=0 female Tg mice; N=3 male Tg mice at time of thesis submission (12 months of age).

3.7 N5 TgCRND8 exhibit increased spontaneous activity during the YM test

Spontaneous activity during the YM test was evaluated longitudinally in N5 TgCRND8 mice and NonTg mice from 2-12 months of age (Fig 15. A). In the YM test, spontaneous activity was assessed using three measures: (1) total distance travelled in centimetres (cm), (2) velocity or the speed of travel (cm/s) and (c) the number of arm transits (i.e., novel and familiar arms) during the second trial. The first level of analysis sought to determine whether N5 TgCRND8 mice exhibited differences in spontaneous activity compared to NonTg mice. N5 TgCRND8 mice demonstrated greater total distance travelled, faster speed of travel and more open arm transits compared to NonTg mice collapsed across age and sex (Fig 15. B-D).

To investigate when this hyperarousal phenotype first manifested, spontaneous activity measures were disaggregated by age and sex (Fig 15. E-P). There was a significant decline in spontaneous activity (i.e., total distance and velocity travelled, frequency of arm transits) in all groups as they aged (Fig 15. E-P). Further, NonTg female mice were more active compared to NonTg male mice collapsed across age (Fig 15. E-M). However, no such sex differences were detected in N5 TgCRND8 mice (Fig 15. H-P). N5 TgCRND8 mice, regardless of sex, moved more, faster and with more arm transits during YM from 2-12 months of age compared to sex-matched NonTg counterparts (Fig 15. E-P).

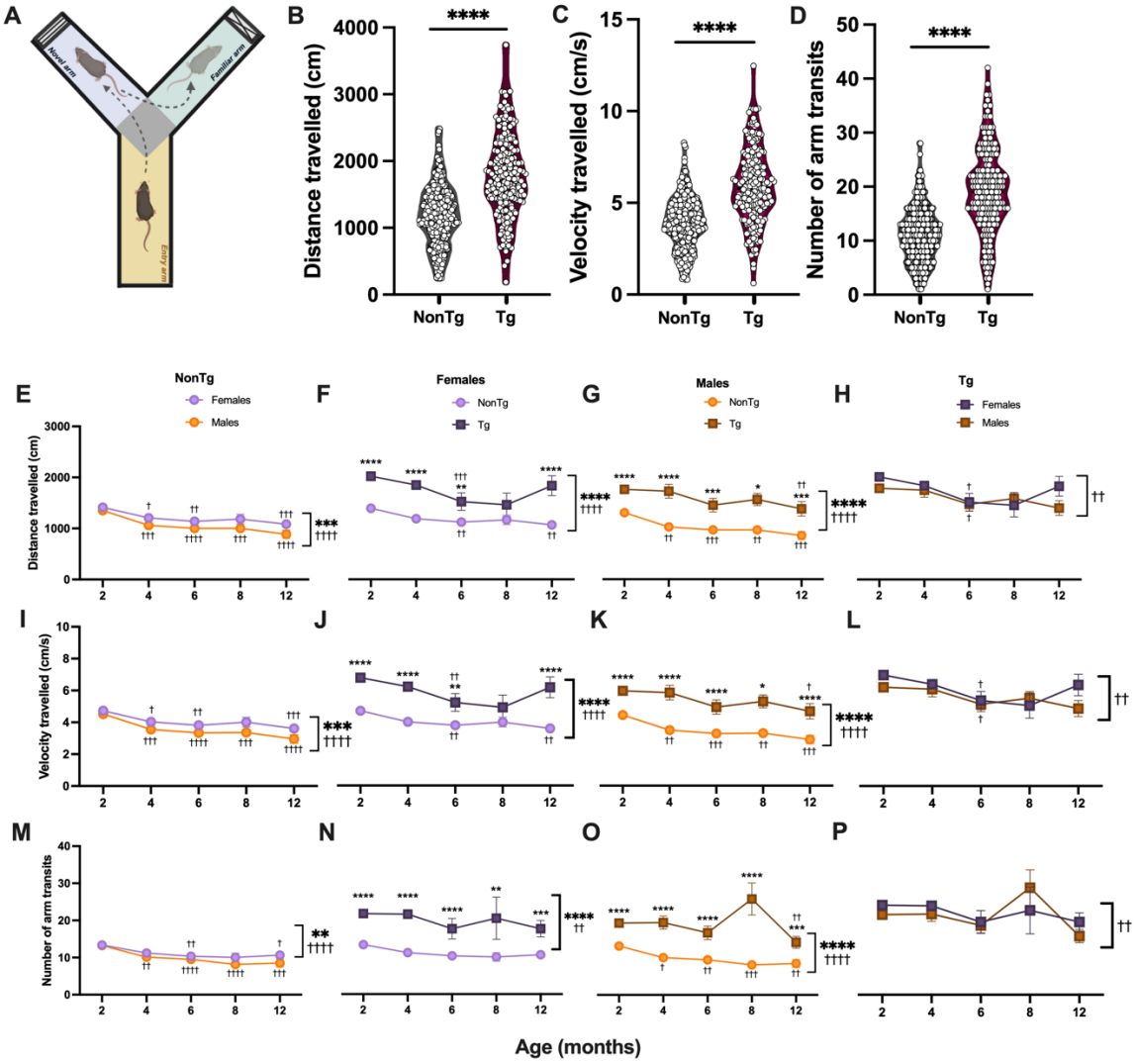

Figure 15 Tg mice, regardless of sex, demonstrate a hyperaroused phenotype from 2-12 months of age in YM. (A) Schematic depicts experimental assessment of activity in mice during the YM test wherein three variables are measured: total distance travelled (cm), speed of travel (cm/s) and number of open arm transits during the second trial. (B) Across all ages from 2 to 12 months of age, N5 TgCRND8 mice regardless of sex demonstrate significant increases in distance and velocity travelled and number of arm transits. To interrogate when hyperarousal in YM first manifests, data were disaggregated by age and sex. (E, I, M) There is an inherent sex difference in YM activity. Both NonTg female and male mice decline in activity as they age, however, NonTg female mice exhibit significant increases in total distance, velocity and number of transits compared to NonTg male mice. Conversely, no such sex difference exists for N5 TgCRND8 mice (H, L, P). (F-G, J-K, N-O) Tg female and Tg male mice exhibit significant increases in all three activity measures (total distance, velocity and number of arm transits) compared to NonTg mice and this activity declines with age. (B-D) Data represent mean ± SEM total of 447 NonTg mice (Females: n = 210; Males n = 237) and 214 Tg mice (Females: n = 109; Males n = 105). Statistics were (B-D) unpaired t-tests or (E-P) two-way mixed-effects ANOVA with *post hoc* Šidák test comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.0001). N= 39-72 mice per sex (2 months of age) N=19-56 mice per sex (4 months of age). N=19-58 mice per sex (6 months of age). N=4-28 mice per sex (8 months of age). N=16-31 mice per sex (12 months of age).

3.8 N5 TgCRND8 exhibit increased spontaneous activity during the MWM test

Spontaneous activity during the MWM test was evaluated longitudinally in N5 TgCRND8 mice and NonTg mice from 2-12 months of age (Fig 16. A). In the MWM test, spontaneous activity was assessed using two measures: (1) total distance travelled (cm) and (2) velocity or the speed of travel (cm/s) across the eight training days. The first level of analysis sought to determine whether N5 TgCRND8 mice exhibited differences in spontaneous activity compared to NonTg mice. N5 TgCRND8 mice moved more and swam faster compared to NonTg mice, regardless of age and sex (Fig 16. B-C).

To investigate when this hyperaroused phenotype in MWM first manifested, spontaneous activity measures were disaggregated by age and sex (Fig 16. D-K). In NonTg mice, regardless of sex, there was no effect of age on total distance and swim velocity (Fig 16. D, H). Female and male N5 TgCRND8 mice moved further and faster than NonTg littermates although this hyperarousal declined in older animals and was more pronounced in older females than males (Fig 16. F-K).

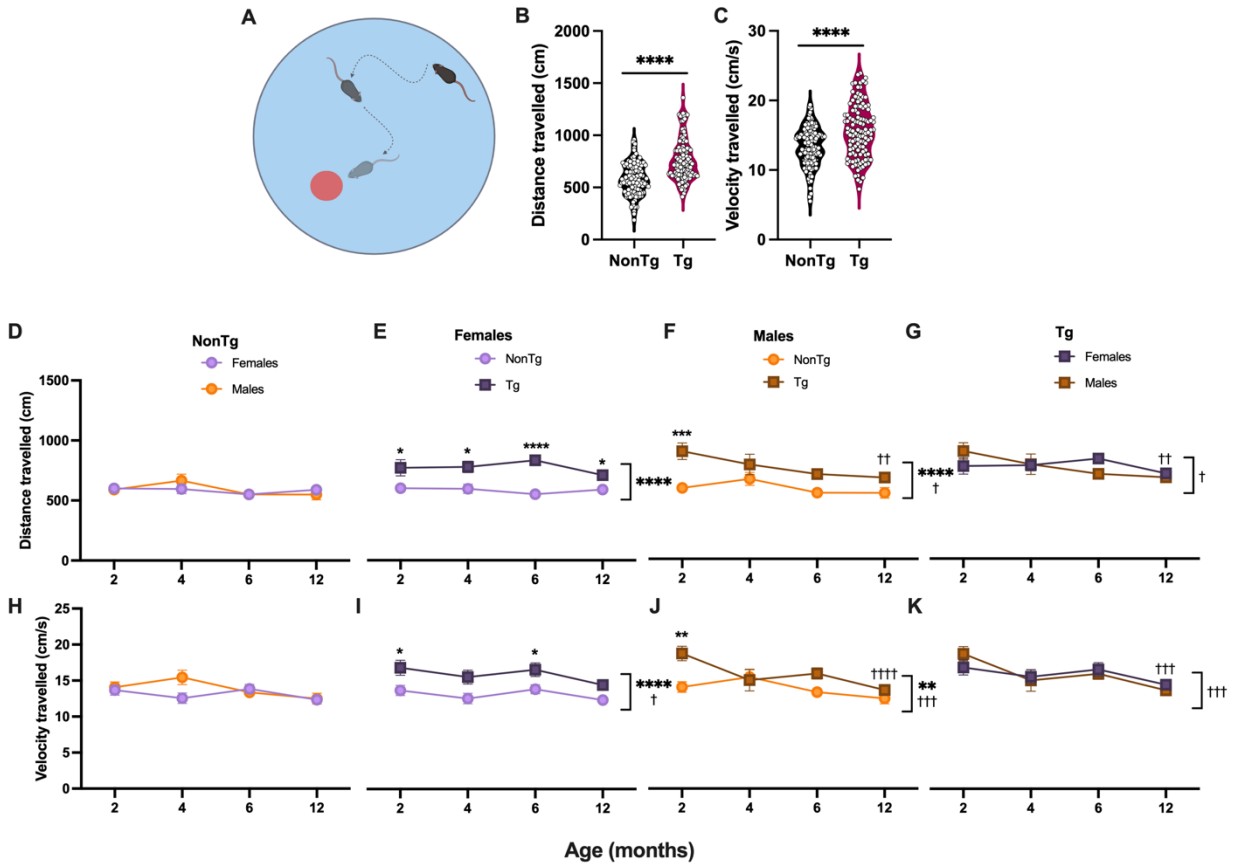

Figure 16 Tg mice, regardless of sex, demonstrate a hyperactive phenotype from 2-12 months of age in MWM. (A) Schematic depicts the experimental assessment of activity in the MWM test. (B-C) Across all ages, Tg mice regardless of sex demonstrate significant increases in total distance and velocity travelled. To interrogate when this hyperactivity in first manifests, data were disaggregated by age and sex. (D, H, G, K) Females and males, regardless of Tg status, do not show differences in MWM activity as they age. However, Tg mice do decline in MWM activity as they age while NonTg mice do not. (E, I, F, J) Tg female and Tg male mice exhibit significant increases in total distance and velocity travelled compared to NonTg female and NonTg male mice, respectively, that declines with age.

3.9 Young N5 TgCRND8 exhibit significant decreases in anxiety during the YM test

Behavioural indices of anxiety during the YM test were evaluated longitudinally in N5 TgCRND8 mice and NonTg mice from 2-12 months of age (Fig 17. A). Here, we refer to behavioural indices of anxiety as the total time spent in the entry arm (arm 1) prior to the first open arm transit during the second trial. In general, mice who are not anxious during the YM test will initiate exploration into the novel and/or familiar arms and will spend less time in the entry arm during the second trial. In contrast, mice who are significantly more anxious will freeze in the entry arm and will not initiate exploration as quickly. The first level of analysis sought to determine whether N5 TgCRND8 mice exhibited differences in anxiety compared to NonTg mice. N5 TgCRND8 mice showed fewer behavioural indices of anxiety, spending significantly less time in the entry arm compared to NonTg mice (Fig 17. B).

