School of Psychology

Depression and Dementia: The Effect of Amyloid Pathology and Therapeutic Interventions on Affective and Cognitive Processes in Mice

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Summary

Preclinical mouse models of Alzheimer's disease capture cognitive impairments which can be attributed to β-amyloid (Aβ) pathology. Aβ pathology may also be an antecedent of depressive symptoms in these mouse models, though this has been less rigorously investigated. This thesis reports a longitudinal investigation of both the depressive symptom of anhedonia (a reduction in pleasurable reactions), and memory of specific object-location associations, in the Tg2576 mouse model of Alzheimer's disease. The effectiveness of sub-anaesthetic ketamine, largely as an anti-depressant treatment, was also examined in anhedonic and cognitively impaired Tg2576 mice. Possible biochemical underpinnings of these deficits were then investigated, namely the glutamate, serotonin and opioid signalling systems. Tg2576 mice displayed an age-dependent reduction in hedonic reactions, consistent with an Aβ-related deficit. While object-in-place testing did not explicitly reveal an age-dependent dysfunction, Tg2576 mice were only impaired later in life, consistent with aging and Aβ underlying their diminished performance. An additional T-maze task revealed that aged Tg2576 mice retained a preference for spatial novelty.

While ketamine treatment increased expression of measures relating to the AMPA receptor GluR1 subunit, it did not improve hedonic or cognitive dysfunction in Tg2576 mice. Tg2576 mice displayed a relative reduction in Y1472 phosphorylation of the NMDA receptor NR2B subunit in the hippocampus, suggesting an NMDAR-mediated dysfunction underlying their object-in-place deficit. Investigation of the opioid system in Tg2576 mice revealed elevations of cortical kappa and hippocampal mu receptor expression. An unbalanced opioid system may therefore diminish hedonic tone in Tg2576 mice. Tg2576 mice also displayed elevated cortical serotonin transporter expression, though the relation of this to behavioural deficits was less clear. This thesis demonstrates that Aβ and its potential effects on the glutamate and opioid systems can underlie both amnesic and affective symptoms in Tg2576 mice, though an attempt to remedy these impairments was unsuccessful.

Acknowledgements

List of Conference Presentations

Adam Brelsford, Jasmine Clarkson, Jessica Hall, Charles Evans, Dominic Dwyer, Mark Good (April 2017). Depression in a preclinical mouse model of Alzheimer's disease: a hedonic deficit not mediated by hippocampal dysfunction. BNA 2017: Festival of Neuroscience. (Based on preliminary experiments which are not presented in this thesis.)

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List of Abbreviations:

3-HAA: 3-hydroxy-anthranilic acid

3-HK: 3-hydroxy-kynurenine

5-HIAA: 5-hydroxyindoleacetic acid

5HT: 5-hydroxytryptamine

α7-nAChR: α7-nicotinic acetylcholine receptor

Aβ: β-amyloid

ACC: Anterior cingulate cortex

AICD: APP intracellular domain

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AMPAR: AMPA receptor

ANOVA: Analysis of variance

APOE: Apolipoprotein E

APP: Amyloid precursor protein

BCA: Bicinchoninic acid

BDNF: Brain-derived neurotrophic factor

BF: Bayes factor

BOLD: Blood oxygenation level-dependent

BPSD: Behavioural and psychological symptoms of dementia

BSA: Bovine serum albumin

CA1: Cornus ammonis 1

CA3: Cornus ammonis 3

Ca^{2+}: Calcium

CaMKII: Calcium/calmodulin-dependent protein kinase II

CSF: Cerebrospinal fluid

DSM: Diagnostic and Statistical Manual of Mental Disorders

ECL: Enhanced chemiluminescence

ELISA: Enzyme-linked immunosorbent assay

fMRI: Functional magnetic resonance imaging

GLT-1: Glutamate transporter 1

HRP: Horseradish peroxidase

ICSS: Intracranial self-stimulation

IDO: Indoleamine 2,3-dioxygenase

IL-1β: Interleukin-1β

ILI: Inter-lick interval

LTP: Long-term potentiation

LPS: Lipopolysaccharide

MAGUKS: Membrane-associated guanylate kinases

MCI: Mild cognitive impairment

Mg^{2+}: Magnesium

mRNA: Messenger RNA

Ms: Millisecond

MSE: Mean square error

NA: Noradrenaline

NFT: Neurofibrillary tangle

NMDA: N-methyl-D-aspartate

NMDAR: NMDA receptor

NPS: Neuropsychiatric symptoms

PAGE: Polyacrylamide gel electrophoresis

PCR: Polymerase chain reaction

PFC: Prefrontal cortex

PKA: Protein kinase A

PKC: Protein kinase C

PSD-95: Postsynaptic density protein-95

PSEN1: Presenilin-1

PSEN2: Presenilin-2

sAPPα: Soluble APP-α

SDS: Sodium dodecyl sulphate

SEM: Standard error of the mean

SERT: Serotonin transporter

SNRI: Serotonin-noradrenaline reuptake inhibitor

SSRI: Selective serotonin reuptake inhibitor

TBST: Tris-buffered saline with Tween20

TEMED: Tetramethylethylenediamine

TNFα: Tumour necrosis factor-α

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Chapter 1: Introduction

1.1 Alzheimer's disease

1.1.1 Background

Alzheimer's disease, first described in 1906 by Alois Alzheimer, is the commonest form of dementia, making up ~62% of dementia cases in the UK (Alzheimer's Society, 2014), and characterised by the presence of β-amyloid (Aβ) plaques and neurofibrillary tau tangles in the brain (Serrano-Pozo, Frosch, Masliah, & Hyman, 2011). Dementia is a syndrome comprising various symptoms, characterised by a marked cognitive decline that is sufficient to impede the independent activities of daily living (Sachdev et al., 2014). Dementia is both a devastating and costly disease syndrome, with no curative treatment currently available. Patients progressively deteriorate over years or decades until death, with a societal cost estimated financially as £26 billion in the UK each year, and $817 billion globally (Alzheimer's Research UK, 2017). The number of people living with dementia is projected to rise from 50 million in 2018 to 152 million in 2050, meaning effective therapies will only become more and more vital (World Health Organisation, 2017). Causes of dementia including Alzheimer's disease place an immense personal and financial burden on caregivers of dementia patients (Förstl & Kurz, 1999; Haro et al., 2014).

Alzheimer's disease is largely an age-related phenomenon; its prevalence rises with age, notably so after the age of 65 (Mayeux & Stern, 2012). Between 60 and 85 years of age, prevalence increases almost 15-fold, and estimates suggest that the incidence rate of Alzheimer's disease roughly doubles every 5 years after the age of 60 (Launer et al., 1999; Mayeux & Stern, 2012). In the absence of effective interventions, the burden of late-onset dementia (the majority of which is due to Alzheimer's disease) is projected to worsen in the UK (Lewis & Torgerson, 2017). Alzheimer's disease is thus a medical condition in urgent need of investigation, an urgency which is only set to increase with time.

The onset of Alzheimer's disease can be early (under the age of 65) or late (at or over 65); early-onset cases tend to be more severe and progress rapidly in comparison to late-onset Alzheimer's disease (Reitz, Brayne, & Mayeux, 2011). While not all the causes of early-onset Alzheimer's disease are known, one underlying cause is inherited autosomal dominant mutations with a high penetrance, in cases referred to as familial Alzheimer's disease (Reitz et al., 2011). Cases of Alzheimer's disease which do not result from heritable high penetrance genetic mutations are known as sporadic Alzheimer's disease. Familial and sporadic Alzheimer's disease share a similar

clinical presentation (Duara et al., 1993), and the same underlying neuropathology (accumulated Aβ and tau formations) (Lippa et al., 1996). Familial Alzheimer's disease cases make up roughly 5% of all Alzheimer's disease cases, and the causative autosomal dominant genetic mutations can occur at three genetic loci – amyloid precursor protein (APP), presenilin 1 (PSEN1) and presenilin 2 (PSEN2) (Bekris, Yu, Bird, & Tsuang, 2010). These genetic mutations either lead to an increase in total Aβ production, or increase production of Aβ42 relative to Aβ40 (Bekris et al., 2010; Citron et al., 1997; Potter et al., 2013; Scheuner et al., 1996). Sporadic Alzheimer's disease cases comprise the majority of Alzheimer's disease patients, with genetic and other risk factors being the underlying causes, most likely due to their affecting either the production or clearance of Aβ. The most significant genetic risk factor is the ε4 polymorphism of the apolipoprotein E (APOE) gene (Corder et al., 1993; Liu, Liu, Kanekiyo, Xu, & Bu, 2013); two copies of the APOEε4 allele confer a greatly increased risk of developing Alzheimer's disease (Liu et al., 2013). Genes associated with a smaller degree of risk include TREM2, PICALM, ABCA7 and BIN1 (Jonsson et al., 2013; Tanzi, 2012). Outside of genetics, biological changes that occur with aging may increase the risk of Alzheimer's disease, such as poorer clearance or degradation of the Aβ protein with age (Silverberg et al., 2010), diminished blood-brain barrier integrity in the elderly (Marques, Sousa, Sousa, & Palha, 2013), or other factors.

1.1.2 Symptoms of Alzheimer's disease

Alzheimer's disease exhibits a wide range of symptoms, which typically worsen with disease progression, with the cardinal and most well-known being memory decline. In addition to this well-recognised memory impairment, Alzheimer's disease patients exhibit a number of psychiatric symptoms, including psychosis and depression (Lopez et al., 2003). While the linkage between disease pathology and impaired memory has been the focus of much research (see, for example, Section 1.1.6), it remains unclear how the pathological processes underlying Alzheimer's disease could cause depression or certain symptoms thereof. The pathological cascade leading to Alzheimer's disease is thought to begin with Aβ (see Section 1.1.4), and a major aim of this thesis is to investigate whether the accumulation of Aβ is sufficient to induce depressive behaviour in a mouse model of Alzheimer's disease. The memory-related symptoms of Alzheimer's disease are discussed immediately below, and the depressive symptoms of Alzheimer's disease are discussed in Section 1.2.2.

1.1.2.1 Memory decline in Alzheimer's disease

In its clinical presentation, Alzheimer's disease typically manifests initially with impairments in anterograde episodic memory (Galton, Patterson, Xuereb, & Hodges, 2000), and (to some extent) semantic memory (Hodges & Patterson, 1995). Memory degradation in early Alzheimer's disease leads to difficulties in performing activities of daily living, as the planning and organising of tasks becomes compromised, leading to the individual requiring some type of support system (Förstl & Kurz, 1999). As the course of the disease progresses, impairments in recent memory worsen, with even early biographical memories being lost by the time the disease reaches the advanced stages (Förstl & Kurz, 1999).

The decline in episodic memory (the conscious recollection of previous experiences (Tulving, 2002)) appears to begin several years prior to diagnosis, in the pre-clinical stage of the disease (Grober et al., 2008; Mickes et al., 2007), and progressively worsens up to and beyond clinical diagnosis (Grober et al., 2008). Other aspects of cognition, including executive function, verbal IQ, and visuospatial ability display sharp declines closer to the time of diagnosis, and also continue to worsen throughout the disease (Grober et al., 2008; Johnson, Storandt, Morris, & Galvin, 2009). One crucial site of pathology thought to contribute to the memory decline in Alzheimer's disease is the temporal lobe (see Section 1.1.6).

1.1.3 Neuropathology of Alzheimer's disease

The primary pathological hallmarks of Alzheimer's disease are plaques and neurofibrillary tangles (NFTs). Plaques are composed of insoluble Aβ, and NFTs are formed from hyperphosphorylated and misfolded tau protein (Serrano-Pozo et al., 2011). The presence of both these proteinopathies is required for a definitive post-mortem diagnosis of Alzheimer's disease, and each lesion has a characteristic topographical progression through distinct regions of the brain, allowing for staging of neuropathological severity to be established (Braak & Braak, 1991; Thal, Rüb, Orantes, & Braak, 2002). In brief, Aβ plaques are deposited first in the neocortex, then allocortical regions including the hippocampal formation, and eventually reach brainstem nuclei and cerebellum (Thal et al., 2002). In contrast, NFTs begin in the transentorhinal region, and eventually spread to the hippocampal formation and then isocortical association areas (Braak & Braak, 1991). These major features of Alzheimer's disease neuropathology, along with other commonly observed aspects, will be discussed below.

1.1.3.1 Amyloid plaques

Aβ plaques are extracellular deposits of fibrillary, insoluble, Aβ peptide, typically comprising the two common Aβ species Aβ40 and Aβ42, of which the latter is the major plaque component (Mann et al., 1996). Plaques can be characterised as 'diffuse' or 'dense core'; it is the 'dense core' Aβ plaques, typically associated with neuritic dystrophy, reactive astrocytes, activated microglia and synapse loss, which are indicative of Alzheimer's disease (Serrano-Pozo et al., 2011). Whilst diagnostically essential, Aβ plaques in and of themselves do not seem to be critical to the pathological process, as they are not the best correlate of cognitive impairment in Alzheimer's disease (Terry et al., 1991), and preclinical rodent research has shown Aβ can be synaptotoxic in the absence of plaque deposition (Hsia et al., 1999; Mucke et al., 2000). It is currently thought that pre-plaque Aβ species (i.e. soluble Aβ oligomers) are critical to the disease process (see Section 1.1.4.3.2). Whilst not the principal contributor to the pathological process, Aβ plaques may still have some impact. As plaques can sequester soluble forms of Aβ, it has been speculated that they may represent a protective structure (Esparza, Gangolli, Cairns, & Brody, 2018). However, it has also been found that plaques may act as a reservoir of damaging soluble Aβ (Koffie et al., 2009). Aβ plaques are therefore diagnostically highly useful, and may indirectly affect the disease process in Alzheimer's disease, but are not thought to be a critical pathological event in their own right.