The next level of analysis sought to determine at what age N5 TgCRND8 mice exhibited this behavioural phenotype. There was an inherent sex difference. Older NonTg male spent more time in the entry arm than NonTg females (Fig 17. C). N5 TgCRND8 mice, regardless of sex, were significantly less anxious compared to NonTg sex-matched counterparts (Fig 17. D-E). However, a significant age interaction was evident in that N5 TgCRND8 female mice were significantly less anxious at 2 and 4 months of age compared to NonTg female mice (Fig 17. D) yet 12 month old N5 TgCRND8 female mice were significantly more anxious compared to NonTg female mice (Fig 17. D). This behavioural switch was not observed in N5 TgCRND8 male mice (Fig 17. E).

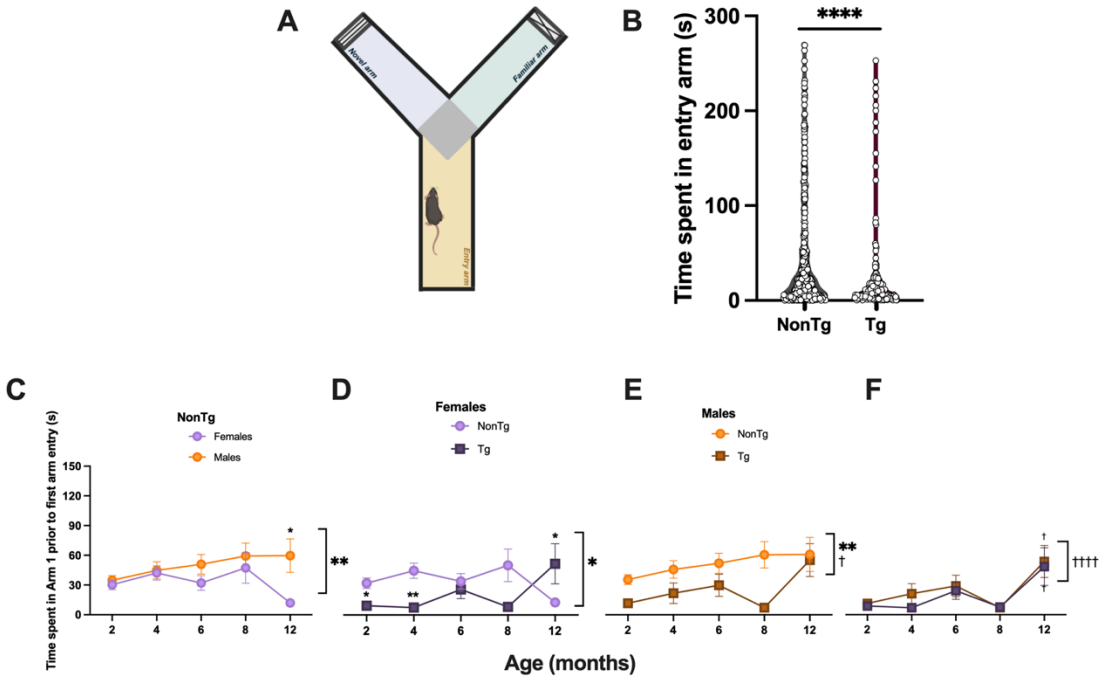

Figure 17 Young Tg mice exhibit significantly lower levels of anxiogenic-like behaviour in YM. (A) Schematic depicts behavioural assessment of anxiety in mice during the YM test wherein time spent in the entry arm (s) prior to the first open arm transit is measured. Mice demonstrating anxiogenic-like behaviour will freeze for a significant period of time in the entry arm prior to the first open arm entry. (B) Across all ages from 2-12 months of age, N5 TgCRND8 mice spend significantly less time in the entry arm prior to the first open arm transit in YM. To interrogate at which time point this phenotype manifests, data were disaggregated by age and sex. (C) There is an inherent sex difference in anxiety during YM. NonTg male mice spend significantly more time in arm 1 prior to the first transit compared to NonTg female mice at 12 months of age. Importantly, this sex difference is not observed in N5 TgCRND8 mice (F). (D-E) N5 TgCRND8 mice, regardless of sex, demonstrate significant decreases in time spent in the entry arm prior to the first open arm transit compared to NonTg sex-matched counterparts. Tg females, but not Tg males, demonstrate an age-dependent switch in anxiety-like behaviour and become significantly more anxious at 12 months of age compared to NonTg female mice. (B) Data represent mean $\pm$ SEM total of 447 NonTg mice (Females: n = 210; Males n = 237) and 214 Tg mice (Females: n = 109; Males n = 105). Statistics were (B) unpaired t-test or (C-F) two-way mixed-effects ANOVA with *post hoc* Šidák test comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.0001). N= 39-72 mice per sex (2 months of age) N=19-56 mice per sex (4 months of age). N=19-58 mice per sex (6 months of age). N=4-28 mice per sex (8 months of age). N=16-31 mice per sex (12 months of age).

3.10 N5 TgCRND8 mice show no evidence of differences in anxiety in the MWM compared to NonTg mice

Behavioural indices of anxiety during the MWM test were evaluated longitudinally in N5 TgCRND8 mice and NonTg mice from 2-12 months of age (Fig 10. A). Here, we refer to behavioural indices of anxiety as percentage of time spent in the thigmotactic zone (% Thigmotaxis) across the eight training days. Thigmotaxis was defined as the percentage of time mice spent swimming within a 15 cm periphery of the pool wall (Fig 10. A). Mice too anxious to perform the MWM test will spend > 60% of time in the thigmotactic zone across the eight training days and are excluded from the analysis.

The first level of analysis sought to determine whether N5 TgCRND8 mice exhibited differences in percent thigmotaxis across the eight training days. No differences in percent thigmotaxis between NonTg and Tg mice were detected (Fig 10. B), although the percentage of N5 TgCRND8 mice excluded due to the thigmotaxis exclusion criteria (i.e., >60% across the training days) was significantly lower than NonTg mice (Fig 10. C).

To investigate the effects of age and sex on thigmotactic behaviour, the percentage of time spent in thigmotaxis was disaggregated by age and sex (Fig 10. D-G). In all groups, anxiety indices in the MWM increased significantly with age (Fig 10. D-G). However, the percent thigmotaxis was comparable between groups across all ages (Fig 10. D-G), indicating no difference in anxiety between genotypes when considering age.

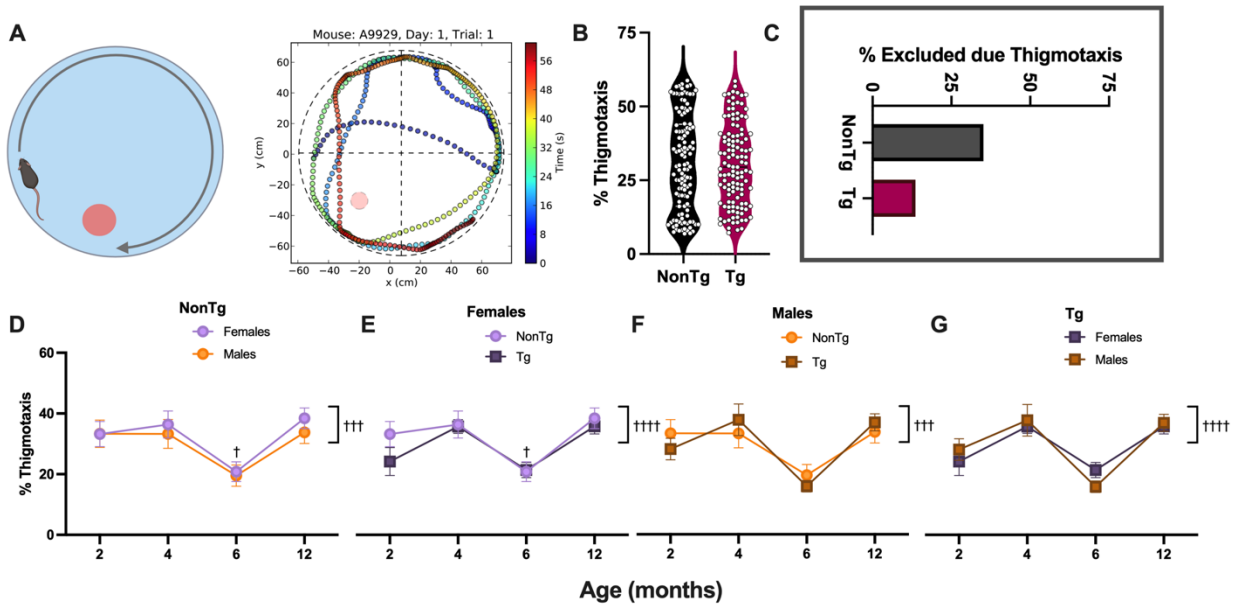

Figure 18 Tg mice are not significantly more anxious in MWM from 2-12 months of age. (A) Schematic depictions of thigmotaxis measure in MWM. Thigmotaxis is calculated throughout the eight training days wherein a mouse spends a given percentage of time swimming within a 15 cm periphery of the pool wall. (B) Across all ages, Tg mice regardless or sex demonstrate comparable percentages of time in the thigmotactic zone compared to NonTg mice. However, the percentage of mice excluded due to thigmotaxis (i.e., > 60% of time spent in the thigmotactic zone throughout the eight training days) is higher in NonTg animals compared to Tg mice from 2 to 12 months of age (C). To interrogate whether there are age and/or sex-specific differences in thigmotaxis, data were disaggregated by age and sex. (D-G) From 2 to 12 months of age, there are no differences in percentage of time spent in thigmotaxis between NonTg and Tg animals regardless of sex.

3.11 N5 TgCRND8 female mice demonstrate earlier spatial working memory impairment compared to N5 TgCRND8 male mice

Y-maze (YM) is a behavioural measure of visuospatial working memory in mice. The main measure of this test is the total time spent in the novel arm compared to the familiar arm, or the percentage of time spent in the novel arm during the second trial (Fig 19. A). Visuospatial working memory was assessed in N5 TgCRND8 mice and NonTg mice longitudinally from 2-12 months of age. The first level of analysis sought to determine whether N5 TgCRND8 mice demonstrate differences in visuospatial working memory. N5 TgCRND8 mice spent a significantly lower percentage of time in the novel arm relative to the familiar arm compared to NonTg mice across age and sex (Fig 19.B).

To determine at what age visuospatial working memory deficits occurred in N5 TgCRND8 mice and whether it is impacted by sex, data were disaggregated by age and sex (Fig 19. C-K). At 2 and 4 months of age, all groups spent significantly more time in the novel arm relative to the familiar arm during the second trial and exhibit visuospatial working memory (Fig 19. C). Visuospatial working memory was impaired at 6 months of age in N5 TgCRND8 female mice (Fig 19. E) and continued to decline with age (Fig 19.1). Male N5 TgCRND8 male mice did not exhibit spatial working memory impairment until 12 months of age (Fig 19. G, J).

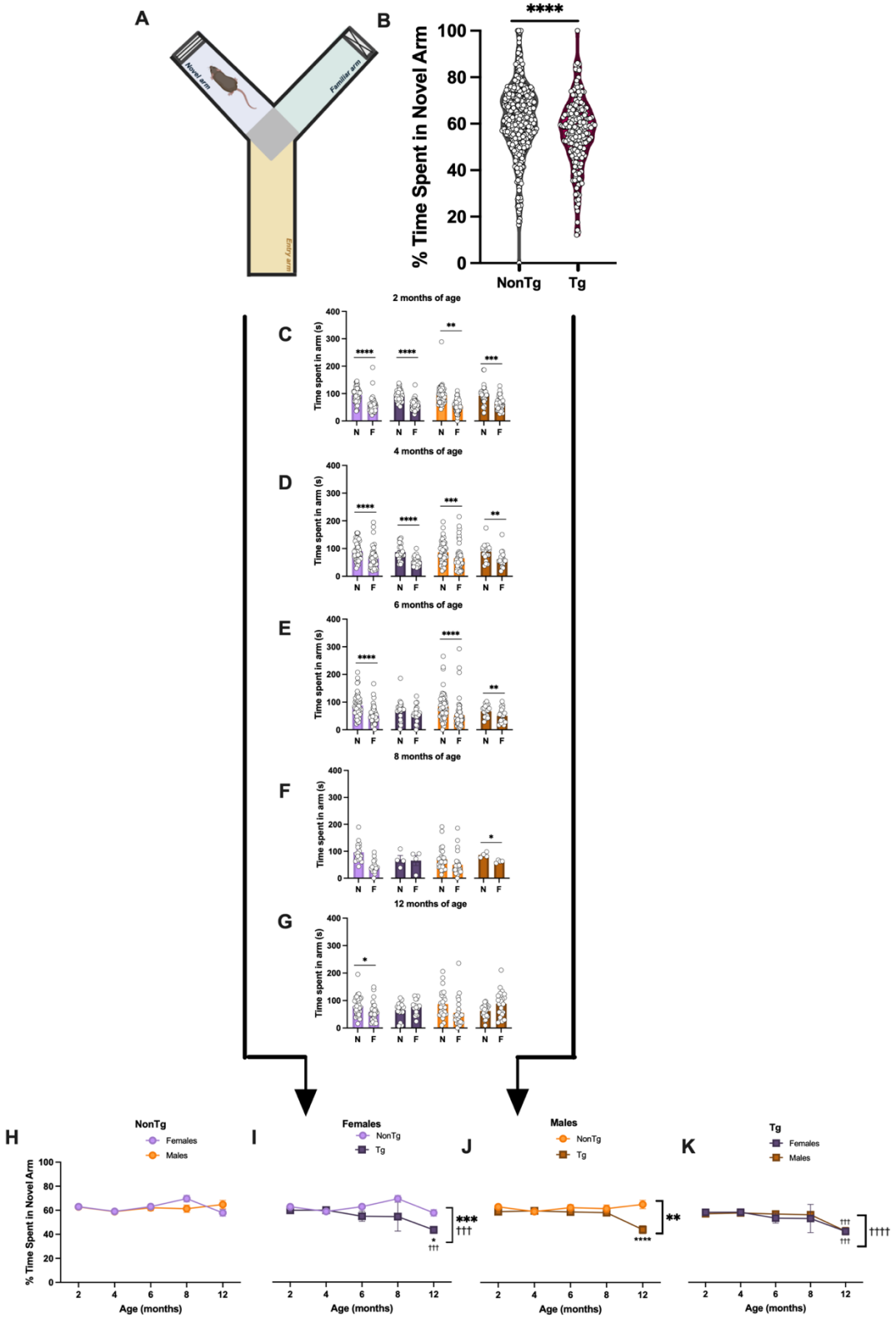
A
Novel arm
Familiar arm
Entry arm
B
****
% Time Spent in Novel Arm
NonTg
Tg
2 months of age
4 months of age
6 months of age
8 months of age
12 months of age
C
D
E
F
G
Time spent in arm (s)
N
F
H
NonTg
Females
Males
I
Females
NonTg
Tg
J
Males
NonTg
Tg
K
Tg
Females
Males
Age (months)

Figure 19 Tg mice exhibit sex differences in behavioural onset of visuospatial working memory impairment. (A) Schematic representation of the behavioural assessment of working memory during the YM test. The duration of time mice spend in the novel and familiar arms is quantified during the second trial of YM. A cognitively healthy mouse can distinguish between novel and familiar arms with the aid of visual cues and will spend more time in the novel or unexplored arm. (B) Across all ages from 2-12 months of age, N5 TgCRND8 mice spend a significantly lower percentage of time in the novel arm during the second trial compared to NonTg mice which is indicative of working memory impairment. To interrogate when these deficits manifest, data were disaggregated by age and sex. (C-D) At 2 and 4 months of age, NonTg and Tg mice regardless of sex demonstrate significant increases in time spent in novel arm compared to familiar during the second trial. (E-F) Tg female mice, but not Tg male mice, spend an insignificant amount of time in the novel arm compared to the familiar arm at 6 and 8 months of age. Conversely, Tg male mice spend significantly more time in the novel arm compared to the familiar arm at 6 and 8 months of age (E-F). At 12 months of age, all groups except for NonTg female mice spend an insignificant amount of time in the novel arm compared to the familiar arm. To further assess genotypic differences in working memory impairment and its interaction with age and sex, the percentage of time spent in the novel arm was assessed longitudinally (H-K). (H, K) Female and male mice, regardless of genotypic status, spend comparable percentages of time in the novel arm from 2-12 months of age. (J, K) Female and male Tg mice demonstrate significant decreases in percentage of time spent in the novel arm compared to Female and male NonTg mice at 12 months of age, respectively. (B) Data represent mean $\pm$ SEM total of 447 NonTg mice (Females: n = 210; Males n = 237) and 214 Tg mice (Females: n = 109; Males n = 105). Statistics were (B) unpaired t-test or (C-D) paired t-tests or (H-K) two-way mixed-effects ANOVA with *post hoc* Šidák test comparing the indicated conditions (*$p<0.05$,**$p<0.01$,***$p<0.001$,****$p<0.0001$). N= 39-72 mice per sex (2 months of age) N=19-56 mice per sex (4 months of age). N=19-58 mice per sex (6 months of age). N=4-28 mice per sex (8 months of age). N=16-31 mice per sex (12 months of age).

3.12 N5 TgCRND8 female mice demonstrate a more severe spatial learning and memory impairment compared to N5 TgCRND8 male mice at 6 months of age

Morris water maze (MWM) is a behavioural measure of visuospatial learning and memory in mice. A composite visuospatial memory score reflects three MWM measures: (1) Escape latency, which is the time the mouse takes to escape onto the hidden platform (Fig 20. A); (2) the percentage of time during the probe trial that the mouse spent in the pool quadrant where the platform was previously located on the eight training days; and (3) the percentage mean spatial strategy, which is the incidence of spatial strategy used throughout the eight training days to find the hidden platform.

Visuospatial learning and memory was assessed in N5 TgCRND8 mice and NonTg mice longitudinally from 2-12 months of age. The first level of analysis sought to determine whether N5 TgCRND8 mice demonstrate differences in visuospatial learning and memory. Escape latencies were significantly longer for N5 TgCRND8 mice (Fig 20. B-C). This was accompanied by a significant decrease in mean spatial strategy (Fig 20. E) and the spatial composite score (Fig 20. F).

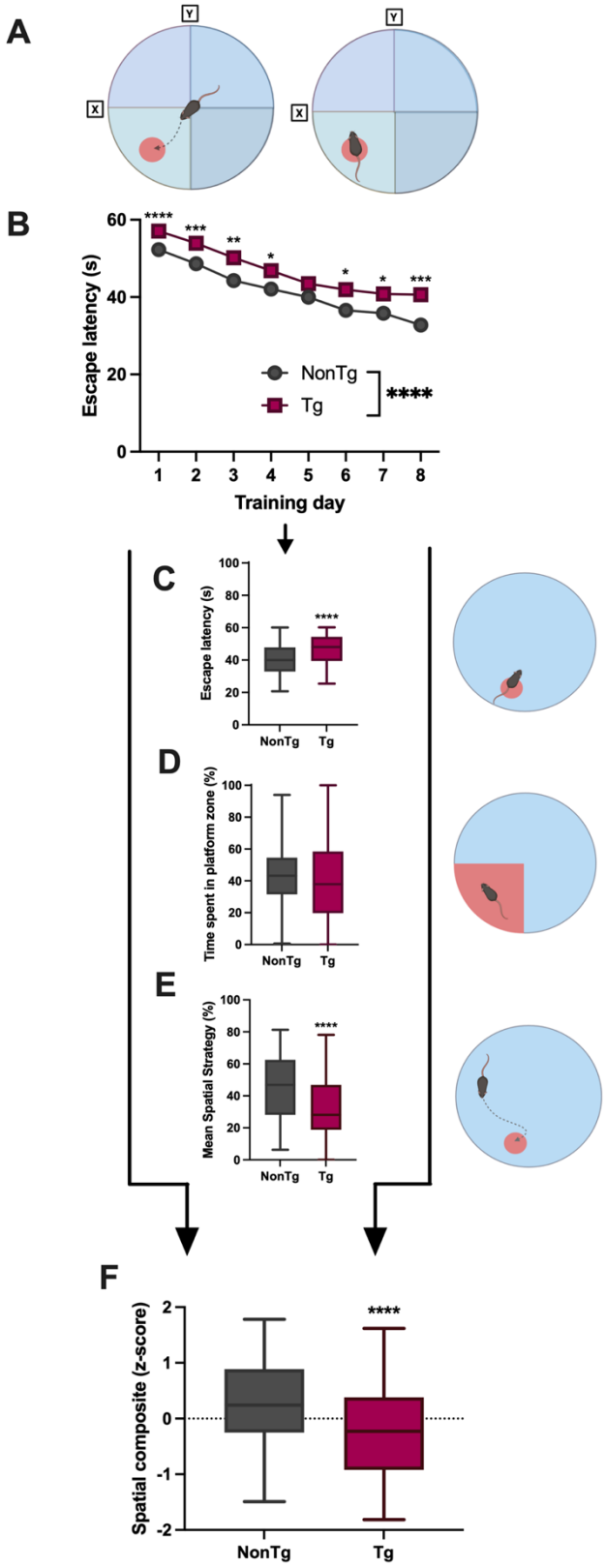
A
Y
X
B
Escape latency (s)
Training day
NonTg
Tg
****
C
Escape latency (s)
D
Time spent in platform zone (%)
E
Mean Spatial Strategy (%)
F
Spatial composite (z-score)
NonTg
Tg

Figure 20 Tg mice demonstrate visuospatial learning and memory impairments across age from 2-12 months of age. (A) Schematics depict the experimental assessment of mice during MWM training days (Day 1-8) across all ages (2-12 months of age). The duration of time mice take to escape onto the hidden platform over the course of eight training days was quantified. (B, C) N5 TgCRND8 mice (from 2 to 12 months of age) take significantly more time to escape onto the hidden platform compared to NonTg mice. The percentage of time mice spent in the pool quadrant where the platform was located during the eight training days was measured on day nine. (D) Across all ages (2 to 12 months of age), N5 TgCRND8 mice spend comparable percentages of time in the platform zone compared to NonTg mice. To interrogate whether impairments in EL reflect changes in effective search strategies, MWM strategies adopted during the training days was assessed in N5 TgCRND8 mice. Mice can adopt one of three strategies during each trial of MWM in order to find the hidden platform: systematic, spatial or repetitive looping. (E) Across the training days, N5 TgCRND8 mice adopt spatial strategy significantly less than NonTg mice from 2-12 months of age regardless of sex. To interrogate further, these three measures were combined into a composite memory score, wherein a score below 0 is indicative of spatial learning and memory deficits (i.e., average escape latency, percentage of time spent in platform zone and mean percentage of spatial strategy). (F) N5 TgCRND8 mice exhibit significantly lower spatial composite scores across all timepoints compared to NonTg mice independently of sex. (B-F) Data represent mean $\pm$ SEM total of 120 NonTg mice (Females: n = 64; Males n = 56) and 121 Tg mice (Females: n = 67; Males n = 54). Statistics were (B) two-way repeated measures ANOVA with *post hoc* Šidák test or (C-F) unpaired t-tests comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.0001). N= 11-18 mice per sex (2 months of age) N=7-16 mice per sex (4 months of age). N=10-19 mice per sex (6 months of age). N=19-24 mice per sex (12 months of age).

To assess at what age visuospatial memory impairments in MWM first occur and whether this impairment is sex-specific, data were disaggregated by age and sex (Fig. 21-24). At 2 months of age, neither female nor male N5 TgCRND8 mice show evidence of impaired visuospatial memory in MWM (Fig 21. A-K). At 4 months of age, N5 TgCRND8 male but not female mice demonstrated visuospatial memory deficits (Fig 14 A-K). At 6 months of age, females but, surprisingly not males, exhibited visuospatial memory impairment (Fig 16. A-K) that reversed again by 12 months of age with males but not females impaired (Fig 17 A-K).