1.1.3.2 Tau tangles

Tau tangles (or NFTs) are intra-neuronal inclusions of microtubule-associated tau filaments which have undergone misfolding and abnormal hyperphosphorylation (Serrano-Pozo et al., 2011). NFT presence is thought to lead to axonal and dendritic degradation, and NFTs can be graded as diffuse, mature or extra-neuronal (in order of ascending severity) (Serrano-Pozo et al., 2011). NFT burden provides a better correlate of dementia severity than Aβ plaque burden (Arriagada, Growdon, Hedley-Whyte, & Hyman, 1992; Bierer et al., 1995), though this may simply reflect the fact that tau pathology is proposed to be downstream of Aβ in the disease process (see Section 1.1.4). As with Aβ plaques, current research now suggests that soluble, pre-tangle, forms of tau may be the overtly damaging species (see Section 1.1.5.2), with NFTs themselves perhaps simply serving as a marker of already inflicted neuronal damage, or potentially representing a protective mechanism (Gendreau & Hall, 2013). In light of this newer focus on soluble forms of tau, NFTs themselves are useful for post-mortem diagnostic purposes, but may not have the most relevance to the pathological events underlying Alzheimer's disease symptoms.

1.1.3.3 Other features

A major feature of Alzheimer's disease is synapse loss (Serrano-Pozo et al., 2011). While not diagnostically necessary, loss of synapses appears to be the best correlate of cognitive decline in Alzheimer's disease (Terry et al., 1991), to the extent that Alzheimer's disease been characterised as a synaptic failure (Selkoe, 2002). Synapse loss contributes to disconnectivity in the brain (Scheff & Price, 2006), and occurs even in early Alzheimer's disease, in areas such as the hippocampus, a region critical to memory (Zola-Morgan, Squire, & Amaral, 1986). Importantly, soluble forms of both Aβ and tau have various deleterious effects at the synapse (see Sections 1.1.4.3.2 and 1.1.5.2). To the extent that Alzheimer's disease is a failure of synapses, soluble Aβ and tau are sensible candidates to examine in terms of mechanistic understanding and therapeutic interventions.

Neuronal loss is another major feature (or consequence) of Alzheimer's disease neuropathology (Serrano-Pozo et al., 2011). The pattern of neuronal loss parallels NFT appearance, but correlates with dementia severity better than NFTs (though not as well as synapse loss) (Serrano-Pozo et al., 2011). Neuronal loss, like synapse loss, contributes to impaired connectivity in the brain (deToledo-Morrell, Stoub, & Wang, 2007), and causes tissue atrophy, resulting in loss of volume in key brain structures such as the hippocampus (De Leon et al., 1997). Neuronal loss is essentially the terminal event in the sequence of Alzheimer's disease pathology, and as such is unlikely to be the most productive stage for targeting interventions.

The inflammatory component of Alzheimer's disease is another important facet of pathology (Heppner, Ransohoff, & Becher, 2015), and involves the microglia and astrocytes of the immune system (Prokop, Miller, & Heppner, 2013; Verkhratsky, Olabarria, Noristani, Yeh, & Rodriguez, 2010). Pro-inflammatory cytokines, such as tumour necrosis factor-α (TNFα) and interleukin-1β (IL-1β), appear to be raised in Alzheimer's disease (Blum-Degen et al., 1995; Tarkowski, Andreasen, Tarkowski, & Blennow, 2003), though this is not a consistent finding (Brosseron, Krauthausen, Kummer, & Heneka, 2014). The innate immune system has been implicated in the development of Alzheimer's disease, as mutated genes encoding for TREM2 (Jonsson et al., 2013), CD33 (Naj et al., 2011), and CR1 (Lambert et al., 2009) are associated with an increased risk. Potential mechanisms underlying inflammatory and immune contributions in Alzheimer's disease include production of inflammatory cytokines via Aβ binding to microglial receptors (El Khoury et al., 2003), as well as potential loss of normal microglial function (Krabbe et al., 2013). Interestingly, an inflammatory 'footprint' can also be found in at least some cases of depression (Raison & Miller, 2011), and microglia have been suggested as a point of connection between depression and Alzheimer's disease (Santos, Beckman, & Ferreira, 2015).

1.1.4 Amyloid cascade hypothesis of Alzheimer's disease

1.1.4.1 History of the hypothesis

After the Aβ protein was identified from brain tissue of Alzheimer's disease patients (Glenner & Wong, 1984), the initial hypothesis was that deposition of insoluble Aβ was the critical pathological event in Alzheimer's disease (Hardy & Allsop, 1991). The 'cascade' of events ending in neurodegeneration and dementia was said to begin with Aβ deposition, with Aβ plaques leading to both neuronal damage in their own right, and to NFTs with their own damaging effects on neurons (Hardy & Allsop, 1991). This seemed to be supported by APP mutations causing Aβ overproduction in familial Alzheimer's disease, and Aβ plaques appearing before NFTs and tissue atrophy in Down's syndrome (in which chromosome 21, harbouring the APP gene, is triplicated). However, since the initial formulation of the amyloid cascade hypothesis, it has become understood that Aβ deposition itself is unlikely to begin the pathological cascade; more recent research suggests that soluble Aβ oligomers are the relevant species (Hayden & Teplow, 2013), and the hypothesis has been revised in light of this (Selkoe & Hardy, 2016). The characterisation of the secretase enzymes and presenilins, and the study of the interplay between Aβ and tau, have also helped refine the amyloid cascade hypothesis. Before discussing the amyloid cascade hypothesis in more detail, some of the basic biology around APP and Aβ will be examined.

1.1.4.2 APP and Aβ

Aβ is the product of a series of enzymatic cleavage processes, and is ultimately derived from the parent molecule APP. APP is a type I transmembrane glycoprotein comprising a large N-terminal ectodomain (lying outside the cell membrane), a transmembrane region and a short C-terminal domain (the cytoplasmic tail) (Ling, Morgan, & Kalsheker, 2003). APP is genetically encoded in humans by one gene found on chromosome 21; 18 exons encode for APP, of which exons 7, 8 and 15 (encoding for extracellular domain regions) allow for alternative splicing (Ling et al., 2003). Thus APP isoforms of varying amino acid lengths can exist, the 3 most common being the 695, 751 and 770 amino acid forms, the former being mainly expressed in the central nervous system (Selkoe, 1998).

For Aβ to be generated, APP is first cleaved by β-secretase at the N-terminal ectodomain to liberate a secreted molecule, sAPPβ (Haass, Kaether, Thinakaran, & Sisodia, 2012). The 99 amino acid APP C-terminus (C99) is then cleaved by γ-secretase in the cell membrane lipid bilayer, generating a liberated extracellular Aβ40 or Aβ42 peptide, depending upon the cleavage site, as well as the cytoplasmic APP intracellular domain (AICD) (Haass et al., 2012). There is also a non-

amyloidogenic enzymatic pathway, in which α-secretase cleaves APP within the Aβ domain to release sAPPα and retain the C83 C-terminal fragment, following which γ-secretase activity produces the p3 peptide, AICD, and no Aβ (Selkoe, 1998). These two APP processing pathways are illustrated in Figure 1.1.
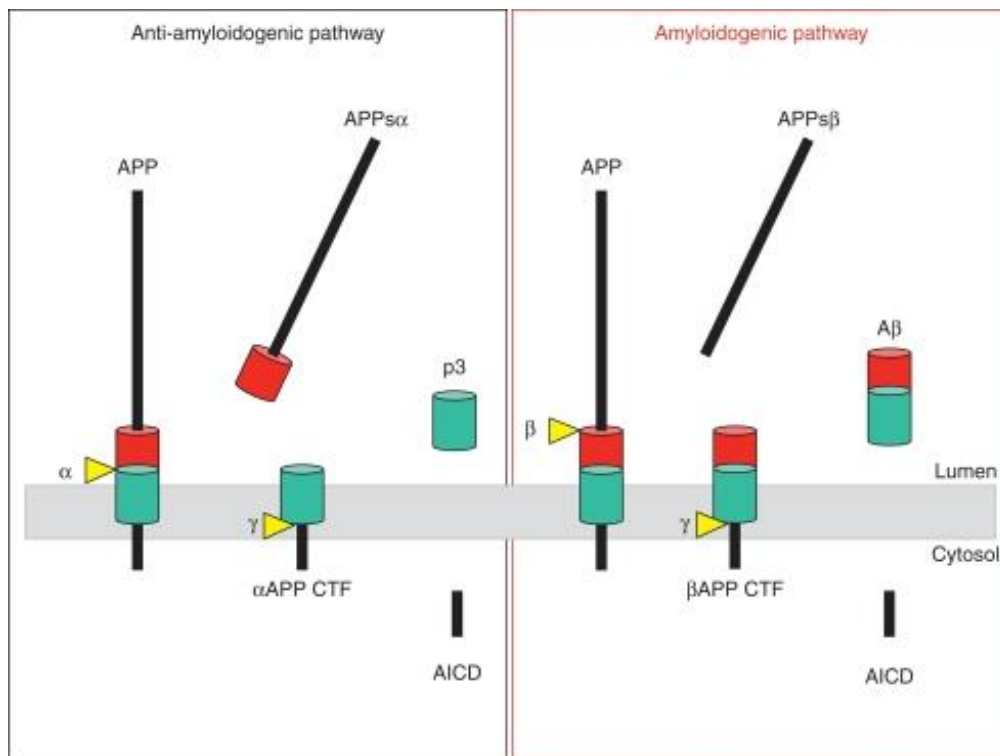
Figure 1.1. APP Processing and Aβ production (Haass, Kaether, Thinakaran, & Sisodia, 2012).

There is some variability in the generation of Aβ by γ-secretase cleavage, resulting in a range of Aβ peptides with differing C-terminals; the Aβ40 species comprises most of the output, with Aβ42 constituting most of the remaining peptides (Thinakaran & Koo, 2008). Of these, it is the Aβ42 peptide which is thought to be the most relevant to Alzheimer's disease, as it is more prone to aggregation (Mann et al., 1996; Suzuki et al., 1994) and forms a number of damaging oligomers (see Section 1.1.4.3.2). APP and Aβ are also thought to serve physiological functions under normal conditions (Dawkins & Small, 2014; Pearson & Peers, 2006), but the focus of this thesis is the pathological effects of Aβ.

1.1.4.3 Evaluation of the amyloid cascade hypothesis

1.1.4.3.1 Genetics

The major evidence in favour of the amyloid cascade hypothesis is principally genetic: all known genetic mutations conferring autosomal dominant familial Alzheimer's disease relate either to APP or presenilins, both of which are involved in Aβ production (Tanzi, 2012). Bolstering this are the facts that duplication of the APP gene also leads to Alzheimer's disease (Rovelet-Lecrux et al., 2006), and Down's syndrome (trisomy 21) includes Alzheimer's disease neuropathology and cognitive deterioration among its consequences (Mrak & Griffin, 2004). There is even a version of trisomy 21 in which the chromosomal segment containing the APP gene is not repeated; individuals with this mutation do not go on to develop Alzheimer's disease (Prasher et al., 1998). In addition, there is a coding mutation (A673T) in the APP gene which confers robust protection against developing Alzheimer's disease (Jonsson et al., 2012). Though the relevance of other APP fragments cannot be definitively ruled out, Aβ is the common molecule linking all known genetic causes of Alzheimer's disease, and as such represents the most likely initiator of the disease. In addition to the convincing genetic evidence, biomarker modelling of the pathological cascade in Alzheimer's disease indicates that accumulation of Aβ42 is the earliest event to occur (Jack et al., 2013).

1.1.4.3.2 Pathological relevance of Aβ

Since being first isolated, a number of toxic effects of Aβ have been identified. Early studies identified a number of ways in which Aβ was broadly toxic to neurons (see Mattson, 1997 for review). For example, Aβ decreases neuronal survival (Yankner, Duffy, & Kirschner, 1990), and damages neurons in a number of ways, including: apoptosis (Loo et al., 1993; Nakagawa et al., 2000), sensitisation to glutamatergic neurotoxicity (Mattson et al., 1992), impairing glucose transport (Mark, Pang, Geddes, Uchida, & Mattson, 1997), oxidative stress (Behl, Davis, Lesley, & Schubert, 1994; Bruce, Malfroy, & Baudry, 1996), and plasma membrane interaction (Hertel et al., 1997). While somewhat informative, these initial toxicity studies used a variety of different Aβ species and aggregation states, concentrations, exposure times, and various primary and secondary cell cultures derived from different species. The absolute physiological relevance of these studies is not clear, though they make the point that Aβ, under certain conditions, can be harmful to neurons. The mechanisms by which Aβ is damaging at the cellular level, in ways which could contribute to the cognitive, and potentially mood, symptoms of Alzheimer's disease, are now understood to a more detailed extent. These are thought to relate primarily to soluble Aβ oligomers, which have a variety of disruptive effects on neurons.