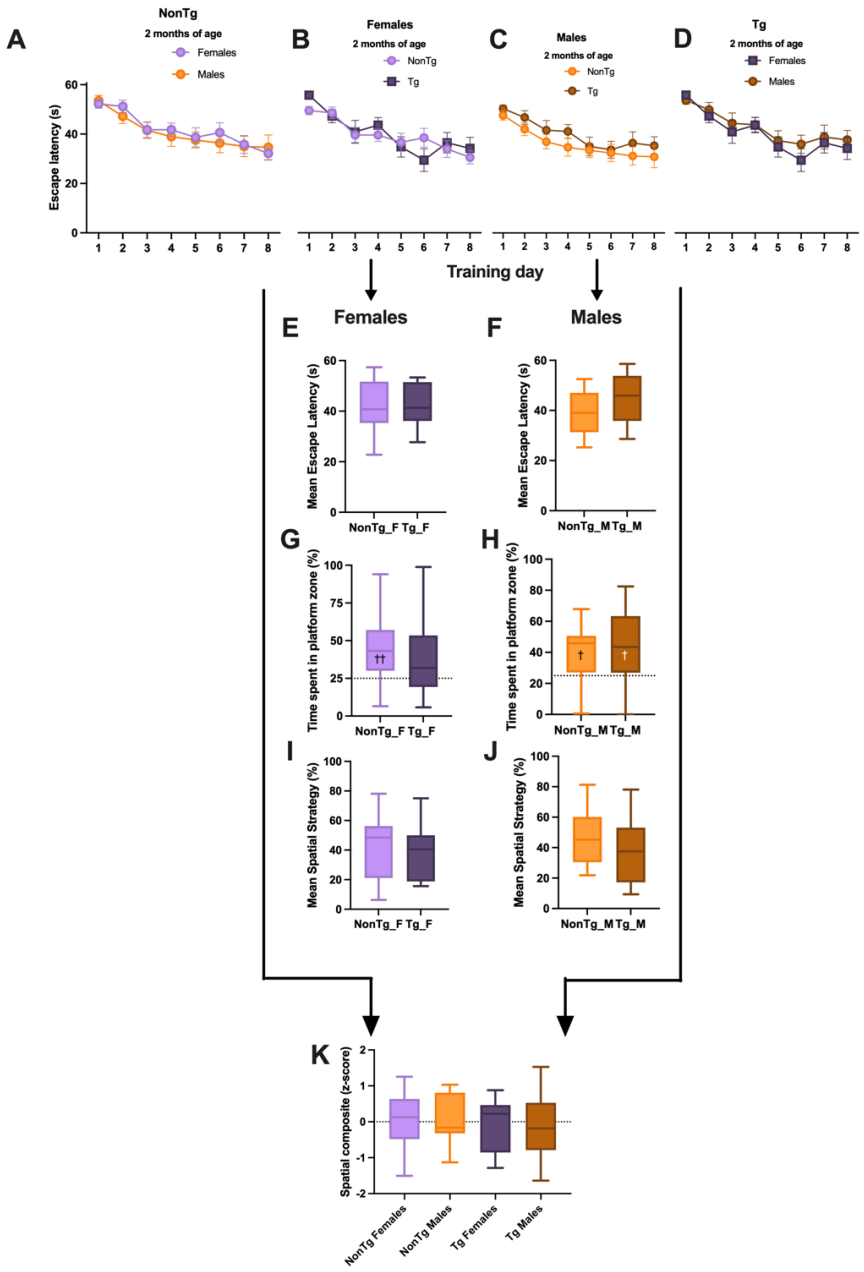

Figure 21 Tg mice do not demonstrate visuospatial learning and memory impairments at 2 months of age. (A, D) There is no sex difference observed in escape latency. Females and males, regardless of Tg status, take comparable amounts of time to escape onto the hidden platform during the eight training days. (B-C, E-F) Tg females and Tg males exhibit comparable escape latencies compared to NonTg female and NonTg male mice, respectively. (G-H) Tg females and males spend comparable percentages of time in the platform zone compared to NonTg mice. However, Tg females, but not Tg males, spend an insignificant percentage of time in the platform zone than what is due to chance. (I-J) N5 TgCRND8 females and males have comparable mean spatial strategies during the training days. (K) No sex or genotypic differences in spatial composite scores observed. Statistics were (A-D) two-way repeated measures ANOVA with *post hoc* Šidák test or (E-J) unpaired t-tests or (K) one-way ANOVA comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.0001). N= 11-18 mice per sex.

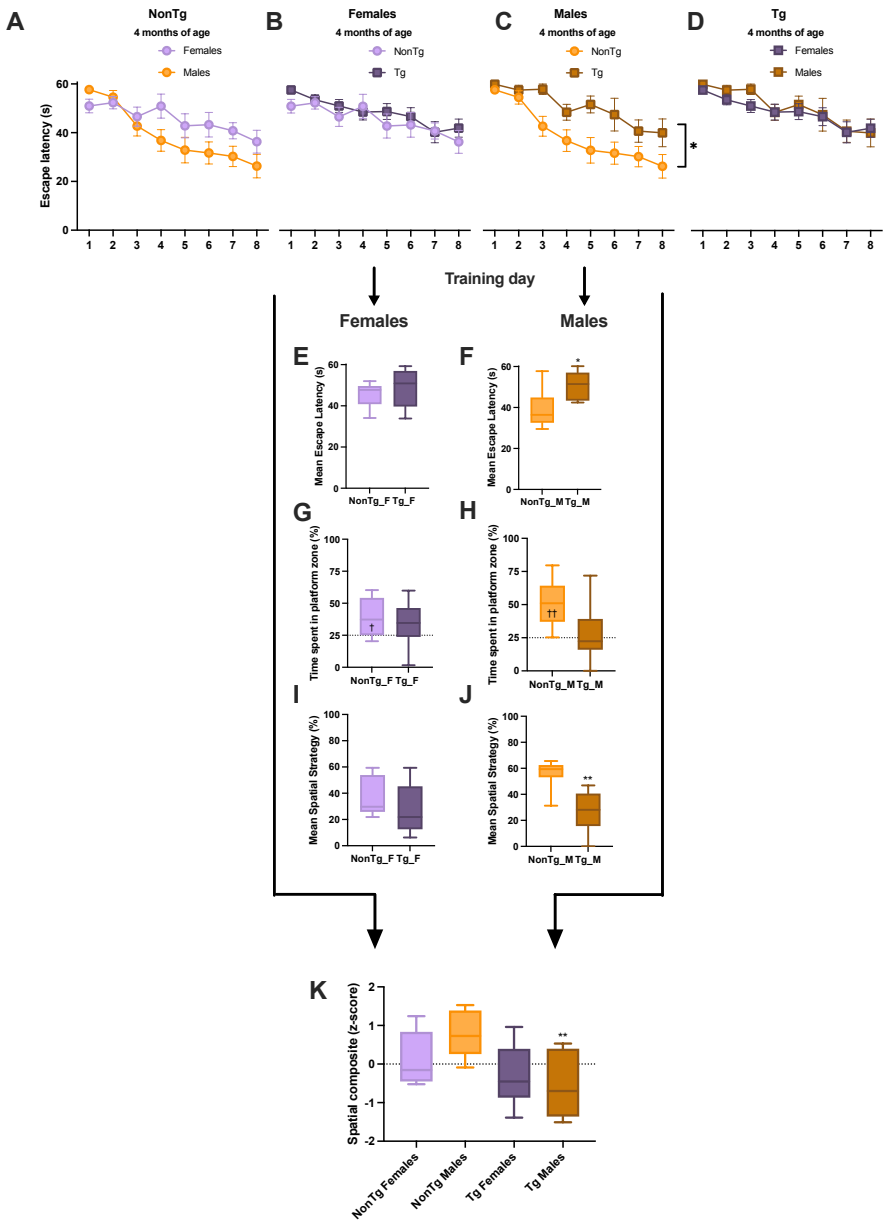

Figure 22 Tg male mice, but not Tg female mice, take longer to escape onto the hidden platform than NonTg male littermates at 4 months of age. (A) No inherent sex differences in escape latency are observed in mice at 4 months of age. (B-D; E-F) Male Tg mice, but not female Tg mice, demonstrate significantly longer escape latencies compared to NonTg male mice at 4 months of age. However, there is no sex difference in escape latency observed in Tg mice across the training days (D). (G-H) Both Tg female and Tg male mice do not spend significantly more time in the platform zone than what is due to chance (i.e., 25%). (I-J) Tg male mice, but not Tg female mice, demonstrate a significant decrease in the mean percentage of spatial strategy used across the training days compared to NonTg male mice. (K) Tg male mice, but not Tg female mice, exhibit significantly lower spatial composite scores compared to NonTg male mice. Statistics were (B) two-way repeated measures ANOVA with *post hoc* Šidák test or (C-F) unpaired t-tests comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.0001). N=7-16 mice per sex.

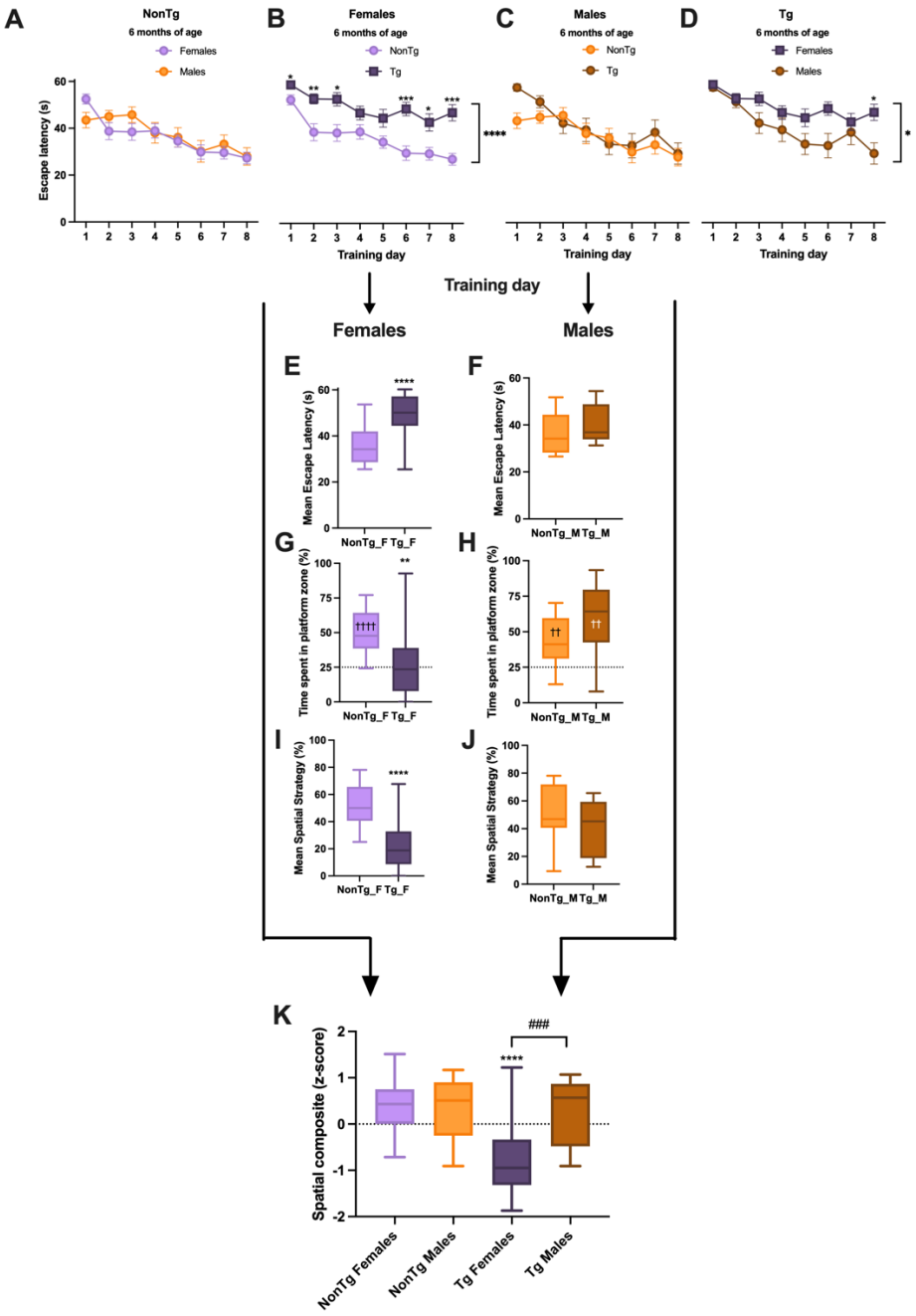

Figure 23 Tg female mice, but not Tg male mice, demonstrate severe visuospatial learning and memory impairment at 6 months of age. (A) No inherent sex difference in escape latency is observed in NonTg mice at 6 months of age. (B-C, E-F) Tg female mice, but not Tg male mice, exhibit significantly higher escape latencies compared to NonTg female mice at 6 months of age. (D) A sex difference is observed in Tg mice at 6 months of age, wherein Tg female mice take significantly longer to escape onto the hidden platform compared to Tg male mice. (E-F) Tg female mice, but not Tg male mice, spend an insignificant amount of time in the platform zone than what is due to chance (i.e., 25%) and exhibit significantly less mean spatial strategy (I-J). (K) Tg female mice exhibit significantly lower spatial composite scores compared to both NonTg female mice and Tg male mice. Statistics were (B) two-way repeated measures ANOVA with *post hoc* Šidák test or (C-F) unpaired t-tests comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.000. N=10-19 mice per sex.