Aβ oligomers of various sizes are thought to be the molecular culprit responsible for memory dysfunction in early Alzheimer's disease (Gong et al., 2003; Lacor et al., 2004). An important site of Aβ oligomer activity is the synapse, where interactions with a number of receptors, including α7-nicotinic acetylcholine (α7-nACh) and NMDA receptors, could contribute to synaptic dysfunction and impaired memory (Dinamarca, Ríos, & Inestrosa, 2012). For instance, naturally secreted human Aβ oligomers induce a reduction in dendritic spine density and synapse loss in rat hippocampal slices, through a mechanism which includes NMDA receptor inhibition (Shankar et al., 2007). Aβ oligomers also block hippocampal long-term potentiation (LTP), a synaptic plasticity-based experimental paradigm for learning and memory (Bliss & Collingridge, 1993), as shown in a number of studies. For example, human Aβ oligomers from cultured cells block LTP when administered to rats (Walsh et al., 2002), and Aβ trimer-enriched culture-medium fractions are potent inhibitors of LTP in mouse hippocampal slices (Townsend, Shankar, Mehta, Walsh, & Selkoe, 2006). Extracts containing soluble Aβ oligomers taken from human Alzheimer's disease brain also impair LTP in mouse hippocampus, an effect not seen when the extracts are depleted of Aβ (Shankar et al., 2008). Soluble cortical extract from human Alzheimer's disease brain is also able to disrupt memory processing *in vivo*, as administration to rats worsened performance in a step-through passive avoidance task, compared against Aβ-depleted extract (Shankar et al., 2008). A specific Aβ oligomeric formation, the Aβ dodecamer (termed Aβ*56), has been identified in transgenic mouse brain (Lesné et al., 2006), and correlates negatively with performance on a spatial memory task. When extracted from transgenic mouse brain and administered to young rats, Aβ*56 impaired performance on probe trials in the Morris water maze task (Lesné et al., 2006).

Taken together, these findings demonstrate that naturally occurring Aβ oligomers, including those derived directly from human Alzheimer's disease brain, are capable of disrupting synaptic function in electrophysiological studies, as well as memory performance in living animals. Particularly damaging Aβ oligomeric formations may include trimers and dodecamers. These various Aβ assemblies are thus a crucial target in the disease process of Alzheimer's disease, as they could underlie early memory symptoms, and represent an early event in the pathological cascade (Selkoe & Hardy, 2016). In addition to their relevance to the memory symptoms of early Alzheimer's disease, Aβ oligomers could theoretically contribute to symptoms of depression. As depressive symptoms appear early in the course of Alzheimer's disease (see Section 1.2.2), this would be consistent with the idea that Aβ oligomers may play a role in the mood disturbances common in Alzheimer's disease also.

1.1.4.3.3 Aβ as a therapeutic target

Pre-clinical studies using transgenic mice provide a useful platform for testing therapies, and have demonstrated that, in principle, anti-amyloid therapies can reverse memory deficits (Götz et al., 2004; Lee et al., 2006; Morgan et al., 2000; Wilcock et al., 2004). However, this has yet to translate to a fully proven therapy for humans. A lingering criticism of the amyloid cascade hypothesis is that it has so far failed to provide a beneficial treatment for Alzheimer's disease in humans. Some of the clinical trials investigating anti-amyloid agents were halted due to adverse reactions (Orgogozo et al., 2003), and others failed to show clinical benefit (Doody et al., 2013). However, as Alzheimer's disease is thought to have a protracted pre-clinical phase during which Aβ levels accumulate (Jack et al., 2013), an explanation of this failure is that therapies were given too late in the disease course to have a clinical impact. This defence appears to have merit, as results from a phase 2 clinical trial of an anti-Aβ protofibril monoclonal antibody (BAN2401) in early Alzheimer's disease patients show a slowing of cognitive decline (Swanson et al., 2018), consistent with the centrality of Aβ to Alzheimer's disease. Though it may be too early to reach definitive conclusions, this may well address the major remaining criticism of the amyloid cascade hypothesis. Thus Aβ is not only a critical peptide to target in Alzheimer's disease, but would appear to require addressing as early as possible in order to produce tangible clinical benefits. Beyond its relevance to cognitive decline, as Aβ could contribute to depressive symptoms in Alzheimer's disease (see Section 1.2.2), therapies targeting Aβ could potentially address mood alterations also.

1.1.5 Tau in Alzheimer's disease

1.1.5.1 Fibrillar tau

Fibrillar tau aggregates arise from hyperphosphorylated tau self-assembling into paired helical filaments (Alonso, Grundke-Iqbal, & Iqbal, 1996). These paired helical filaments coalesce to form NFTs, one of the two major lesions used in post mortem diagnosis of Alzheimer's disease (Serrano-Pozo et al., 2011). NFT count provides a better correlate of cognitive decline than Aβ plaque load (Arriagada et al., 1992), thus it was suspected that fibrillar tau assemblies were damaging to neurons. However, the role of fibrillar tau formations in Alzheimer's disease is now disputed, with some researchers suggesting certain tau aggregates could be protective rather than damaging (Cowan & Mudher, 2013). Evidence from animal studies questions the toxicity of NFTs: a manipulation that increases tau aggregation in a rat tauopathy model decreases neuronal loss (d'Orange et al., 2018), and the presence of NFTs in neurons does not cause gross neuronal dysfunction in a transgenic mouse model (Kuchibhotla et al., 2014). Thus despite NFTs being

diagnostically necessary, insoluble fibrillar forms of tau may not be the most relevant to the disease process in Alzheimer's disease.

1.1.5.2 Soluble tau

Pre-fibrillar, soluble species of tau, known to occur in Alzheimer's disease brain (Lasagna-Reeves, Castillo-Carranza, Sengupta, Sarmiento, et al., 2012; Maeda et al., 2006), are under investigation as formations which could contribute to pathology in Alzheimer's disease (Kopeikina, Hyman, & Spires-Jones, 2012). Transgene suppression in a transgenic tauopathy mouse model, for example, reduces soluble tau but not NFTs, restoring neuronal responsiveness to environmental stimuli and reducing hippocampal neuron loss (Fox et al., 2011). Soluble human tau has also been found to reduce synapse number in mouse dentate gyrus, and impair performance in a cognitive task (Bolós et al., 2017). Soluble tau oligomers extracted from human Alzheimer's disease brain induce both LTP disruption and memory impairment in rodents (Lasagna-Reeves, Castillo-Carranza, Sengupta, Guerrero-Munoz, et al., 2012). Studies such as these suggest that soluble tau species are more pathologically relevant than fibrillar tau, and are more likely to contribute to the symptoms of Alzheimer's disease.

1.1.5.3 Synergistic effects of Aβ and tau

The chain of pathological events in Alzheimer's disease is thought to begin with the occurrence of Aβ oligomers, which are then able to induce toxic tau species by promoting tau hyperphosphorylation (De Felice et al., 2008; Jin et al., 2011). The interplay between Aβ and tau is an important facet of Alzheimer's disease, as at least some of the disturbances Aβ can cause at the synapse appear to depend on the co-occurrence of tau (Ittner & Götz, 2011; Spires-Jones & Hyman, 2014). In addition, presence of Aβ and tau biomarkers points to a synergistic effect between the two in driving the conversion from mild cognitive impairment (MCI) (the pre-clinical precursor of Alzheimer's disease) to Alzheimer's disease (Pascoal, Mathotaarachchi, Shin, et al., 2017), and in causing metabolic decline in the brains of cognitively normal elderly (Pascoal, Mathotaarachchi, Mohades, et al., 2017). The view that Aβ and tau are both critical players in Alzheimer's disease pathology is thus a sensible one, and the precise relation of each to both the disease process and one another are important topics. However, it remains the case that Aβ is the molecule which appears to be the initiator of the pathological process, and as such potentially represents the most appropriate intervention point in the disease cascade.

1.1.6 The hippocampus in Alzheimer's disease

The hippocampus is a structure located in the medial temporal lobe, a brain region known to be vital to mnemonic processes since the 1950s, when a patient with bilateral medial temporal lobe removal was found to exhibit profound anterograde amnesia (Scoville & Milner, 1957). Later studies demonstrated that organic damage localised to the hippocampus in humans was sufficient to cause pronounced and persistent memory impairment (Victor & Agamanolis, 1990; Zola-Morgan et al., 1986), revealing the importance of the hippocampus per se to memory. The importance of the hippocampus to functional memory holds true in non-human primates and rodents, as well as humans (Squire, 1992).

One important site of pathology in Alzheimer's disease is the hippocampus, which is known to both accumulate small Aβ species (Funato, Enya, Yoshimura, Morishima-Kawashima, & Ihara, 1999), and contain deposited Aβ plaques and NFTs (Price, Davis, Morris, & White, 1991). The hippocampus is compromised early in the course of Alzheimer's disease, experiencing synapse loss which correlates with lowered performance on tests of cognition (Scheff, Price, Schmitt, DeKosky, & Mufson, 2007; Scheff, Price, Schmitt, & Mufson, 2006). Early in Alzheimer's disease, the hippocampus also displays decreased activation after stimulus presentation (Small, Perera, DeLaPaz, Mayeux, & Stern, 1999). Beyond these early changes, the hippocampus undergoes frank neurodegeneration in Alzheimer's disease, with neuronal loss leading to tissue atrophy and volume reduction (Barnes et al., 2009; Seab et al., 1988; Simić, Kostović, Winblad, & Bogdanović, 1997). Pathological changes in Alzheimer's disease also impair hippocampal connectivity with other brain areas, such as cortical and limbic regions (Allen et al., 2007; Hyman, Van Hoesen, Damasio, & Barnes, 1984).

Notably, the hippocampus is a structure rich in NMDA and cholinergic receptors (Breese et al., 1997; Rubboli et al., 1994; Ułas, Brunner, Geddes, Choe, & Cotman, 1992). Both of these receptor types are important to cognition and memory (Hasselmo, 2006; Morris, 2013), are affected by Aβ (Oz, Lorke, Yang, & Petroianu, 2013; Shankar et al., 2007), and are altered in Alzheimer's disease (Kuhl et al., 1996; Mishizen-Eberz et al., 2004; Nordberg, 2001; Ułas et al., 1992). NMDA and cholinergic receptors are also the targets of licensed medications used in Alzheimer's disease (see Section 1.1.7); the fact that these medicines provide partial relief from the memory symptoms of Alzheimer's disease suggests the contribution of these receptors to impaired memory. Given that synapse loss in the hippocampus correlates with diminished cognitive performance, that the hippocampus accumulates Aβ, and that Aβ both perturbs synapses and acts on receptors known to subserve memory function, the hippocampus is an important site of pathology in Alzheimer's disease, and is likely to contribute to its amnesic symptoms.

1.1.7 Treatment of Alzheimer's disease

Currently licensed therapies for treating Alzheimer's disease are limited to two classes of compounds: cholinesterase inhibitors, and NMDA receptor antagonists. The cholinesterase inhibitors include the drugs donepezil, galantamine and rivastigmine, all of which are recommended in the UK for use in mild to moderate Alzheimer's disease (National Institute for Health and Care Excellence, 2018b). These agents provide some degree of improvement in cognitive function versus placebo (Birks, Chong, & Grimley Evans, 2015; Birks & Harvey, 2018; Loy & Schneider, 2006), though they do not halt disease progression, and patients still decline over time (Johannsen, 2004). Broadly speaking, the mechanism of action of donepezil and galantamine is inhibition of the enzyme acetylcholinesterase, reducing the breakdown of acetylcholine, increasing acetylcholine levels and improving nicotinic cholinergic neurotransmission (Shinotoh et al., 2001; Woodruff-Pak, Vogel, & Wenk, 2001). Rivastigmine inhibits both acetylcholinesterase and butyrylcholinesterase to improve cholinergic neurotransmission (Eskander, Nagykery, Leung, Khelghati, & Geula, 2005; Kaasinen et al., 2002). The facilitation of cholinergic neurotransmission appears to be the major mechanism by which cholinesterase inhibitors provide clinical benefit, though other mechanisms such as increasing nicotinic receptor density have been suggested (Wilkinson, Francis, Schwam, & Payne-Parrish, 2004).

The NMDA receptor antagonist class of therapies currently comprises one agent, memantine, which is recommended in the UK for moderate and severe Alzheimer's disease (National Institute for Health and Care Excellence, 2018b). Memantine has a small beneficial effect on cognitive function in moderate to severe Alzheimer's disease (McShane, Sastre, & Minakaran, 2006), and acts as a non-competitive antagonist of the NMDA receptor (Parsons, Danysz, & Quack, 1999). The utility of this antagonism is thought to depend upon the NMDA receptor occupancy profile of memantine; it is thought to block the NMDA receptor channel under background pathological conditions, such as synaptic presence of Aβ oligomers, whilst still leaving the channel when a strong pre-synaptic signal arrives, better allowing the synaptic plasticity underpinning learning and memory to occur (Danysz & Parsons, 2012). As with cholinesterase inhibitors, memantine does not arrest the disease process itself, and while patients display an initial cognitive improvement from baseline, they still decline over time (Atri et al., 2015).

In addition to improving cognitive symptoms, cholinesterase inhibitors provide a small benefit in alleviating neuropsychiatric or behavioural symptoms in Alzheimer's disease (Trinh, Hoblyn, Mohanty, & Yaffe, 2003; Wang et al., 2015). Whether these agents address depression specifically is less clear: donepezil may fully or partially resolve depression in a number of Alzheimer's disease patients, though this is based on a secondary analysis with no placebo comparison group (Cummings, McRae, & Zhang, 2006). A pooled analysis of galantamine studies did

not reveal any difference from placebo in changing depression score on the Neuropsychiatric Inventory (Herrmann, Rabheru, Wang, & Binder, 2005), and rivastigmine use produced a poorer depression subscale score when compared with a historical control cohort (Frankfort et al., 2006), though an open-label study has suggested rivastigmine may alleviate depression in mild Alzheimer's disease (Spalletta et al., 2013). Memantine may also provide a small benefit in improving behavioural symptoms of Alzheimer's disease (Matsunaga, Kishi, & Iwata, 2015), although this may be due to its effect on symptoms such as agitation and irritability, rather than depression (Grossberg, Pejović, Miller, & Graham, 2009). As these symptomatic treatments of Alzheimer's disease are not clearly effective in reducing depression, other agents with antidepressant activity should be investigated (see Section 1.2.4).

1.2. Depression and anhedonia in Alzheimer's disease

1.2.1 Defining depression and anhedonia

Depression is a heterogeneous mood disorder, comprising varying combinations of psychological and physical signs and symptoms, central to which are depressed mood and anhedonia (a loss of interest or pleasure from events normally found enjoyable) (American Psychiatric Association, 2013). The constellation of the other signs and symptoms of depression can include weight or appetite changes, insomnia or hypersomnia, loss of energy/fatigue, feelings of guilt or worthlessness, psychomotor retardation or agitation, impaired concentration, and thoughts of death or suicidal ideation or attempts (American Psychiatric Association, 2013). From the late 1950s through to the 1970s, depression was sub-classified into various forms (Raskin & Crook, 1976), though arguably the most significant division was that of distinguishing 'bipolar depression' from 'unipolar depression' (Depue & Monroe, 1978). The term depression from this point onwards will refer to 'unipolar' rather than 'bipolar' depression. Depression can also be categorised by its responsiveness to treatment: depression that remits following antidepressant therapy is treatment-responsive, whereas depression that fails to remit given an appropriate antidepressant agent is considered treatment-resistant (at least as the term is commonly understood) (Fava, 2003).

While the prominent diagnostic guidelines for depression vary in symptom number and duration required for a clinical diagnosis, anhedonia is a core theme in them all. For example, in the Diagnostic and Statistical Manual of Mental Disorders (5th edition) (DSM-5), depressed mood and/or anhedonia are essential for a major depressive disorder diagnosis (American Psychiatric Association, 2013), and the 10th iteration of the International Classification of Diseases (ICD-10) considers loss of enjoyment or interest to be one of the typical depression symptoms (World Health Organization,

1992). In the United Kingdom, clinical guidance issued by the National Institute for Health and Care Excellence on recognising and managing depression in adults uses the previous DSM (DSM-4) guidance for diagnosis (National Institute for Health and Care Excellence, 2018a). Under DSM-4 guidance, core symptoms of a depressive episode are either depressed mood or significant diminishment of pleasure or interest in most or all activities. (American Psychiatric Association, 1998). Some guidelines cleave depression into major or minor episodes, the former comprising 5 or more symptoms and the latter comprising between 2 and 4 symptoms; both entities must feature either depressed mood or loss of interest or pleasure (O'Connor, Whitlock, Gaynes, & Beil, 2009). Regardless of the specific clinical diagnostic guidance implemented, it is clear that a loss or decrease in experiential pleasure (anhedonia) is a major component of depression.

Hedonic deficits feature in various psychiatric conditions, including depression and schizophrenia (Pelizza & Ferrari, 2009), as well as neurodegenerative conditions such as Alzheimer's disease and Parkinson's disease (Lemke, Brecht, Koester, Kraus, & Reichmann, 2005; Lopez et al., 2003). In the context of depression, anhedonia appears to be fairly prevalent amongst depressed individuals (Balsis & Cully, 2008; Haarasilta, Marttunen, Kaprio, & Aro, 2001; Pelizza & Ferrari, 2009), and predictive of a poorer outcome (Spijker, Bijl, de Graaf, & Nolen, 2001). Questionnaire-based studies have found that depressed patients taking antidepressants still report feeling reduced positive emotions, including less enjoyment from hobbies, interests and music (Goodwin, Price, De Bodinat, & Laredo, 2017; Price, Cole, & Goodwin, 2009). Whether this represents a side effect of common antidepressants as some have suggested (Price et al., 2009), or simply a failure of these drugs to improve hedonic deficits, the point remains that conventional antidepressant treatments may not be particularly suitable for addressing anhedonia, a core component of depression. In treatment-resistant depression, the presence of anhedonia is predictive of longer time to remission, fewer depression-free days, and minimal response to transcranial magnetic stimulation (Downar et al., 2014; McMakin et al., 2012). Again, this suggests that anhedonia is a highly important and clinically meaningful aspect of depression, which warrants deeper investigation and targeted treatment.

1.2.2 Depressive symptoms in Alzheimer's disease

Symptoms of depression appear early in the course of Alzheimer's disease, including in mild cognitive impairment (Gabryelewicz et al., 2004; Visser, Verhey, Ponds, Kester, & Jolles, 2000), and appear to be relatively persistent, at least over a 12-16 month time frame (Garre-Olmo et al., 2003; Starkstein et al., 1997). The early appearance of depression in Alzheimer's disease is noteworthy,

suggesting that early pathological events are sufficient to produce depressive symptoms, and pointing towards the relevance of Aβ as a potential antecedent. The symptom profile of depression in Alzheimer's disease is broadly similar to that of elderly depressed patients (e.g. sadness, guilt, loss of interest) (Chemerinski, Petracca, Sabe, Kremer, & Starkstein, 2001).

The prevalence of depression in Alzheimer's disease is unclear and ranges from the fairly low (Newman, 1999; Weiner, Doody, Sairam, Foster, & Liao, 2002), to moderately high (Ballard, Bannister, Solis, Oyebode, & Wilcock, 1996; Migliorelli et al., 1995). This uncertainty is likely due to, among other things, the use of different diagnostic criteria, and the study of differing patient populations (e.g. home residence versus nursing home residence, mixing possible and probable Alzheimer's disease patients, etc.). Despite this lack of clarity from individual studies, a meta-analysis has found that the prevalence of depression in Alzheimer's disease is roughly 40% (Chi et al., 2015). Both major and minor depression exist in Alzheimer's disease (Lyketsos et al., 1997; Starkstein, Jorge, Mizrahi, & Robinson, 2005); when these groups are combined, it appears that roughly 50% of Alzheimer's disease patients experience some level of depression.

Thus depression in Alzheimer's disease appears to be present in the population and emerges early in the disease, is similar in appearance to 'typical' depression in elderly patients, and relatively persistent. The significance of this depression will now be considered.

1.2.3. Significance of depression in Alzheimer's disease

Addressing the non-cognitive features of Alzheimer's disease (sometimes called neuropsychiatric symptoms (NPS) (Lyketsos et al., 2011), or behavioural and psychological symptoms of dementia (BPSD) (Cerejeira, Lagarto, & Mukaetova-Ladinska, 2012)) is an important component of treating Alzheimer's disease. Depression in Alzheimer's disease carries a number of additional negative outcomes (Lyketsos & Olin, 2002), including: an increase in mortality (Burns, Lewis, Jacoby, & Levy, 1991); faster decline in cognition (Zahodne, Ornstein, Cosentino, Devanand, & Stern, 2015); reduced quality of life (González-Salvador et al., 2000); lower ability to perform activities of daily living (Lyketsos et al., 1997); increased likelihood of aggressive behaviour (Lyketsos et al., 1999); increased likelihood of discharge from an assisted living facility to nursing home or hospital (Kopetz et al., 2000); and increased burden and depression for caregivers (Bozgeyik, Ipekcioglu, Yazar, & Ilnem, 2018; Neundorfer et al., 2001).

In principle, depression in Alzheimer's disease could simply be a reactive occurrence due to the distressing and unpleasant reality of living with a neurodegenerative condition. However, early studies suggest – unlike what might be expected if depression is a reaction to dementia and its

symptoms – that the level of insight into cognitive decline does not seem to be associated with an increased occurrence of depression (Cummings, Ross, Absher, Gornbein, & Hadjiaghai, 1995; Migliorelli et al., 1995; Ott et al., 1996; Verhey, Rozendaal, Ponds, & Jolles, 1993). However, a more recent study has suggested that greater insight in Alzheimer's disease is predictive of greater depression severity (Horning, Melrose, & Sultzer, 2014). Regardless, emotional reactivity is unlikely to be the only explanation of depression in Alzheimer's disease, because disclosure of diagnosis is associated with worsening depressive symptoms in only a minority of patients (Mormont, Jamart, & Jacques, 2014). It could therefore be the case that an enhanced awareness of one's condition could be a source of an additional depressive burden, but there is more to depression in Alzheimer's disease than simply a reaction to the negative consequences of the disease itself.

Beyond these externally observed consequences, post mortem examination has revealed that among Alzheimer's disease subjects with a lifetime history of depression, those with concurrent depression at the onset of Alzheimer's disease have a greater Aβ plaque and neurofibrillary tangle burden in the hippocampus than those without (Rapp et al., 2006). Given the manifold problems caused by depression in Alzheimer's disease, improvements in identification and treatment of this depression are a major issue in need of addressing. To the extent that depression in Alzheimer's disease is not purely 'reactive', it may represent a common (though not universal) consequence of the disease process itself. If properly explored, these possibilities could yield valuable insights into underlying mechanisms and thus therapeutic targets which could alleviate this depression. Before considering how the pathological processes of Alzheimer's disease could potentially give rise to depression and anhedonia, their diagnosis and treatment in Alzheimer's disease will be described.

1.2.4 Diagnosis and treatment of depression and anhedonia in Alzheimer's disease

As with depression in the general population, depression in Alzheimer's disease is recognised by means of a clinical survey or questionnaire. Prior to the creation of rating scales or diagnostic criteria which were specific to depression in Alzheimer's disease, clinicians would use methods which generally relied upon patient interview or self-report. Given the fact that memory and communication abilities are impaired in Alzheimer's disease (especially as the disease progresses), then it is clear that interview and self-report measures may not be particularly suitable for use in this specific population. In 1988 the Cornell Scale for Depression in Dementia was introduced, designed to specifically gauge depression in patients with dementia, being administered to both the patient and a reliable informant (Alexopoulos, Abrams, Young, & Shamoian, 1988). Additionally, in 2002 a National Institute of Mental Health-sponsored working group issued

provisional criteria for diagnosing depression in Alzheimer's disease, modifying DSM-IV style criteria for this specific patient population (Olin et al., 2002). Interestingly, these specific instruments for gauging or diagnosing depression in Alzheimer's disease tend to produce higher estimates of depression prevalence (Müller-Thomsen, Arlt, Mann, Maß, & Ganzer, 2005; Teng et al., 2008), which may explain the varying prevalence estimates in the literature, and suggests that perhaps depression in Alzheimer's disease may be more prevalent than once suspected.

Specific anhedonia measurement scales have yet to be applied to Alzheimer's disease, and as they rely upon self-report they would have questionable applicability, though could potentially be used in early stages of the disease. A consequence of this is the true extent of anhedonia as a feature of Alzheimer's disease is still unknown. However, the 2002 provisional diagnostic criteria do consider a decrease in positive affect or pleasure to be a core feature of depression in Alzheimer's disease (Olin et al., 2002). A cross-sectional study of psychiatric symptoms in probable Alzheimer's disease found that, while major depression was less common as dementia advanced, anhedonia became more common, and was present in 72% of individuals with severe cognitive impairment (Lopez et al., 2003). This suggests that anhedonia specifically may be both prevalent in Alzheimer's disease and a particular by-product of the disease process itself.

There are no evidence-based guidelines on how best to treat depression in Alzheimer's disease. Several randomised controlled trials have taken place comparing an antidepressant against either placebo or another antidepressant in this population (Banerjee et al., 2011; Lyketsos et al., 2003; Rosenberg et al., 2010; Weintraub et al., 2010), along with less robustly designed studies (G. M. Petracca, Chemerinski, & Starkstein, 2001; G. Petracca, Tesón, Chemerinski, Leiguarda, & Starkstein, 1996; Reifler et al., 1989). Unfortunately many studies of antidepressants in Alzheimer's disease (and dementia more broadly) have utilised small sample sizes, the largest of which found no difference between either sertraline or mirtazapine and placebo at 13 and 39 weeks (Banerjee et al., 2011), while another trial of sertraline found it was not superior to placebo at 12 and 24 weeks (Rosenberg et al., 2010; Weintraub et al., 2010). Thus the best available evidence from the largest and most recent individual trials is that commonly used classes of antidepressants (selective serotonin reuptake inhibitors (SSRIs) in the case of sertraline, and serotonin and noradrenaline reuptake inhibitors (SNRIs) in the case of mirtazapine) do not appear to be effective in treating depression in Alzheimer's disease. The lack of clear benefit of antidepressants in Alzheimer's disease and dementia is also borne out in meta-analyses investigating this topic (Dudas, Malouf, McCleery, & Dening, 2018; Orgeta, Tabet, Nilforooshan, & Howard, 2017).