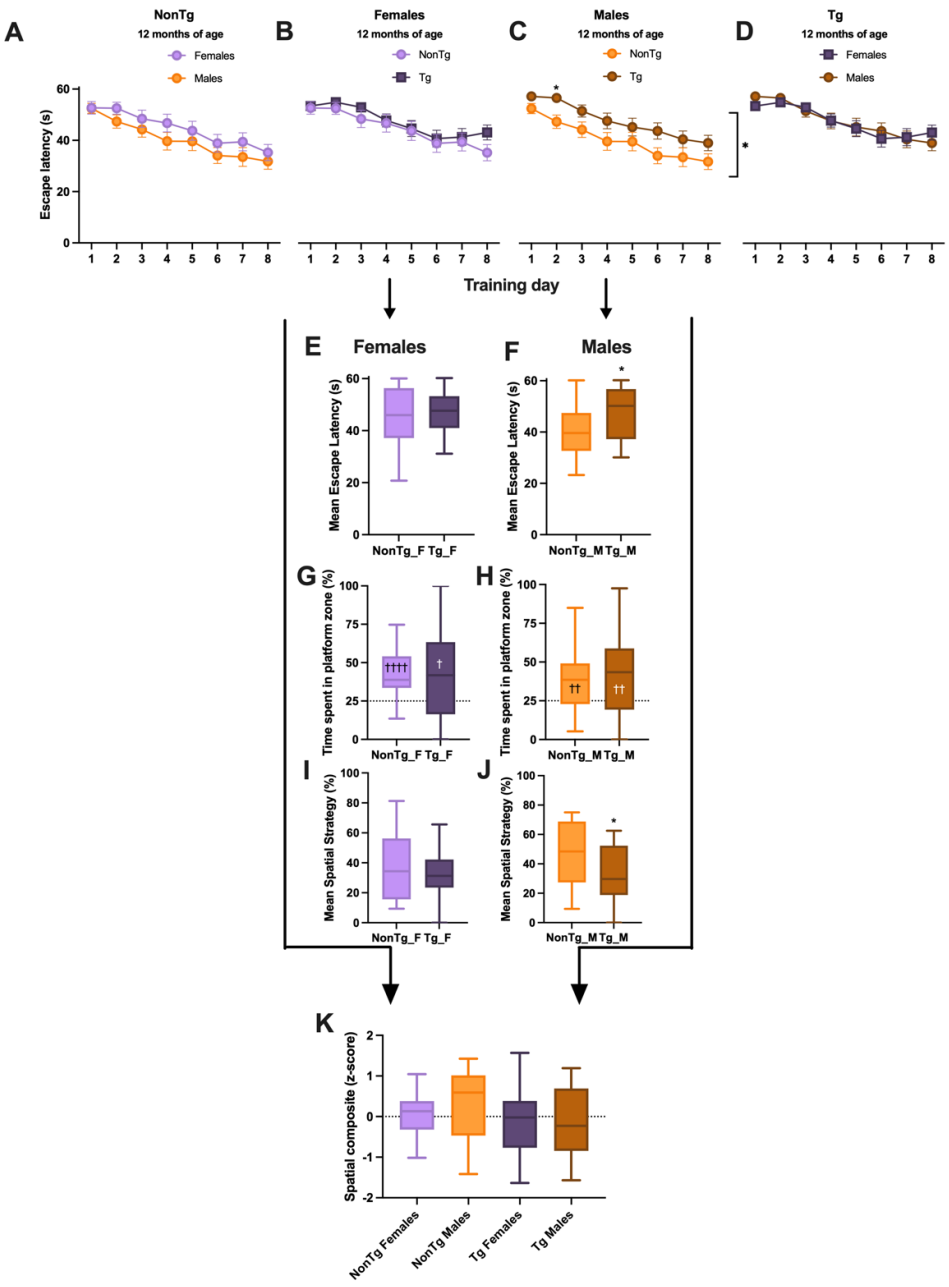

Figure 24 Tg male mice, but not Tg female mice, take longer to escape onto the hidden platform compared to NonTg male littermates at 12 months of age. (A,D) No sex difference in escape latency is observed in mice at 12 months of age regardless of Tg status. (B-C, E-F) Tg male mice, but not Tg female mice, take significantly longer to escape onto the hidden platform compared to NonTg male mice at 12 months of age. (G,H) Both NonTg and Tg mice regardless of sex spend significantly more time in the platform zone than what is due to chance. (I-J) Tg male mice, but not Tg female mice, exhibit a significant decrease in percentage of spatial strategy across the training days. (K) No sex or genotypic differences in spatial composite scores are observed at 12 months of age. Statistics were (B) two-way repeated measures ANOVA with *post hoc* Šidák test or (C-F) unpaired t-tests comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.0001). N=19-24 mice per sex.

3.13 N5 TgCRND8 female mice fail to transition to a spatial strategy and demonstrate lower cognitive reserve compared to N5 TgCRND8 male mice

To assess these differences in more detail, navigation strategy was assessed (Fig 25. A). There are three types of search strategy in MWM: (1) Spatial strategy, defined as the mouse's ability to navigate a direct path to the hidden platform using visual cues placed on the walls of the testing room; (2) Systematic strategy, a less productive strategy wherein the mouse explores the various quadrants of the pool to find the hidden platform and (3) Repetitive looping, a nonproductive search strategy, wherein a mouse continually swims in a looping or circling trajectory (Fig 25. B; Granger et al., 2016; Vorhees et al., 2006).

From 2-12 months of age, NonTg female and NonTg male mice successfully transition from a systematic to a spatial strategy across the MWM training days (Fig 25. B-E). However, at 2 and 4 months of age, N5 TgCRND8 mice, regardless of sex, fail to transition from a systematic to a spatial strategy (Fig 25. B-C). Strikingly, at 6 and 12 months of age, N5 TgCRND8 male mice overcome this initial deficit and transition to an increasingly productive spatial search strategy, while while N5 TgCRND8 female mice fail to make this transition exhibiting more nonproductive repetitive looping strategies (Fig 25. D, E).

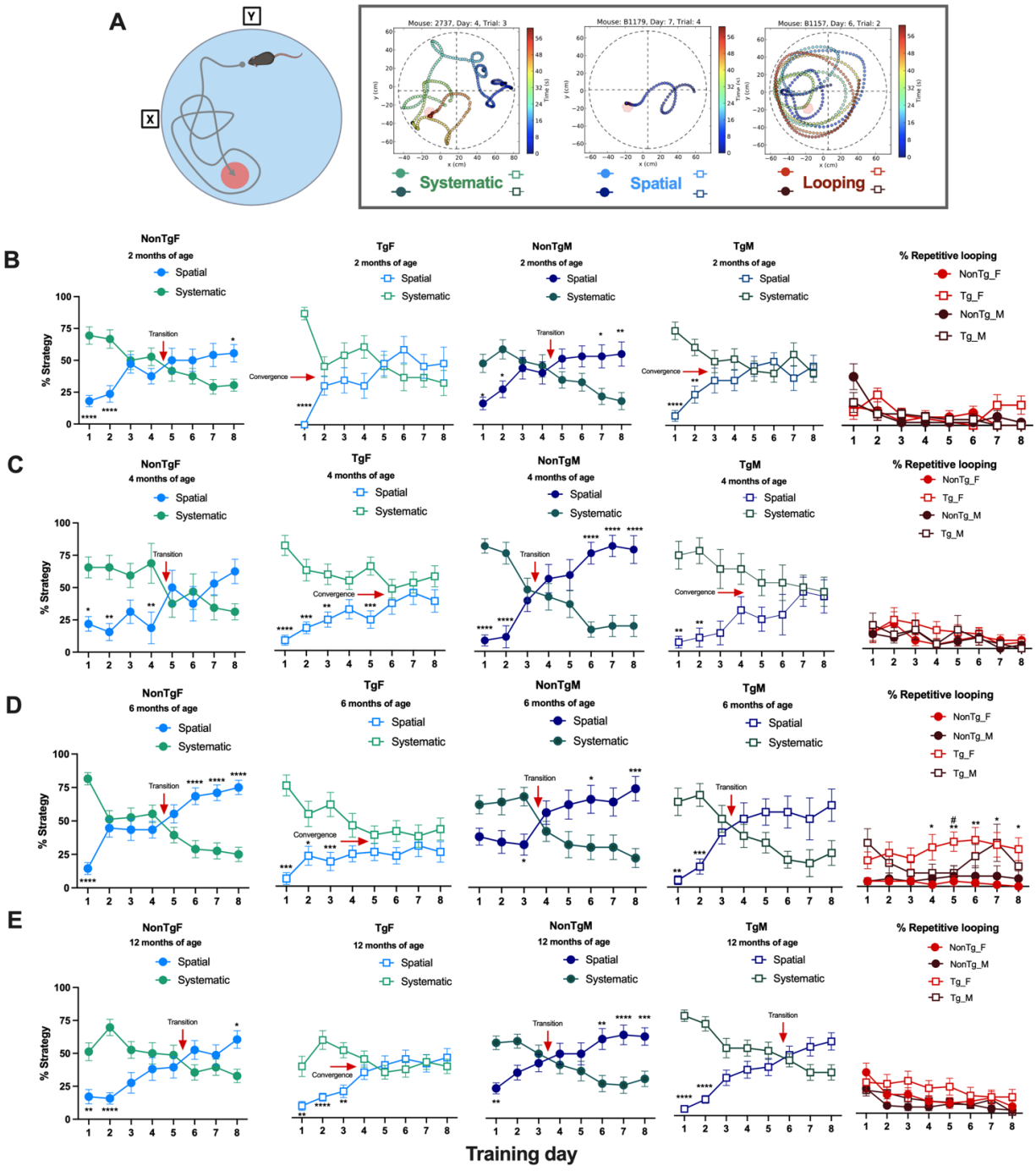
A
Y
X
Mouse: 2737, Day: 4, Trial: 3
Mouse: B1179, Day: 7, Trial: 4
Mouse: B1157, Day: 6, Trial: 2
Systematic
Spatial
Looping
B
C
D
E
NonTgF
TgF
NonTgM
TgM
2 months of age
4 months of age
6 months of age
12 months of age
Spatial
Systematic
% Strategy
Transition
Convergence
% Repetitive looping
NonTg_F
Tg_F
NonTg_M
Tg_M
Training day

Figure 25 Tg mice, regardless of sex, fail to switch to a spatial strategy at 2 months of age; however, Tg males, but not Tg females, overcome this deficit and transition to a spatial strategy at 6 months of age. (A) Schematics depict the experimental assessment of search strategy across the eight training days. The mice adopt one of three strategies on each sixty second MWM trial in order to find the hidden platform: systematic, spatial or repetitive looping. (B-C) NonTg females and NonTg males transition to a spatial strategy between day 4 and 5; however, Tg females and Tg males fail to transition and converge spatial and systematic strategies at 2 and 4 months of age. (B-C) No sex or genotypic differences in repetitive looping are observed at 2 and 4 months of age. (D-E) NonTg mice, regardless of sex, transition to a spatial strategy across the training days at 6 months of age. (D) Tg male mice, but not Tg female mice, transition to a spatial strategy at 6 months of age. Conversely, Tg female mice converge spatial and systematic strategies and subsequently exhibit significant increases in repetitive looping (a nonproductive strategy) compared to NonTg female mice (days 4-8) and Tg male mice (day 5). (E) At 12 months of age, Tg female mice converge systematic and spatial strategies at 12 months of age but do not show significant repetitive looping strategy adoption while Tg males mice transition to spatial. (E) No sex or genotypic differences in repetitive looping are observed at 12 months of age. Data represent mean $\pm$ SEM total of 120 NonTg mice (Females: n = 64; Males n = 56) and 121 Tg mice (Females: n = 67; Males n = 54). Statistics were (B) two-way repeated measures ANOVA with *post hoc* Šidák test or (C-F) unpaired t-tests comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.0001). N= 11-18 mice per sex (2 months of age) N=7-16 mice per sex (4 months of age). N=10-19 mice per sex (6 months of age). N=19-24 mice per sex (12 months of age).

3.14 N5 TgCRND8 mice exhibit higher mortality compared to NonTg littermates as they age

Given the variability in visuospatial memory impairment with aging, we asked if Tg animals exhibited a higher mortality than NonTg animals such that Tg animals with the greatest impairments might die earlier than more resilient Tg animals. N5 TgCRND8 mice demonstrated significant decreases in survivorship compared to NonTg littermates whether or not provided with acute access to running wheels (Fig 26. A). No sex differences were detected. N5 TgCRND8 female and N5 TgCRND8 male mice demonstrated comparable reduced percent survivorship with age (Fig 26. B-D).