In sum, the evidence suggests that antidepressants have little or no beneficial effect on depression in Alzheimer's disease. Importantly, traditional antidepressants do appear to be effective in treating depression in adults and the elderly (Arroll et al., 2005; Kok, Heeren, & Nolen, 2011; Wilson, Mottram, Sivananthan, & Nightingale, 2001), raising the possibility that there is something about Alzheimer's disease (e.g. sites of disease pathology or transmitter systems affected) that renders conventional antidepressants ineffective. Larger high quality studies are needed to strengthen the evidence base before firm conclusions can be drawn either way, but it is apparent that novel antidepressant agents urgently require investigation in the context of Alzheimer's disease. Given that anhedonia is both prevalent and a core component of depression in Alzheimer's disease, a predictor of poorer outcome in depression generally, and may be unaddressed by conventional antidepressants, treatments that could target this specific facet of depression merit particular investigation. The underlying biology of anhedonia in Alzheimer's disease is thus a key issue to be studied in aid of identifying targeted therapies (see Section 1.2.5.2). In the next section, the key pathological changes manifesting during the course of Alzheimer's disease are discussed, in light of how they may account for depression in Alzheimer's disease.

1.2.5 Biology of depression and anhedonia in Alzheimer's disease

1.2.5.1 Biology of depression in Alzheimer's disease

The most influential biological theory of depression is the monoamine hypothesis of depression (Schildkraut, 1965). This has led to the development of various classes of antidepressant drugs, and holds that a deficiency in monoaminergic neurotransmitters (e.g. serotonin and noradrenaline) is the underlying biochemical state of depression, restoration of which should relieve depression, though this has since been refined (Krishnan & Nestler, 2008; Nutt, 2002). While conventional antidepressant agents that target the monoamine transmitter systems are effective in both adults (Arroll et al., 2005), and the elderly (Kok et al., 2011; Wilson et al., 2001), this does not appear to be the case for depression in Alzheimer's disease (Dudas et al., 2018; Orgeta et al., 2017). The lack of clear benefit from antidepressants in Alzheimer's disease could hypothetically be due to altered monoamine or other transmitter systems in Alzheimer's disease, in a manner distinct from 'typical' depression.

A single class of molecules is unlikely to provide a full explication of depression, however, and a number of other molecular hypotheses of depression have been proffered, including glutamatergic (Sanacora, Treccani, & Popoli, 2012), GABAergic (Luscher, Shen, & Sahir, 2011), cytokine (Schiepers, Wichers, & Maes, 2005), and corticosteroid receptor (Holsboer, 2000), as well

as an excitatory synapse hypothesis of depression (Thompson et al., 2015). Importantly, many of these molecular systems (and synapses themselves) are altered during the course of Alzheimer's disease; it would not be unreasonable to speculate that one or more of these narrower hypotheses of depression may be more relevant to depression in Alzheimer's disease. There is no single unified hypothesis providing a comprehensive account for the occurrence of depression in Alzheimer's disease. What have been noted, however, are neurodegenerative consequences of Alzheimer's disease which could contribute to depression (Šimić et al., 2017), as well as biological endpoints shared by depression and Alzheimer's disease which could explain the former emerging in the latter (Caraci, Copani, Nicoletti, & Drago, 2010).

Firstly, the serotonergic and noradrenergic system appear to be compromised in Alzheimer's disease. Markers of serotonergic neurons, such as serotonin (5-hydroxytryptamine; 5HT), its primary metabolite 5-HIAA, and 5HT uptake are reduced in the temporal cortex relative to control brain (Garcia-Alloza et al., 2005; Palmer et al., 1987). The dorsal raphe nucleus, a major source of serotonergic innervation to the forebrain, displays Aβ plaques and NFTs in Alzheimer's disease brain, as well as a loss of large neurons and a decrease in large neuron density (Halliday et al., 1992; Yamamoto & Hirano, 1985). Noradrenaline levels are also decreased in regions of Alzheimer's disease brain, including frontal cortex, temporal cortex and hippocampus (Reinikainen et al., 1988). Aβ plaques and NFTs occur in the locus coeruleus, a major source of noradrenergic neurons, in Alzheimer's disease brains (Cole, Neal, Singhrao, Jasani, & Newman, 1993; German, White, & Sparkman, 1987), along with a substantial loss of noradrenergic neurons themselves (Iversen et al., 1983). The degradation of the serotonergic and noradrenergic systems in Alzheimer's disease may account for the lack of clear effectiveness of antidepressants in this population. It could be the case, for example, that for conventional antidepressants such as SSRIs and SNRIs, which act upon serotonin and noradrenaline reuptake sites, a relatively intact population of serotonergic and noradrenergic neurons is required for their effectiveness. The substantial damage done to these monoaminergic systems in Alzheimer's disease could limit the utility of standard antidepressant agents, and provides a rationale for investigating drugs which target other receptor classes.

There are also a number of biochemical states observed in depression which are also seen in Alzheimer's disease, including alterations in glucocorticoids, inflammatory mediators and neurotrophic factors (Caraci et al., 2010). Depressed patients show, for example, raised salivary cortisol after waking (Bhagwagar, Hafizi, & Cowen, 2005), and atypical cortisol trajectories following stress exposure (Burke, Davis, Otte, & Mohr, 2005). Alzheimer's disease patients also show raised cortisol levels (Popp et al., 2009, 2015), which are associated with a more rapid cognitive decline when present in the pre-clinical stage of the disease (Popp et al., 2015). The precise relevance of

raised cortisol to depression in Alzheimer's disease is unclear, as plasma cortisol level following dexamethasone suppression testing has been found to positively correlate with number of total and major depression symptoms (Greenwald et al., 1986), while CSF cortisol does not appear to differ between depressed and non-depressed Alzheimer's disease patients (Hoogendijk, Meynen, Endert, Hofman, & Swaab, 2006). Similarly, the increase of certain pro-inflammatory cytokines appears to play a role in depression (Schiepers et al., 2005), and Alzheimer's disease also exhibits a profile of raised cytokines (Swardfager et al., 2010), which could in principle contribute to depression pathogenesis in Alzheimer's disease. The neurotrophic factor BDNF is lowered in depressed patients (Sen, Duman, & Sanacora, 2008), and the Val66Met BDNF genetic polymorphism confers an increased risk of depression in Alzheimer's disease (Borroni et al., 2009).

While the above phenomena are interesting and merit further study, there is another aspect of depression in Alzheimer's disease that remains relatively neglected. In light of anhedonia being a critical facet of depression, and a prevalent occurrence in Alzheimer's disease, the functionality of the reward system in Alzheimer's disease is an important topic. Reward circuitry in the brain which underlies pleasurable reactions could plausibly be a site of pathology in Alzheimer's disease, leading to anhedonia. This could theoretically occur by loss of structural integrity or volume, or by more subtle events including alterations in neurotransmitter or receptor function, or both.

1.2.5.2 Biology of anhedonia in Alzheimer's disease

Given that anhedonia is known to occur in Alzheimer's disease, and that dysfunction within the reward system could give rise to this anhedonia, the integrity of the reward system in Alzheimer's disease merits close inspection. In brief, this reward system includes cortical structures such as the orbitofrontal cortex, insula and anterior cingulate cortex, as well as limbic structures such as the nucleus accumbens and ventral pallidum, and brainstem structures such as the parabrachial nucleus (Berridge & Kringelbach, 2013). Brain imaging studies of the sort used to investigate depressed patients, aimed at the reward system during reward-based tasks, are lacking in Alzheimer's disease patients. However, there are several post mortem studies which have investigated reward-related sites in the brain, in the context of the pathological lesions, neuronal loss and neurodegeneration of Alzheimer's disease. In Alzheimer's disease patients, Aβ plaques and NFTs are present in the striatum generally (Braak & Braak, 1990), the nucleus accumbens specifically (Suenaga, Hirano, Llena, Yen, & Dickson, 1990), the orbitofrontal cortex (Van Hoesen, Parvizi, & Chu, 2000), and parabrachial nucleus (German et al., 1987; Parvizi, Van Hoesen, & Damasio, 1998), demonstrating that pathological Aβ accumulation and NFT formation occur in recognised sites of the

reward system (Berridge & Kringelbach, 2015). In addition to the presence of pathological lesions, one MRI study has shown volume reduction in the nucleus accumbens in Alzheimer's disease (Pievani et al., 2013), and gross structural changes to the orbitofrontal cortex have been reported post mortem (Van Hoesen et al., 2000). Cholinergic neuron loss in the ventral striatum and ventral pallidum is also seen in Alzheimer's disease (Lehéricy et al., 1989; Lehéricy, Hirsch, Hersh, & Agid, 1991), although it is not clear how this relates to hedonic processing.

While these findings do not conclusively demonstrate impaired hedonic processing circuitry in Alzheimer's disease, they do show that the brain structures involved in a functional reward system are affected by Alzheimer's disease pathology. It is not an unreasonable assumption, therefore, that changes such as these could produce anhedonia in Alzheimer's disease. Furthermore, to the extent that anhedonia could emerge early in the course of Alzheimer's disease, its antecedents are unlikely to include widespread neuronal loss or tissue atrophy, as these are later occurrences in the pathological chain of events. Events which would better explain anhedonia early in Alzheimer's disease may include either some direct action of Aβ in impairing hedonic processing, or a downstream effect on some particular neurotransmitter or signalling pathway, or both. Given that Aβ is particularly active at synapses and this is thought to contribute to early memory symptoms in Alzheimer's disease (Gong et al., 2003; Lacor et al., 2004), the synapse may also be a sensible location for investigating an early depressive symptom such as anhedonia. Opioidergic neurotransmission is one particularly important mechanism underlying the generation of reward sensations (Castro & Berridge, 2014, 2017), and may merit inspection in the context of anhedonia in early Alzheimer's disease. What would be needed to investigate this in sufficient detail, and to allow for a potential therapy to be trialled, would be a biological system which recapitulated, to some extent, the early (i.e. Aβ-focused) phase of Alzheimer's disease. Mouse models of Alzheimer's disease harbouring human APP mutations offer such a system, and will be explained in the next section.

1.3 Mouse models of Alzheimer's disease

1.3.1 Modelling Alzheimer's disease in rodents

Genetics-based mouse models of Alzheimer's disease first appeared in the mid-1990s, and initially incorporated mutated forms of APP from human familial Alzheimer's disease, which were overexpressed throughout the central nervous system and beyond (Games et al., 1995; Hsiao et al., 1996). Later genetic alterations to these mouse models included the addition of human presenilin mutations to induce a more rapid Aβ accumulation (Radde et al., 2006), and human tau mutations to

reproduce a fuller tau pathology and NFTs (Oddo et al., 2003). These various mouse models serve as a platform for studying many facets of Alzheimer's disease, such as the relative contributions of Aβ and tau to cognitive impairment, and the time course over which cognitive impairment may emerge. Mouse models of Alzheimer's disease also offer the opportunity to investigate whether neuropsychiatric symptoms of Alzheimer's disease, such as depression, may manifest, and to test potential therapeutic compounds to address both cognitive and neuropsychiatric symptoms. An approach to modelling the early pathological changes of Alzheimer's disease, which could potentially account for the emergence of anhedonia, is the use of mice overexpressing mutant APP.

1.3.2 APP overexpressing models

The first successful mouse models of Alzheimer's disease featured overexpression of mutated human APP genes, under the control of various promoters. The first of these was the PDAPP mouse model (Games et al., 1995), which was followed by the Tg2576 model (Hsiao et al., 1996) (and see Section 1.3.2.1), and then by others, such as the APP23 and J20 models (Mucke et al., 2000; Sturchler-Pierrat et al., 1997). The presence of APP mutations in these mice leads to the accumulation of soluble Aβ species in the brain, deposition of Aβ plaques in several brain regions, and the presence of reactive astrocytes and microglia in the vicinity of Aβ plaques, all of which recapitulate aspects of Alzheimer's disease in humans. These mouse models do not, however, capture the full repertoire of pathological changes seen in human Alzheimer's disease; they do not display overt tau pathology in the form of NFTs (Spires & Hyman, 2005), and they do not feature widespread atrophy or neuronal loss (Irizarry, McNamara, Fedorchak, Hsiao, & Hyman, 1997). This incomplete pathological profile demonstrates that these mouse models are not complete or perfect models of Alzheimer's disease. However, because they show synaptic loss and synaptic disturbances due to Aβ oligomers, along with memory impairments, it has been suggested that they provide a model of early or pre-clinical Alzheimer's disease (Zahs & Ashe, 2010). Consequently, therapies found to be beneficial, either biochemically or behaviourally, in these mouse models should be viewed in this context; useful in a model of early, rather than advanced, Alzheimer's disease.

APP overexpressing mouse models provide a useful platform for investigating both memory and depressive symptoms of early Alzheimer's disease. They display memory deficits across a range of cognitive tasks, including the Morris water maze (Kelly et al., 2003; Müller-Schiffmann et al., 2016; Puoliväli et al., 2002; Trinchese et al., 2004), radial arm maze (Balducci et al., 2010; Wright et al., 2013), and other behavioural assays (Karl, Bhatia, Cheng, Kim, & Garner, 2012; Pozueta et al., 2013). In addition to impaired memory, APP overexpressing mice also display depressive behaviour. This has been demonstrated using conventional behavioural tests of depression, including the forced

swim test (Abdel-Hafiz et al., 2018; Filali, Lalonde, & Rivest, 2009), tail suspension test (Iascone et al., 2013), and sucrose preference test (Romano et al., 2015). The appropriateness of these tests for depression, and a novel alternative which may be more suitable for gauging anhedonia, will be discussed briefly in Section 1.3.3, and at more length in Sections 2.2.1 and 3.4. Given that an APP overexpressing mouse model is a useful tool for investigating early depressive symptoms of Alzheimer's disease, such as anhedonia, a generally representative mouse model needs to be selected. This thesis will use the Tg2576 mouse model, which is discussed next.

1.3.2.1 The Tg2576 mouse model

The Tg2576 model was first reported in 1996 (Hsiao et al., 1996), and features a human APP double mutation (K670N/M671L), found in a Swedish family with familial Alzheimer's disease (the 'Swedish' mutation; APP$_{Swe}$) (Mullan et al., 1992). This APP double mutation is expressed in Tg2576 mouse brain at an approximately 5 to 6 times greater level than endogenous mouse APP, under control of the hamster prior promoter (Hsiao et al., 1996). Tg2576 mice display a marked rise in soluble Aβ40 and Aβ42 brain levels beginning around 8 months of age, along with appearance of early Aβ plaques from 7-8 months of age (Kawarabayashi et al., 2001), with Aβ deposition occurring in, but not limited to, frontal, temporal and entorhinal cortex, and the hippocampus (Hsiao et al., 1996). Aβ plaques in Tg2576 mouse brain are eventually associated with microglial and astrocytic involvement, and dystrophic neurites (Hsiao et al., 1996). Despite exhibiting Aβ pathology, NFT formation and extensive neuronal loss are not seen in Tg2576 mice (Irizarry et al., 1997; Spires & Hyman, 2005), though synaptic disturbances, such as decreases in dendritic spine density, are seen from 4 months of age (Dong, Martin, Chambers, & Csernansky, 2007; Jacobsen et al., 2006; Spires-Jones et al., 2007). Thus neuropathologically, Tg2576 mice are a good model of the early changes seen in Alzheimer's disease.

Tg2576 mice show impaired learning and memory abilities across a range of testing paradigms, and potentially show some type of depressive behaviour. Tg2576 mice display memory deficits in the Morris water maze and Y-maze (Hsiao et al., 1996), a T-maze task, (Chapman et al., 1999), and in contextual fear conditioning (Jacobsen et al., 2006), similar to other APP overexpressing models. An aspect of memory in Tg2576 mice that has been investigated in multiple studies is object-in-place memory, that is, the preference for exploring spatially switched objects in an arena (Dix & Aggleton, 1999). Tg2576 mice, at 14 months of age, display intact object novelty and object recency preferences, but show an impairment in their preference for exploring spatially switched objects (Hale & Good, 2005), a deficit also found at 10-12 and 21 months of age (Good & Hale, 2007; Good, Hale, & Staal, 2007). In addition to impaired memory, Tg2576 mice potentially

display some type of depressive behaviour, though this is based on just one study, which found that Tg2576 mice fail to develop a preference for a location paired with chocolate, and consume less chocolate than wild-type mice (Nobili et al., 2017). This was taken to indicate a deficiency in reward processing in Tg2576 mice, suggestive of a 'depression-like' behaviour. This finding could indeed represent some dysfunction of reward processing, though reduced consumption does not necessarily imply a lack of reward (see Section 1.3.3). Behaviourally too, then, Tg2576 mice are representative of APP overexpressing models, displaying memory deficits and some degree of depressive behaviour.

Tg2576 mice are thus a sensible choice for examining anhedonia in pre-clinical Alzheimer's disease. That the depressive behaviour of Tg2576 mice has only been examined in one study, suggests that there is a need for further investigation, and with a technique more sensitive to the core depression symptom of anhedonia. In addition to this, Tg2576 mice offer the opportunity to capture when depressive symptoms might emerge relative to memory impairments, i.e. prior to, alongside, or after a cognitive deficit manifests. This type of dual hedonic-cognitive profile could be informative in many ways. For example, an early anhedonic phenotype relative to cognitive impairment could imply a particular vulnerability of the reward circuitry underlying anhedonia to the damaging presence of soluble Aβ species, over that of hippocampal circuitry. Conversely, a hedonic deficit that emerged relatively late might suggest a certain robustness of reward pathways in the presence of Aβ. The timing of the two deficits could potentially help to clarify the earliest symptoms of pre-clinical Alzheimer's disease; a depressive symptom such as anhedonia occurring prior to a cognitive deficit could lend weight to the suggestion that depression is an important part of prodromal Alzheimer's disease (Sun et al., 2008). A finding such as this, if widely replicated and confirmed in humans, could potentially aid in the diagnosis of Alzheimer's disease. The inclusion of a cognitive test will also allow the investigation of whether a therapeutic compound can benefit cognition, as well as mood, in Tg2576 mice. The issue of testing for depression in rodents, and a novel method for investigating anhedonia, will briefly be discussed next.

1.3.3 Testing for depression in rodents

The commonly used assays of depression are not necessarily appropriate for detecting anhedonia, and in some cases may not be the most appropriate tests for depression in general. Forced swim and tail suspension tests are thought to capture a state of despair (Porsolt, Brossard, Hautbois, & Roux, 2001), which is neither a core component of depression nor related to anhedonia. Sucrose preference testing, in which preference for a sucrose solution over water is calculated based

on amount of each fluid consumed, is presented as sensitive to hedonic changes (Willner, Towell, Sampson, Sophokleous, & Muscat, 1987). However, sucrose preference is a measure derived from amount consumed, and it has been demonstrated that amount of a sweet solution consumed can decrease, while positive hedonic reactions to that solution, when it is experienced, can remain intact (Pelchat, Grill, Rozin, & Jacobs, 1983). Thus a decrease in sucrose preference, which derives from the amount consumed, is not necessarily evidence of anhedonia. A novel method for investigating depressive behaviour, which is sensitive to anhedonia and not derived from gross consumption, is lick cluster analysis (Dwyer, 2012). This is based on objective recordings of the lick microstructure of rodents when consuming palatable solutions (Davis & Smith, 1992), and is thought to capture the hedonic reactions to those solutions, regardless of the precise amount consumed (Dwyer, 2012). This approach is based on the idea that a measurably reduced response to something which is normatively pleasurable would by definition be an example of an anhedonic reaction, and therefore this task should be sensitive to an aspect of anhedonia-related behaviour in rodents. A similar phenomenon of reduced hedonic reactions to a sweet stimulus has been observed in dementia patients (Perl et al., 1992), though reduced palatability has not been observed within depression per se (Scinska et al., 2004, Swiecicki et al., 2015). Thus lick cluster analysis is an ideal method for the longitudinal assessment of anhedonia in Tg2576 mice.

There has been relatively little investigation of the neurobiology responsible for lick cluster size in response to palatable substances. This has, however, been better characterised in the separate but conceptually similar task of taste reactivity testing, in which reward sites such as the nucleus accumbens, orbitofrontal cortex and ventral pallidum, and neurotransmitters such as opioids and endocannabinoids are involved. This material is discussed further in Sections 3.1 and 5.1. This is a relatively brief justification for using lick microstructure analysis; more details are presented in Section 2.2.1, and an evaluation of lick cluster analysis relative to other behavioural tests for depression can be found in Section 3.4.

1.4. Proposal for thesis of the PhD

Depression is a common and early problem in Alzheimer's disease, with multiple negative consequences, and an important component of this depression is anhedonia. A major aim of this thesis is to investigate whether the early pathological event of Aβ build-up could be one cause of anhedonia in Alzheimer's disease. Given that conventional antidepressants do not appear particularly effective in Alzheimer's disease, another aim of the thesis is to investigate whether an

alternative antidepressant agent could reduce Aβ-dependent anhedonia. To this end, the use of an antidepressant thought to target anhedonia will be of particular interest.

A good way to model the early, Aβ-related, pathological changes of Alzheimer's disease is the use of APP overexpressing mice, of which Tg2576 mice are a representative model. Tg2576 mice can thus be used to investigate the contribution of Aβ accumulation to anhedonia. As Aβ levels in Tg2576 mice are initially low and increase with age, a sensible investigation of anhedonia would be the longitudinal profiling of anhedonia as these mice age. Standard behavioural tests used in assessing depression in rodents either do not measure anhedonia, or are not necessarily indicative of anhedonia; a novel method of gauging anhedonia known as lick cluster analysis will thus be used for the longitudinal assessment of anhedonia in Tg2576 mice.

An additional aim of this thesis is to profile memory impairment in Tg2576 mice alongside the longitudinal measurement of anhedonia. The object-in-place memory deficit in Tg2576 mice has been replicated, and appears to be robust and stable, as its presence has been demonstrated over a range of several months. This would suggest that object-in-place testing is appropriate for characterising the memory decline in Tg2576 mice. A longitudinal profile of this deficit has not been previously characterised, and will be a novel finding in its own right. In addition, this could provide further behavioural context into which a potential anhedonic profile of Tg2576 mice can be placed.

In summary, the aims of this thesis are to longitudinally investigate anhedonia and memory impairment in Tg2576 mice, by the use of lick cluster analysis and object-in-place testing, respectively, and to trial a novel antidepressant agent whose activity may reduce anhedonia. The biochemical bases of any hedonic or cognitive deficits, or antidepressant effects, will also be investigated. In particular, opioidergic and NMDA receptor-related changes, which may relate to hedonic or cognitive behavioural changes, respectively, will be examined.

Chapter 2: Methods

2.1 Housing, breeding and genotyping of Tg2576 mice

2.1.1 Housing of Tg2576 mice

All mice were housed in a dedicated holding room in the Behavioural Neuroscience Laboratory, Cardiff School of Psychology. Housing conditions included a 12 hour light/dark cycle (08:00 – 20:00), and regulated temperature (21 ± 2°C) and humidity (55% ± 10%). Holding cages of dimensions 48×15×13 cm (D×W×H) contained sawdust and cotton squares for bedding, and environmentally enriching items (wooden chew sticks and cardboard tubes). Standard mouse chow and a bottle of clean drinking water were freely available to caged mice, except when noted otherwise (i.e. food restriction as part of an experimental design). The health and well-being of mice were regularly monitored by JBIOS animal unit technicians and the Named Animal Care and Welfare Officer. Mouse cages were cleaned, and fresh chow and water provided, on a weekly basis. All behavioural testing occurred in the light phase of the light/dark cycle, and all experiments were conducted in accordance with the Animals (Scientific Procedures) Act 1986.

Tg2576 and wild-type mice forming the experimental cohorts were initially housed in groups of between 2 and 5. However, by around 4-5 months of age, aggression between mice was often seen. As a result, many mice were eventually separated and single-housed. By the end of behavioural testing the majority of mice were single-housed, such that it affected both genotypes to a roughly equal extent. Where this was not the case at the start of behavioural testing, further separation was carried out in order that one genotype was not disproportionately affected over the other. Two colonies of Tg2576 and wild-type mice were generated for behavioural experiments, numbering 42 transgenic and 41 wild-type mice at the start of behavioural testing, and spaced roughly 6 weeks apart in date of birth. This provided both a populous sampling group for the initial lick cluster studies, in which group numbers typically number a minimum of 16 each (Lydall, Gilmour, & Dwyer, 2010; McNamara, Davis, Dwyer, John, & Isles, 2016), and allowed for a between-subjects drug manipulation after the lick cluster age profile had been obtained, even with some degree of cohort attrition.

2.1.2 Breeding schedule and weaning

The Tg2576 line was acquired by the Cardiff School of Psychology in 1998, and was initially founded by a C57Bl/6j x SJL F3, then crossed twice into C57Bl/6j (Hsiao et al., 1996). Further

generations were crossed to C57Bl/6j × SJL F1 (Chapman et al., 1999). Since acquisition, Tg2576 mice have been maintained at the Cardiff School of Psychology, generally as follows. Adult male heterozygous Tg2576 mice (up to 10-12 months of age) derived from in-house breeding were paired with 8-10 week old female BL6SJL mice acquired from Jackson laboratories. The female BL6SJL mice had acclimated to the Cardiff Behavioural Neuroscience Laboratory housing conditions over a 2 week period, after which they were paired one to one with male Tg2576 mice in a dedicated breeding room, under housing conditions as previously described. The paired male and female mice were separated after a 2 week period, with male Tg2576 mice being returned to their cages, and female BL6SJL mice remaining in the breeding room to give birth. Once litters were born, the offspring were monitored and eventually separated between 21 and 28 days of age, dependent on their level of development. Offspring were initially group-housed in cages containing between 2 and 5 mice. Due to the additional variability the oestrus cycle of mammals can introduce to behavioural and biochemical results, only male offspring were retained when creating the experimental cohorts of mice. These male mice were ear clipped for identification purposes, with the resultant ear tissue being used to genotype the mice.

2.1.3 Genotyping

Mouse ear tissue was collected in 1.5mL nuclease-free microcentrifuge tubes, and kept on dry ice immediately prior to DNA extraction. DNA extraction and genotyping were carried out using the Phire Tissue Direct PCR Master Mix kit (Thermo Scientific). Each ear tissue sample was mixed with 20μL Dilution Buffer and 0.5μL DNARelease Additive, vortexed and briefly centrifuged, then left to react for 5 minutes at room temperature. Three further vortex and centrifugation cycles were carried out, punctuated by two periods of heating at 98°C (lasting 3 and 2 minutes, respectively). Samples were then stored at -20°C until being genotyped.