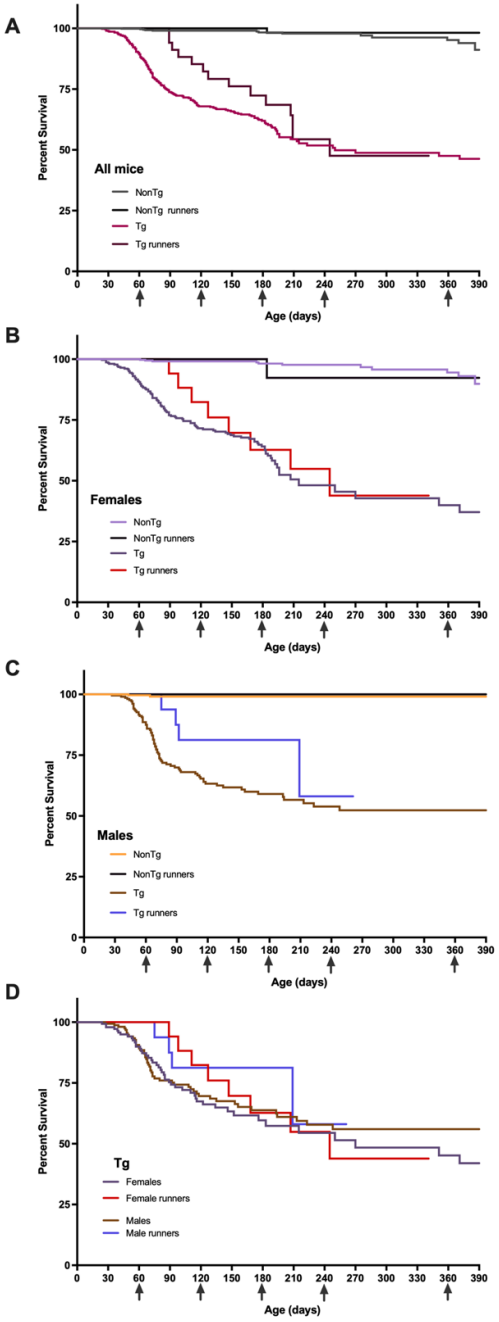

Figure 26 Survival is decreased in female and male N5 TgCRND8 mice compared to NonTg littermates. (A) N5 TgCRND8 mice (n = 527) have a higher mortality rate than NonTg (n = 746) mice. Median survival of Tg mice was 250 days compared to 624 for NonTg mice c^2=234.2, p < 0.0001. Median survival was not altered by acute access to running wheels p > 0.05. (B) Female Tg mice have lower median survival rate (215 days, n = 316) than NonTg females (624 days, n = 444) c^2=161.4, p < 0.0001. Acute access to running wheels extended median survival to 245 days but this extension was not statistically significant p < 0.05. (C) Male Tg mice reached 53% survival by 259 days (n=211) with significantly earlier mortality than NonTg males (n=302) c^2=86.6, p<0.0001. Acute access to running wheel had no impact on survival p < 0.05. (D) No sex difference in survival was detected between Tg animals (p>0.05). Statistics were Mantel-Cox log-rank tests comparing survival of the indicated groups.

3.15 N5 TgCRND8 mice do not exhibit sexually dimorphic differences in Aß42/40 burden in the temporal cortex and hippocampus as they age

Human Aß40 and Aß42 levels in the temporal cortex and hippocampus of N5 TgCRND8 mice were quantified and assessed longitudinally from 2-12 months of age (Fig 27). N5 TgCRND8 mice, regardless of sex, exhibit significant increases in the levels of Aß40 and Aß42 as they age, with Aß42 detected at higher levels from 2-8 months of age (Fig 27. A-B). No sex differences were detected (Fig 28. A-B). No cross-reactivity with murine Aß was detected in NonTg mice (Fig 27. B,C). We could not attribute the increase in mortality to a higher Aß load. Human Aß40 and Aß42 levels in the temporal cortex and hippocampus of N5 TgCRND8 mice who died pre-maturely were comparable to that of surviving Tg animals (Fig 27. A-B).

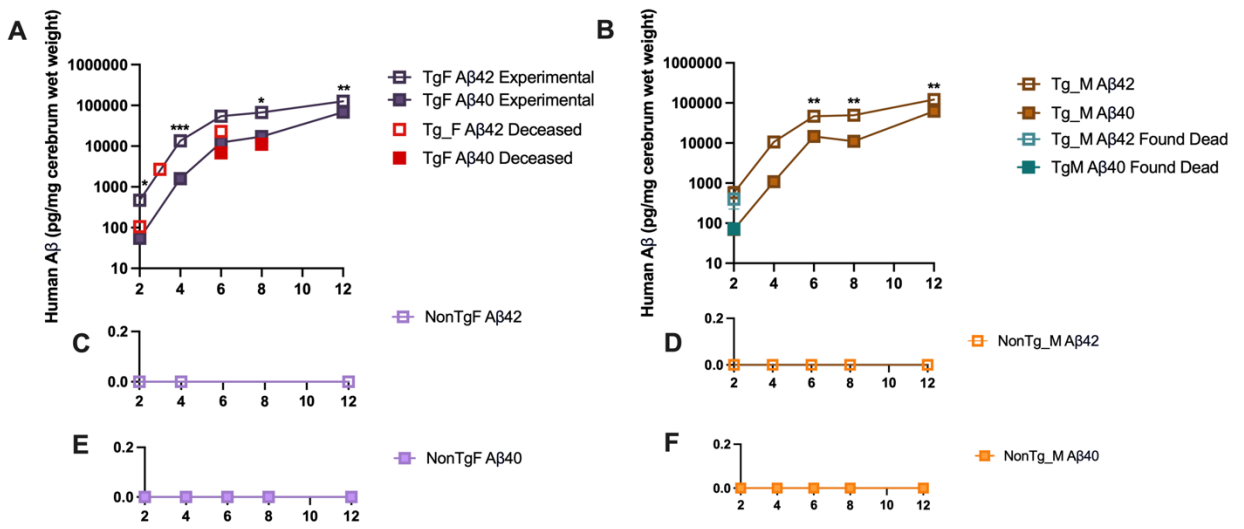

Figure 27 Human Aβ40 and Aβ42 levels in the temporal cortex and hippocampus of N5 TgCRND8 mice increase significantly with age. Human Aβ40 and Aβ42 peptides were quantified in the temporal cortex and hippocampus of N5 TgCRND8 mice by solid-phase sandwich Enzyme-Linked immunosorbent assays (ELISA Aβ40 and Aβ42). (A-B) Tg females and males exhibit significant increases in Aβ40 and Aβ42 deposition as they age from 2 to 12 months of age. Regardless of sex, Tg mice exhibit enhanced Aβ42 accumulation relative to Aβ40. (C-F) NonTg control samples yielded results below the detectable level of quantification (0 pg/mg). (A-B) Statistics were two-way mixed-effects ANOVA with *post hoc* Šidák test comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.0001). N= 4-5 Tg mice per sex (2 months of age) N=3-6 Tg mice per sex (4 months of age). N=5-6 Tg mice per sex (6 months of age). N=5-6 Tg mice per sex (12 months of age).

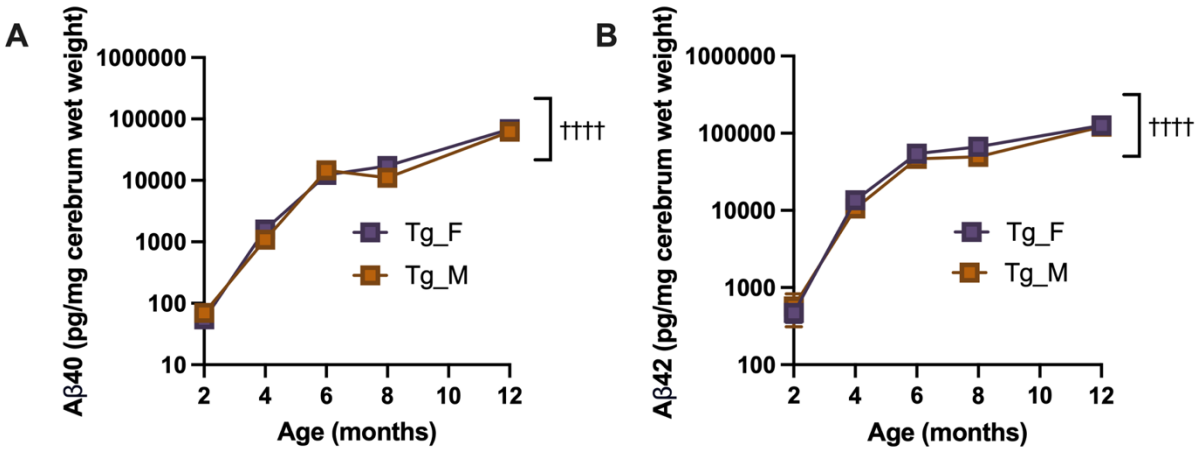

Figure 28 There is no sex difference in human Aβ40 and Aβ42 levels in the temporal cortex and hippocampus of N5 TgCRND8 mice as they age. (A-B) Female and male N5 TgCRND8 mice exhibit comparable levels of human Aβ40 and Aβ42 deposition in the temporal cortex and hippocampus as they age from 2-12 months of age. Human Aβ40 and Aβ42 levels increase significantly with age. (Statistics were two-way mixed-effects ANOVA with *post hoc* Šidák test comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.0001). N= 4-5 Tg mice per sex (2 months of age) N=3-6 Tg mice per sex (4 months of age). N=5-6 Tg mice per sex (6 months of age). N=5-6 Tg mice per sex (12 months of age).

3.16 N5 TgCRND8 mice are smaller and weigh less than NonTg mice

Body weights (g) were assessed in N5 TgCRND8 mice and NonTg littermates longitudinally from 2-12 months of age (Fig 29. A-D). NonTg male mice exhibited significantly higher body weight than NonTg females (Fig 29. A-D). Female and male N5 TgCRND8 mice weighed less than NonTg mice collapsed across age (Fig 29. A-D).

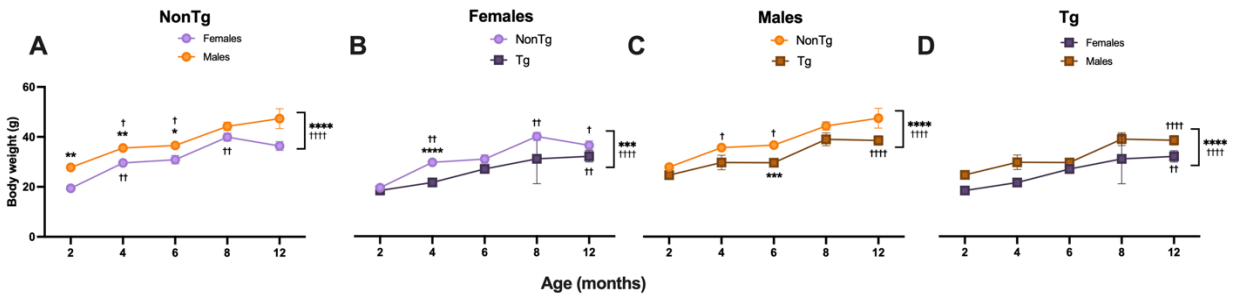

Figure 29 N5 TgCRND8 mice exhibit reduced total body weight compared to NonTg littermates. (A) NonTg male mice exhibit significantly greater total body weight compared to NonTg female mice. (B-C) N5 TgCRND8 mice, regardless of sex, demonstrate significant decreases in total body weight compared to sex-matched NonTg counterparts collapsed across age. (D) N5 TgCRND8 male mice exhibit significantly higher total body weight collapsed across age compared to N5 TgCRND8 female mice. (Statistics were two-way mixed-effects ANOVA with *post hoc* Šidák test comparing the indicated conditions (*p<0.05,**p<0.01,***p<0.001,****p<0.0001). N= 5-14 mice (2 months of age) N= 4-15 mice (4 months of age). N= 6-17 mice (6 months of age). N= 3-26 mice (8 months of age). N= 9-15 mice (12 months of age).