Genotyping samples were prepared in 0.5mL nuclease-free PCR tubes at 4°C. Each sample comprised 3μL extracted DNA sample and 20μL master mix (made up of nuclease-free water, Phire Tissue Direct PCR Master Mix, and 3 oligonucleotide primers). Primer sequences for amplifying endogenous murine prion protein, present in all mice, were 1501 (5'-AAGCGGCCAAAGCCTGGAGGGTGGAACA-3', 50pmol/μl) and 1502 (5'-GTGGATAACCCCTCCCCCAGCCTAGACCA-3, 10pmol/μl). Primer sequences for amplifying APPSwe, the transgene specific to Tg2576 mice, were 1502 and 1503b (5'-CTGACCACTCGACCAGGTTCTGGGT-3', 10pmol/μl). All primers were sourced from Eurofins Genomics. A positive control sample (known transgenic Tg2576 mouse), negative control sample (known wild-type mouse) and water blank

(nuclease free water in master mix) were also made. Samples were then heated via a PCR thermal cycling machine, as described in Table 2.1.

Step	Temperature	Time
1	98°C	5 minutes
2	92°C	5 seconds
3	72°C	5 seconds
4	72°C	3 minutes
Repeat steps 2-4 x39		
5	72°C	5 minutes
6	4°C	∞ (until electrophoresis)

Table 2.1. PCR thermal cycling programme used for genotyping Tg2576 and wild-type mice.

After thermal cycling completion, 20µL of sample was mixed with 4µL Novel Juice (GeneDireX), which contains 3 tracking dyes to visualise DNA bands and migration (Bromophenol Blue, Xylene Cyanol FF and Orange G). Samples and Novel Juice were mixed on aluminium foil cleaned with 100% ethanol. 20µL of each sample-Novel Juice mixture was then loaded onto a 1% agarose gel (TopVision Agarose, Thermo Scientific) made with, and submerged in, diluted Tris-acetate-EDTA 50X buffer (Thermo Scientific). To confirm the base pair number of DNA bands, 10µL of a 50 base pair DNA ladder (GeneDireX) was mixed with 2µL Novel Juice and loaded on to the gel. The loaded samples were electrophoresed at 100 volts applied for 1 hour, and the gel was then visualised using a Syngene G:BOX Chemi XX6. Samples from transgene-positive Tg2576 mice produced two bands of approximately 300 and 600 base pairs, while transgene-negative wild-type mice produced one band of approximately 600 base pairs (see Figure 2.1 below for an exemplar image).
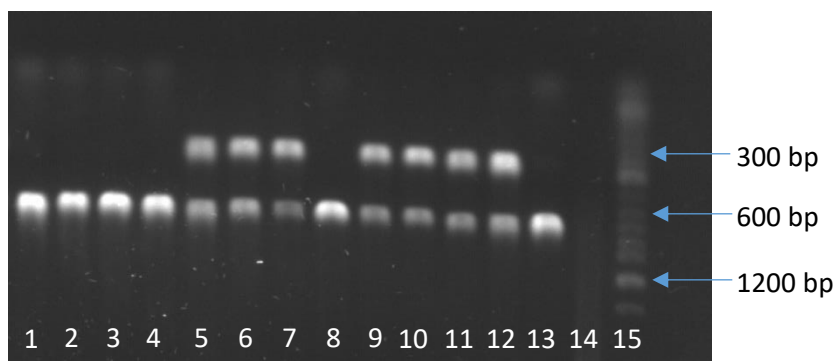
Figure 2.1. Tg2576 mouse genotyping gel electrophoresis image. Lanes 1 - 4 and 8 represent wild-type mice. Lanes 5 - 7 and 9 - 11 represent transgenic Tg2576 mice. Lanes 12, 13, 14 and 15 represent transgene-positive Tg2576 control, transgene-negative wild-type control, water blank and 50bp ladder, respectively.

2.2 Lick microstructure analysis.

2.2.1 Background

      The measuring of lick cluster size in this thesis involves recording the licking behaviour of mice when given access to a palatable solution. This approach builds on pioneering research by J.D. Davis and others, who found that rats consume fluids in rhythmic runs (or clusters) of licks, separated by pauses (Davis & Smith, 1992). A lick cluster is defined as a sequence of licks separated by an inter-lick interval (ILI) of no more than a given interval, where an ILI exceeding this number signifies the beginning of a new lick cluster. ILIs are distributed regularly within a lick cluster, but are irregularly distributed between lick clusters; in rat studies, a pause criterion of 0.5s effectively cleaves ILIs into those falling within and without lick clusters (Davis & Perez, 1993; Davis & Smith, 1992). Though the microstructure of the licking behaviour of mice has not been characterised to the extent it has been in rats, it appears largely similar, albeit mice tend to have shorter ILIs within clusters (Boughter, Baird, Bryant, St John, & Heck, 2007). The significance of this is that a shorter pause criterion for grouping ILIs into within clusters and between clusters, such as 0.33s, is probably more appropriate (though this would not materially alter conclusions drawn from lick cluster size much of the time, as most ILIs longer than 0.33s will also be longer than 0.5s).

      The mean number of licks in a cluster (or lick cluster size) is not an arbitrary figure, but is lawfully related to the nature of the ingested solution: lick cluster size generally increases monotonically with the concentration of palatable solutions (e.g. sucrose) and decreases monotonically as the concentration of unpalatable solutions (e.g. quinine) increases (Davis & Smith, 1992; Hsiao & Fan, 1993). Conditioned taste aversion experiments have shown that when a palatable solution is paired with lithium chloride (which induces gastric malaise), lick cluster size is reduced as a consequence (Dwyer, 2009). Moreover, lick cluster size is not merely a proxy for consumption, because the two measures can dissociate (for a review see Dwyer, 2012). In addition, drug manipulations which enhance hedonic responses in humans (e.g. benzodiazepines), also augment lick cluster size (Higgs & Cooper, 1998). These findings relating to changes in lick cluster size derive from rat studies and, while this level of investigation has yet to occur in mice, lick cluster size seems to behave similarly in the latter species. For example, increases in sucrose concentration produce increases in lick cluster size in mice (Clarkson, Dwyer, Flecknell, Leach, & Rowe, 2018; Davies et al., 2015), and the dissociation between consumption and hedonics seen in rats can also be observed in mice (Davies et al., 2015). In general, pleasant substances produce higher lick cluster sizes, and unpleasant substances produce lower lick cluster sizes. Importantly, the fact that lick cluster size can change without the solution changing suggests that it reflects the animal's reaction to the solution rather than being a fixed property of the solution itself. Given this, a lower lick cluster

size in response to a normatively positive substance would represent a diminished reaction to that pleasant substance, analogous to anhedonia. This has been observed in mice (Austen, Sprengel, & Sanderson, 2017; McNamara et al., 2016).

Interpreting lick cluster analysis does depend on the measuring of other behavioural variables which can influence lick cluster size, such as the ILI and the amount consumed per lick. An interpretation in favour of a hedonic deficit can be made if inter-lick interval and amount per lick can be discounted as, or are unlikely to be, major influences on lick cluster size. Overall amount consumed is another response to palatable solutions which can be recorded in lick cluster studies, which can dissociate from the hedonic reaction to that solution (as described in Chapter 1).

2.2.2 Experimental details

Lick cluster analysis necessarily requires some minimum level of consumption to occur. In order to incentivise consumption to obtain a reliable measure of lick cluster size, mice were mildly food-deprived prior to pre-training and testing sessions, except where noted. Food was removed in the morning and returned roughly 6-8 hours later, after testing had finished. Mice were weighed each pre-training and testing day; baseline weight was recorded at the time of food deprivation, and post-deprivation weight was recorded immediately prior to behavioural testing. Any mouse whose body weight fell below 85% of its initial baseline weight and did not recover, had access to food immediately restored, though this was a rare occurrence. Outside of the 6-8 hour food deprivation period, animals had access to food and water ad libitum. In addition, when testing at 16 months of age, mice alternated between food-deprived days and free-feeding days, in order to examine whether deprivation itself influenced lick cluster size (Section 3.2.4.5).

The material conditions of testing occurred as described in previous lick cluster studies (Davies et al., 2015; Lydall et al., 2010). Briefly, testing occurred in a room housing 16 drinking chambers, which were semi-translucent plastic boxes with dimensions of 30×13×12.8 cm (D×W×H), metal grid floors and metal lids. Sucrose solutions were presented in 50mL plastic cylinders with stainless steel drinking spouts, which were inserted into the right side of a drinking chamber at the start of each experimental session. A touch-sensitive lickometer noted the time of each lick to the nearest 0.01 second; this was recorded via MED-PC software (Med Associates Inc., St. Alban's, VT, USA) on a Windows-enabled PC.

At all time points, a pre-training period occurred prior to experimental drinking sessions, in which mice were given access to an 8% w/w sucrose solution, and of sufficient length (4-10 days) for

consistent levels of consumption to be reached. Following this pre-training period, experimental sessions occurred in two phases of equal length (see Chapter 3 for precise details); in the first phase, roughly half of each genotype received 4% sucrose, with the other half receiving 16%; in the second phase, animals which had received 4% sucrose in phase 1 received 16% sucrose, and vice versa. All drinking sessions were started approximately 3 hours after the animals' food was first removed, and all sucrose solutions were made on the day of testing, using deionised water. Pre-training and experimental drinking sessions lasted 10 minutes, during which time the experimenter was absent from the testing room. The weight of fluid consumed by each animal per session was determined by weighing its drinking bottle before and after that session. As well as recording baseline weight, weight change following food restriction, and sucrose consumption each day, average ILI within clusters and amount consumed per lick were also derived from the recorded data. Data were aggregated across all test days at each concentration, except days where there was evidence of failure of either lick recording or drinking spout, or where extremely little drinking occurred (i.e. less than 50 licks in a session). Any mouse with less than two days' drinking data at one or both phases was also removed from analysis, as this could not be taken as a reliable measure of its behaviour. For information on cleaning of data at each time point, see Chapter 3. Data were analysed using 0.33s as the pause criterion for defining a new cluster beginning, though data were also collected using 0.25s, 0.5s, 0.66s and 1s pause criteria (the pattern of results was not materially different between pause criteria, and thus the data at criteria other than 0.33s will not be presented or discussed further).

2.3 Object-in-place testing

2.3.1 Background

        For the purposes of this thesis, the object-in-place cognitive test has several advantageous features over other test paradigms and object-based assays. Other conventional approaches to testing rodent memory include the Morris water maze, radial arm maze and T- or Y-mazes (Vorhees & Williams, 2014). The Morris water maze features an aversive element as it involves placing rodents in water (Morris, 1984), and, although this is not procedurally or environmentally identical to the forced swim test (Porsolt, Le Pichon, & Jalfre, 1977), repeated water maze tests will likely still produce some level of distress. Given that a major aim of this thesis is to profile a depression-related behaviour over time, interpreting results of lick cluster studies would be complicated by the repeated use of a distressing task, which could in its own right potentially contribute to or influence a depressive phenotype. Maze tests typically include an appetitive component such as sucrose

reward (Vorhees & Williams, 2014); both reward and satiety processing may be aberrant in Tg2576 mice (Ishii, Wang, Racchumi, Dyke, & Iadecola, 2014; Nobili et al., 2017), both of which may alter motivation towards and consumption of such rewards. As Tg2576 mice may not experience rewarding substances in the same way as wild-type mice, a test of cognition which does not include reward is preferable. In addition, appetitive maze tasks typically involve some degree of food deprivation to ensure animals are motivated towards finding food rewards (Vorhees & Williams, 2014). Pre-existing reward or satiety processing alterations in Tg2576 mice may mean that food deprivation produces unequal motivational states in Tg2576 and wild-type mice, which could complicate interpretation of results. A cognitive test that does not rely on food deprivation to induce motivation is thus also preferable. Object-related cognitive tests provide such an assay, as they rely on the natural tendency of rodents to spontaneously explore objects, in a non-aversive, non-rewarded, and non-deprived setting (Dix & Aggleton, 1999; Ennaceur & Delacour, 1988).

There are a number of possible object exploration-based tests which could be used to profile cognition in Tg2576 mice. These tests broadly investigate recognition memory, that is, the remembering that an item or event has been previously encountered (Mandler, 1980). Specifically, recognition memory tests can investigate object novelty per se (Ennaceur & Delacour, 1988), objects in novel locations (Ennaceur, Neave, & Aggleton, 1997), and specific object-location associations (Dix & Aggleton, 1999), among other manipulations. Typically, rodents will display a preference for exploring novel objects, locations, and specific object-location associations in the previously mentioned tests. Importantly, these facets of recognition memory have all been investigated in Tg2576 mice (Good & Hale, 2007). Tg2576 mice display an intact preference for exploring novel objects, and familiar and novel objects in new locations, yet are unable to integrate specific object-location associations, showing a diminished recognition of objects that exchange positions in a 4 object array (Good & Hale, 2007). This deficit in object-in-place memory has been detected at 10-12 (Good, Hale, et al., 2007), 14 (Hale & Good, 2005), and 16 and 21 months of age (Good & Hale, 2007), and appears to be of a roughly similar magnitude at these time points. Given that this deficit appears robust and has been revealed in multiple studies, the object-in-place task is a suitable choice for profiling cognitive decline in the Tg2576 mouse. This deficit and its potential neural underpinnings will be discussed in more detail in Chapter 4. The methodology used for the object-in-place task in this thesis is discussed below, and in Chapter 4.