4 DISCUSSION

In this thesis, I assessed behavioural indices of ADL (i.e., nesting, sleeping, eating, drinking), BPSD (i.e., sleep-wake cycle, motoric hyperarousal, anxiety) and cognition (learning, memory, and strategy/cognitive reserve) in the N5 TgCRND8 model of Aß deposition from 1 to 12 months of age (Fig. 30, 31) with the intent of elucidating how age and sex interact to elicit different trajectories of behavioural decline. Surprisingly, I found that impairments, associated with ADL (i.e., NB, resting, food foraging and water-seeking behaviour) manifested early in life and improved when mice reached age equivalents of mature adults (i.e., 4-6 mo), before declining again in middle age (i.e., 12-14 mo) (Flurkey et al., 2007). Sex differences were task-specific. Middle aged females were more severely impaired than males in NB, food foraging behaviours while middle aged males were more severely impaired than females in resting and water-seeking behaviours. Similarly, behavioural indices of BPSD were evident early in life. Female and male Tg mice showed motoric hyperarousal in YM and MWM as early as 2 months of age and continued to show this phenotype across their lifespan. When provided with running wheels, males showed earlier hyperarousal than females attributed to an inherent sex difference wherein young NonTg females run more than young NonTg males, a behaviour that declines with age. Both sexes showed decreased anxiety compared to NonTg littermates, associated with increased activity and exploration in novel environments. Finally, female and male Tg mice showed sex differences in visuospatial working memory, with females being impaired earlier than males at age equivalents of mature adult, however, they showed comparable age-dependent decline. Importantly, Tg males at age equivalents of mature adult were able to transiently overcome visuospatial deficits by adopting more productive navigation strategies (i.e., cognitive reserve). Accumulation of human Aß40 and Aß42 levels increased with age in N5 TgCRND8 mice without any sexual dimorphism.

Taken together, these data provide evidence of sex and age-dependent biological differences in behavioural response to accumulating Aß, independent of Aß load.

The early onset of behavioural impairment seen in this thesis was surprising. Initial AD has been assumed to be clinically silent in humans thus is defined biologically by increasing neuropathological Aß burden in the brain that begins decades prior to the onset of cognitive symptoms. Here, I show that in a rapid onset model of Aß accumulation behavioural changes can indeed be seen prior to the onset of cognitive deficits. It should be noted that the TgCRND8 mouse is an aggressive model of Aß deposition, thus at 2 months of age levels are comparable to that observed in late adult humans, enabling us to accelerate "biological" AD (Chisti et al., 2001; Granger et. al., 2016). Alzheimer described the first AD patient case in 1906, and yet after nearly one hundred years of scientific investigations we still do not understand the underlying sex-specific biological mechanisms by which initial AD leads to the disproportionally rapid deterioration of cognitive status in women. Thus, the trajectories investigated in this thesis uniquely show capacity of males to transiently overcome cognitive correlates of Aß burden by adopting different search strategies in the MWM and transiently improving ADL-like behaviours. This preclinical investigation further supports the concept that AD is a *sex-specific* biological disease of aging. We cannot develop successful AD therapeutics that rely on the assumption that age and sex act in isolation to enhance AD risk when it is their *intersection* that drives clinical AD progression. The female sex is one of the strongest risk factors for AD dementia, yet we fail to consider that AD may mean something biologically different in women compared to men as modeled in this thesis.

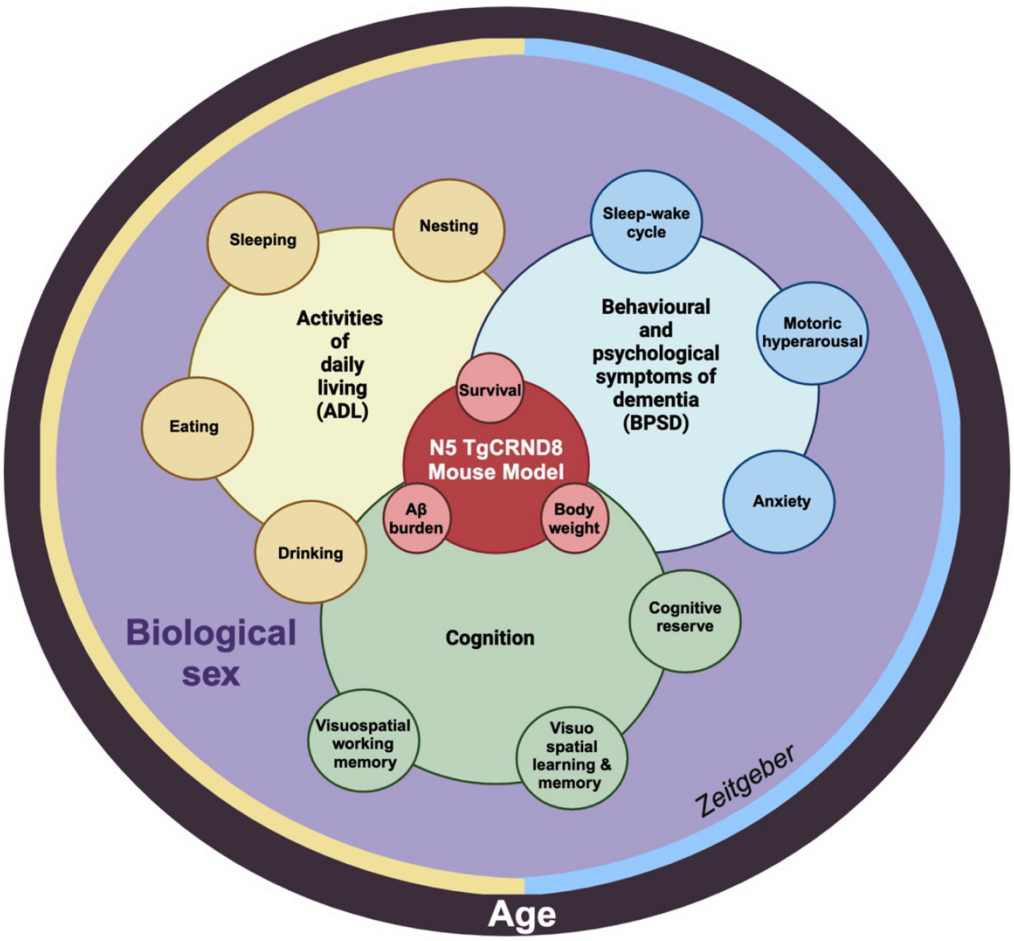

Figure 30 Variables studied in the N5 TgCRND8 mouse model. Behavioural indices of ADLs (i.e., nesting, sleeping, eating, drinking), BPSDs (i.e., sleep-wake cycle, motoric hyperarousal, anxiety) and cognition (i.e., visuospatial working memory, visuospatial learning and memory and cognitive reserve) were assessed in N5 TgCRND8 mice (i.e., decreased survival, increased Aß40/42 burden) at the intersection of age and biological sex. Created with BioRender.com

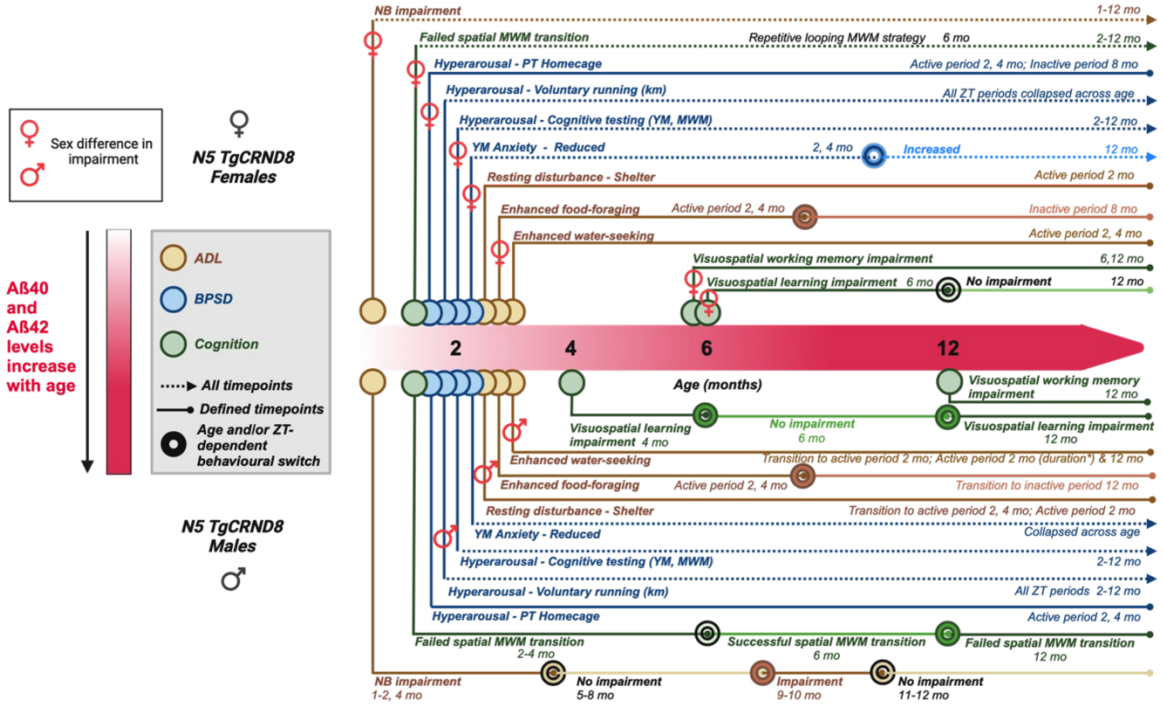

Figure 31 N5 TgCRND8 mice exhibit age-dependent sexual dimorphisms in behavioural indices of cognitive decline despite identical behavioural and gross phenotypic presentation. Behavioural indices of ADL impairment in Tg mice manifested as the inability to construct quality nests, restlessness, enhanced food foraging and water-seeking behaviours. Behavioural indices of BPSD in young Tg mice manifest as hyperarousal and reductions in anxiety when introduced to novel environments. Tg female mice demonstrate impairments in NB, visuospatial working memory and visuospatial learning and memory either earlier and/or more severe than Tg male mice at 6 months of age despite identical Aß40/42 load. Created with BioRender.com

4.1 N5 TgCRND8 female mice demonstrate more severe and/or accelerated trajectories to behavioural indices of ADL impairment

The progressive decline in the ability to carry out ADLs is characteristic of AD (Edemekong et al., 2024; Graessel et al., 2009; Palacios-Navarro et al., 2022). NB is a complex home cage behaviour in mice that reflects human ADLs (Fig 1.A) (Deacon, 2006). The ability of mice to construct high quality nests depends on healthy hippocampal function and engages executive functions, such as planning and organization (Deacon & Rawlins, 2005; Filali et al., 2009; Guenther et al., 2001). In this thesis, I report that N5 TgCRND8 female mice demonstrate earlier and/or more severe behavioural indices of ADL impairment compared to N5 TgCRND8 male mice. In humans, subjective memory loss (SMC) combined with iADL impairment is an early preclinical sign of dementia (Cloutier et al., 2021; Dubbelman et al., 2020; Vidoni et al., 2010; Yam & Marsiske, 2013). While few studies have addressed potential sex differences in ADL decline, women are more likely to report early SMC compared to men and SMC severity has been linked with impairments in iADLs and depressive symptoms (Cordier et al., 2019; J. Li et al., 2022; Ryu et al., 2016). It is interesting that in serotonin depleted mice, nesting behaviour is disrupted and in humans, women are twice as likely to be diagnosed with depression compared to men (Albert, 2015; Lerch-Haner et al., 2008). Further, there is a strong relationship between hippocampal lesion volume and the ability to carry out ADLs in humans (Ardekani et al., 2016; Peng et al., 2014; Vidoni et al., 2010). These findings are consistent with the possibility that severe ADL impairment in N5 TgCRND8 female mice may be due to faster hippocampal atrophy rates observed in women compared to men.

The ADL-associated disturbances in resting, food- and water-seeking behaviours observed in this thesis may indicate early, underlying hypothalamic dysfunction in N5 TgCRND8 mice. In this thesis, I found that N5 TgCRND8 mice, regardless of sex, demonstrated a restless and enhanced food- and water-seeking phenotype exclusively in the transition to and/or during the active period at the earliest age of longitudinal inquiry (i.e., 2 months of age). Changes in appetite and/or eating behaviour are common in AD and have been hypothesized to play a role in the unintentional body weight loss observed in AD patients (Gillette-Guyonnet et al., 2000; Mungas et al., 1990; Sfera et al., 2016). Further, chronic dehydration may be an important risk factor for AD (Gharibzadeh & Hoseini, 2007; Manaugh et al., 2022; Stoothoff & Johnson, 2001). It is fascinating that while N5 TgCRND8 mice, regardless of sex, seek food and water more often early in age, they exhibit markedly reduced body weight across their lifespan. It is well-documented that hypothalamic atrophy is an early neuropathological manifestation of AD that precedes cognitive decline (Ishii & Iadecola, 2015; Loskutova et al., 2010; Tao et al., 2021). Among its many functions, the hypothalamus is responsible for regulating the sleep-wake cycle, thirst and hunger; all of which were behaviourally evaluated in N5 TgCRND8 mice (Brown et al., 2015; Hopkins et al., 2000; Morton et al., 2014; Ono & Yamanaka, 2017; Sfera et al., 2016; Yeo & Heisler, 2012). It is fascinating that in anticipation and/or response to the lights OFF cue, N5 TgCRND8 mice become restless, yet in response to the lights ON cue they did not show this phenotype. Restless behaviour in N5 TgCRND8 mice is thus facilitated by an environmental switch (i.e., light to dark), which is linked biologically to the entrained, endogenous circadian cycle.

The hypothalamus regulates circadian sleep-wake cycles through the SCN, which has been reported to degenerate early in AD and is linked with sleep fragmentation (Harper et al., 2005; Singer & Alia, 2022). Hypothalamic-Pituitary-Adrenal (HPA) axis hyperactivity, in particular

increased cortisol levels, have also been reported early in AD and promote the initiation of wakefulness (Csernansky et al., 2006; Ennis et al., 2017; Lupien et al., 1999; Ouanes & Popp, 2019; Rothman & Mattson, 2010). Interestingly, increased morning cortisol levels have been found in AD patients, which coincides with restlessness observed in N5 TgCRND8 mice during the transition to active period (Yao et al., 2021). During this restless period, N5 TgCRND8 mice demonstrate enhanced food- and water-seeking behaviours. It appears that restlessness and enhanced sensations of appetite and thirst interact during the active period to promote increased wakefulness in N5 TgCRND8 mice. The behavioural findings of this thesis are consistent with the possibility that the SCN degenerates early in N5 TgCRND8 mice and that this is linked to endogenous circadian disturbance (i.e., sleep-wake cortisol secretion) which may underlie and/or promote cognitive decline at later ages of longitudinal inquiry.

4.2 N5 TgCRND8 mice demonstrate a ZT-specific, hyperaroused phenotype that is enhanced when provided with a productive and repetitive physical exercise task

Motoric hyperactivity is a common BPSD (Csernansky et al., 2006; Lupien et al., 1999; Ouanes & Popp, 2019; Yao et al., 2021). It manifests as increased spontaneity of movements, such as pacing, wandering, agitation and/or repetitive, purposeless behaviours (Cerejeira et al., 2012; Khachiyants et al., 2011). The biological relationship between motoric hyperactivity, sleep-wake dysfunction and Aß neuropathology has yet to be fully elucidated. However, current scientific evidence supports that motoric hyperactivity occurs early in AD and precedes significant Aß burden (Ambrée et al., 2006; T. Wang et al., 2022).

In this thesis, I found that young N5 TgCRND8 mice demonstrate a hyperaroused phenotype that is greatly exacerbated across age and over the ZT cycle when provided with a

productive, repetitive running wheel task. It is important to discern hyperarousal from restlessness. Here, I refer to 'restlessness' as the inability to rest and represents a behavioural indice of ADL-associated sleep (i.e., shelter behaviours across the ZT cycle), whereas the term 'hyperarousal' refers to increased spontaneity (i.e., total number of zone transits) or intensity (i.e., distance ran on the running wheel) of movements. While restlessness and hyperarousal can occur concurrently, in this thesis I argue that the hyperaroused state of N5 TgCRND8 promotes restlessness, which may contribute to and/or underlie sleep-wake dysfunction. In a homecage-like environment, young N5 TgCRND8 mice are hyperaroused exclusively in the transition to and/or during the active period, as evidenced by increased spontaneity of zone transits. However, when they are provided with a repetitive, high-intensity physical exercise task, the hyperaroused state of N5 TgCRND8 mice extended to the transition to inactive period and inactive period. Importantly, this suggests that altering the home cage environment plays a role in promoting restlessness in N5 TgCRND8 mice, an important point of consideration in the home care of persons with AD.

4.3 Young N5 TgCRND8 mice exhibit markedly reduced anxiogenic-like behaviours that may indicate early dysfunction in limbic neurocircuitry

Anxiety is a common neuropsychiatric symptom in preclinical AD and the severity of anxiety symptoms have been linked to faster cognitive decline (Cerejeira et al., 2012; Pietrzak et al., 2015). Further, women are significantly more likely to be diagnosed with an anxiety disorder compared to men (McLean et al., 2011). In this thesis, I found that young N5 TgCRND8 mice exhibit markedly reduced anxiogenic-like behaviours, associated with their increased hyperarousal, that is accompanied by an age- and sex-dependent behavioural switch wherein increasingly anxiogenic behaviours are observed in older N5 TgCRND8 female mice. It is

fascinating that when young N5 TgCRND8 mice are introduced to an unfamiliar environment (i.e., Y-maze), they initiate rapid exploration of the area through a hyperaroused state. It appears that young N5 TgCRND8 demonstrate a reduced behavioural response to detect for possible threats, which is a biological response regulated by the amygdala (Hopper & Vogel, 1976). Importantly, amygdala atrophy has been reported to occur early in AD and may underlie preclinical neuropsychiatric symptoms such as anxiety and aggression (Hopper & Vogel, 1976; Poulin et al., 2011). The behavioural findings of this thesis are consistent with the possibility that limbic neurocircuitry is dysfunctional early in N5 TgCRND8 mice and the severity of this dysfunction may be linked to increased anxiogenic-like behaviours in older N5 TgCRND8 female mice.

4.4 N5 TgCRND8 female and N5 TgCRND8 male mice exhibit age-dependent sexual dimorphisms in behavioural indices of cognitive decline despite identical gross phenotypic presentation

The N5 TgCRND8 mouse model behaviourally recapitulates sex differences observed in human AD (Hua et al., 2010; Mielke et al., 2014; Sachdev et al., 2009). While men have a higher incidence of non-amnestic MCI, women disproportionally transition to AD and exhibit accelerated trajectories to cognitive decline (Ardekani et al., 2016; Hua et al., 2010; Mielke et al., 2014). Non-amnestic MCI involves intact memory with one or more cognitive domains being negatively affected, such as language and/or executive functions (Csukly et al., 2016). Persons with non-amnestic MCI may have trouble making sound decisions and/or performing the sequence of steps needed to complete a complex task (Csukly et al., 2016). In this thesis, I report that the N5 TgCRND8 mouse model exhibits age-dependent sexual dimorphisms in behavioural indices of visuospatial working memory, visuospatial learning and memory and cognitive reserve despite

statistically identical longitudinal Aß40/42 challenge and survivorship. Two-month old N5 TgCRND8 female and N5 TgCRND8 male mice had defined cognitive and gross phenotypic presentations. Young N5 TgCRND8 mice were impaired in their ability to carry out ADLs (i.e., NB), fail to adopt a spatial MWM search strategy and exhibit markedly increased Aß40 and Aß42 brain levels. Yet at six months of age, sex became a determining factor of vastly different cognitive trajectories in N5 TgCRND8 mice. Six-month old N5 TgCRND8 female mice were severely impaired in behavioural indices of spatial working memory, visuospatial learning and memory and cognitive reserve (i.e., the ability to switch to more productive navigation strategies) compared to N5 TgCRND8 male mice. There must be a sex-specific, metabolic underpinning to the finding that six-month old N5 TgCRND8 male mice are able to overcome initial deficits in visuospatial learning and memory, while N5 TgCRND8 female mice fail to do so under identical Aß brain burden and associated mortality. The behavioural findings of this thesis are consistent with the possibility that biological AD means something different metabolically for women that underlies Aß neuropathology. Sex-specific vulnerabilities in AD (e.g., genetic, immune, vascular and hormonal related factors) have been linked to aberrant lipid metabolism and indicates the need for sex-specific therapeutic investigations (Ancelin et al., 2013; González-Domínguez et al., 2022; Strefeler et al., 2023) thus future investigation should examine sex-specific metabolic responses that may underlie these differences.

4.5 Conclusion

In this thesis, behavioural indices of ADLs (i.e., nesting, sleeping, eating, drinking), BPSD (i.e., motoric activity, anxiety, perseveration) and cognition (i.e., spatial working memory, visuospatial learning and memory, cognitive reserve) were characterized longitudinally in N5 TgCRND8 mice alongside Aß40 and Aß42 levels in the temporal cortex and hippocampus from birth to death (i.e., 1-12 months of age) (Fig 31). The primary findings of this thesis are two-fold. Impairments in behavioural indices of ADL, BPSD and cognitive reserve (i.e., ability to adopt more productive MWM navigation strategies) manifested early in life in N5 TgCRND8 mice at human age equivalents of pre-mature adults (Flurkey, 2007). In contrast, sex-specific impairments in visuospatial working memory, visuospatial learning and memory and cognitive reserve were detected in N5 TgCRND8 mice later in life at human age equivalents of mature adults under identical longitudinal Aß (Aß40 and Aß42) challenge between females and males. Thus, the N5 TgCRND8 mouse model is a valuable preclinical tool that has provided insight into how age and sex interact with initial (biological) AD neuropathology to elicit early ADL and BPSD behavioural impairment in N5 TgCRND8 mice that later manifests as sex-specific cognitive decline. Future investigations would benefit from using N5 TgCRND8 mice as a preclinical, sex-specific AD model wherein therapeutics that target Aß vulnerabilities exhibited by females can be tested.

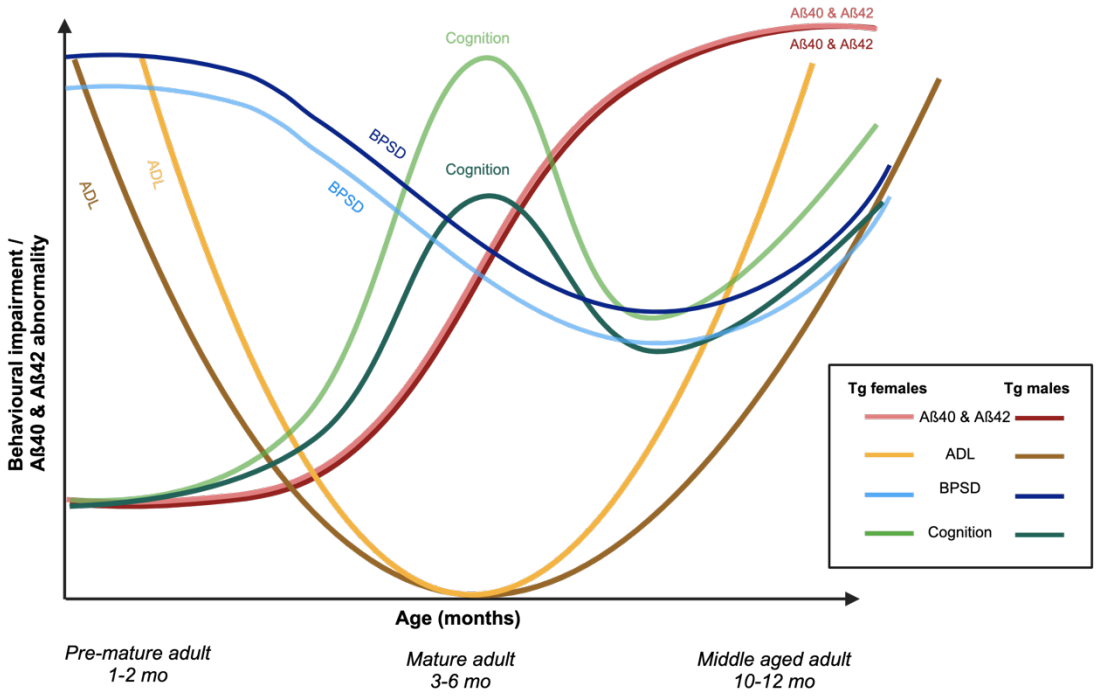

Figure 32 Summary of the age- and sex-dependent progression of behavioural indices of ADL impairment, BPSD onset and cognitive decline in the N5 TgCRND8 mouse model. Tg females and Tg males exhibited statistically comparable Aß40 and Aß42 load in the temporal cortex and hippocampus as they aged. ADL impairment (i.e., nesting, resting, food foraging and water-seeking behaviours) manifested early in life at human age equivalents of pre-mature adults and were generally more severe in Tg females. Similarly, behavioural indices of BPSD (i.e., wake dysfunction/restlessness, hyperarousal and anxiety) also manifested at an early age in N5 TgCRND8 mice but were generally more pronounced in males. Behavioural indices of cognitive impairment were detected in N5 TgCRND8 mice later in age at human age equivalents of mature adults and Tg females demonstrated more severe impairment. Importantly, mild visuospatial learning and memory impairments were detected in N5 TgCRND8 mice regardless of sex as early as two months of age. (Flurkey, 2007) Created with BioRender.Com

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