2.3.2 Experimental details

Testing occurred in a well-lit and quiet room, featuring distinct visual cues of different shapes and colours on the walls. A Perspex arena (60×60×40cm), with a grey floor and transparent walls covered with white paper, was positioned in the centre of the room and at just below waist height. Activity in the arena was watched and recorded via a camera directly above, at ceiling height, which was connected to a monitor and a Philips DVDR recorder. All objects chosen for habituation and object-in-place experiments had been used in previous experiments, were composed of non-porous material, and built so as to withstand the investigative behaviour of mice. During all habituation and test sessions, contact with objects was defined as a mouse oriented towards an object within a distance of 1cm, and sniffing or otherwise investigating the object. Climbing or sitting atop an object was not recorded as contact time. Object contact was manually recorded using stopwatches, with the experimenter sitting out of sight of the mice in the arena. Prior to all habituation and test sessions, between mice, and between sample and test phases, objects and the arena were wiped clean with 70% isopropyl alcohol wipes, in order to remove odour cues, urine and excrement.

Mice were run in separate squads for object-in-place testing, in which each squad went through a habituation phase then a test phase, which were taken from a prior PhD thesis which investigated visuospatial memory in Tg2576 mice (Hale, 2007). The habituation protocol was as follows: at the first time point, mice spent 10 minutes on 2 consecutive days in the empty arena, followed by 10 minutes on 2 consecutive days with 2 identical objects placed 10cm apart in the arena centre, which they could freely explore. At every time point thereafter, habituation consisted solely of the latter object-presenting portion. A new pair of identical objects was used for habituation at each time point. The test phase consisted of a sample trial in which mice could freely explore an array of 4 objects, a 2 minute inter-trial interval, and a 10 minute test trial in which 2 of the 4 previously encountered objects exchanged positions (mismatched objects), along a diagonal plane (see Figure 2.2 for a diagrammatic example). Objects in the sample and test trial were placed in the centre of the arena, approximately 15cm apart. The sample trial was 10 minutes for transgenic mice, and of variable length (up to 10 minutes) for wild-type mice, as a yoked protocol was adopted in order to match object sampling time between genotypes (described in more detail below). At each time point, every mouse was tested on 2 separate and distinct 4 object arrays on 2 consecutive days; object set and diagonal shift (top right-bottom left versus top left-bottom right) were counterbalanced across genotype and day. Further counterbalancing details are included in Chapter 4.

As prior experiments have revealed that Tg2576 mice tend to display lower contact times with objects than wild-type mice (Good & Hale, 2007; Hall et al., 2016), a yoking procedure was employed in the object-in-place sample trials. Each wild-type mouse was yoked to a Tg2576 mouse, and was allowed to accumulate the same amount of total object contact time in the sample phase that its transgenic counterpart had accumulated over 10 minutes. Yoking was achieved by the experimenter manually timing the total object contact a wild-type mouse accumulated in the sample phase, and ending the sample phase once total object contact time had reached that of the prior Tg2576 mouse. Yoking was only applied to the sample phase of object-in-place testing; there was no criterion set for object contact times mice needed to accumulate in the test phase, outside of the minimum level of exploration noted below. If a wild-type mouse happened to display less contact time than its transgenic counterpart, then it was given the full 10 minute sample phase. While the yoking procedure used may lower the performance of wild-type mice in absolute terms, it ensures that the sampling of objects is equivalent between genotypes, and that any deficits seen are not simply due to differences in overall levels of exploration in the sample phase.

Test phase data were initially collected as raw contact times, and also converted to discrimination ratios, by the following formula: mismatched object exploration (sec)/(familiar object exploration (sec) + mismatched object exploration (sec)). Discrimination ratios significantly greater than 0.5 indicate a preference for exploring mismatched objects, while ratios not significantly different from, or lower than, 0.5 indicated no discernible preference for exploring mismatched objects. Data were averaged to mean contact times over the 2 test days, and mean discrimination ratios over the 2 test days, prior to analysis. Any mouse showing low contact times (either <1s total object contact or no exploration of either familiar or mismatched objects) had that day's data removed. The specific details of data cleaning at each time point are covered in Chapter 4.

Sample trial
10 minutes (TG)
Matched contact (WT)

Inter-trial interval (2 minutes)

Test trial
10 minutes (TG & WT)
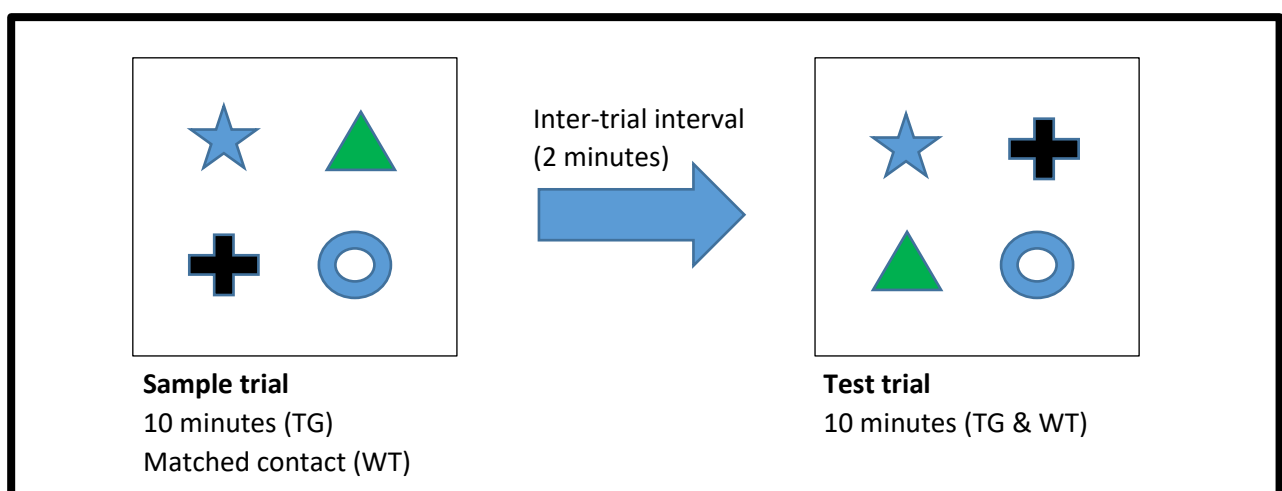
Figure 2.2. Schema representing object-in-place testing protocol, displaying a 4 object array in an arena, within which two objects exchange locations in the test trial. TG = Tg2576 mice, WT = wild-type mice.

A schema illustrating the full behavioural testing schedule which both mouse cohorts underwent is shown below.
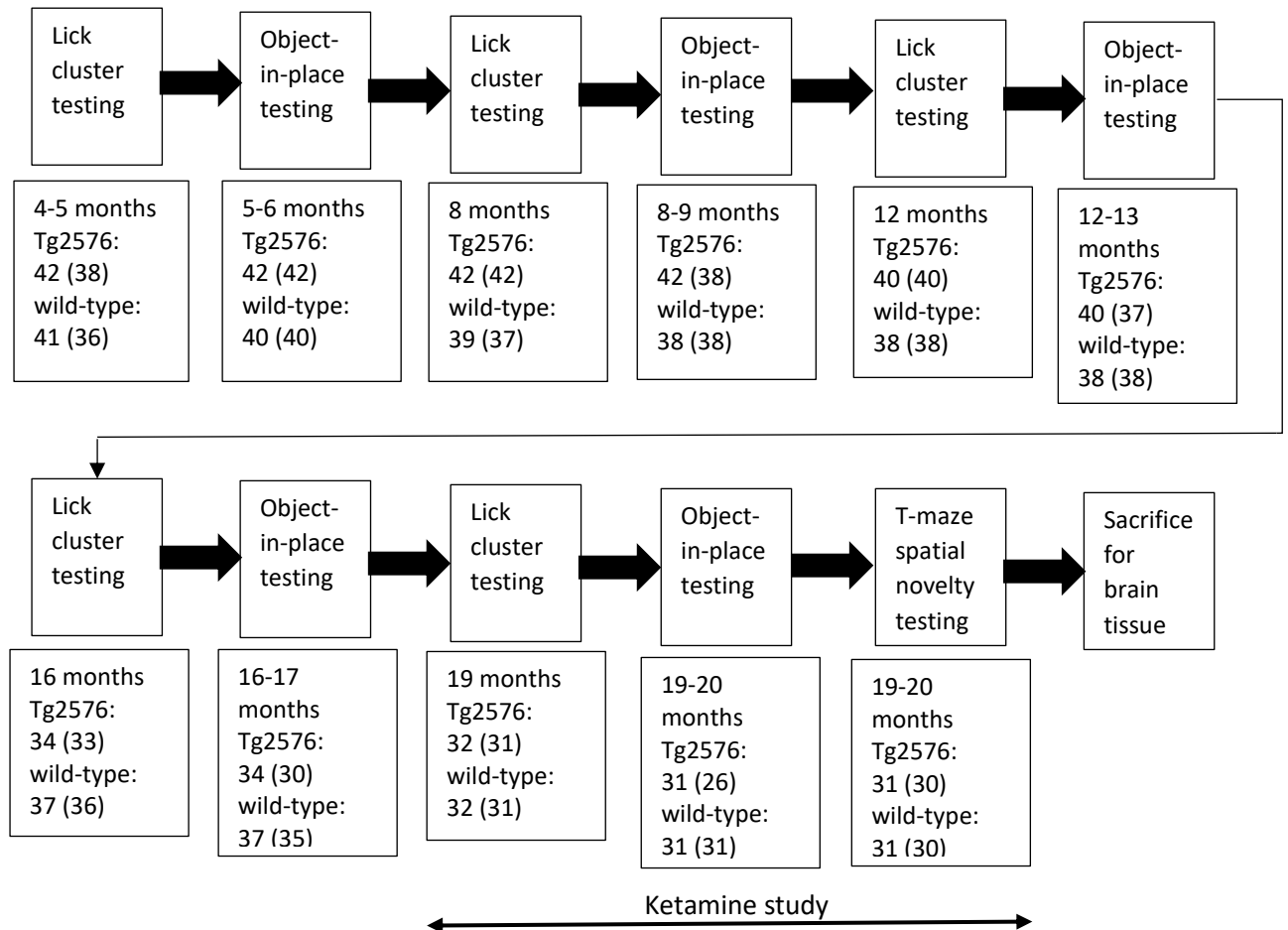
Figure 2.3. Schema illustrating the behavioural testing schedule the mouse cohorts passed through. Initial numbers reflect the numbers of each genotype entered into each behavioural test; bracketed numbers are the numbers of mice in each genotype analysed after data cleaning.

2.4 Tissue collection and sample preparation

Mice were killed by cervical dislocation, and the brain was removed. Left and right hippocampi and the surrounding left and right cortical tissue were dissected, collected separately in 0.5mL tubes, and snap-frozen in liquid nitrogen. Samples were then stored at 80°C until sample preparation occurred.

Right hippocampus and cortex were used for human Aβ ELISAs. Samples underwent extractions that isolated first soluble Aβ, then insoluble Aβ. During these extraction procedures, samples were kept cool at 4°C. Frozen tissue samples were transferred to pre-weighed 2mL homogenisation tubes (Precellys CK28, 03961-1-002), re-weighed and the tissue weight was calculated. 2% SDS containing a protease inhibitor cocktail (set III EDTA-free, Calbiochem) at a 1:100 ratio was added to the tissue samples at 75mg of tissue per mL of solution. Samples were homogenised at 6000 RPM using a Precellys 24-Dual homogeniser for two 30 second periods separated by a 30 second pause, then transferred to polycarbonate centrifuge tubes. Samples were centrifuged using a Beckman Optima Ultracentrifuge, at 100,000 x g for 1 hour at 4°C. After centrifugation, the supernatant (containing soluble Aβ) was transferred to a new 1.5mL microcentrifuge tube. 5µL of this fraction was retained for use in a protein assay, and the remaining fraction was aliquoted into microcentrifuge tubes, and diluted 1:5 with EC sodium buffer (20mM Na_2HPO_4/NaH_2PO_4, 0.2mM EDTA, 0.4M NaCl, 0.2% (w/v) bovine serum albumin (BSA), 0.05% (w/v) CHAPS, 0.05% (w/v) NaN_3 at pH 7). Soluble fraction samples were then stored at -20°C until used in ELISAs. The remaining pellet was re-suspended in 70% formic acid (Sigma-Aldrich) at 150mg of tissue per mL of solution, and centrifuged as described previously. The supernatant (containing insoluble Aβ) was transferred to a new 1.5mL microcentrifuge tube, then aliquoted into microcentrifuge tubes, and diluted 1:20 with a neutralising buffer (1M Tris, 0.5M Na_2HPO_4, at pH 11). Insoluble fraction samples were then stored at -20°C until used in ELISAs.

Left hippocampus and cortex were used for Western blot experiments. As synaptic receptor expression was of particular interest, samples underwent a synaptic protein extraction procedure. During this extraction procedure, samples were kept cool at 4°C. Frozen tissue samples were transferred to pre-weighed 1.5mL microcentrifuge tubes, re-weighed and the tissue weight was calculated. Syn-PER™ Synaptic Protein Extraction Reagent (Thermo Scientific) containing a protease inhibitor cocktail (set III EDTA-free, Calbiochem) at a 1:100 ratio, and a phosphatase inhibitor cocktail (set V 50X, Millipore) at a 1:50 ratio, was added to the tissue samples at a volume of 10µL per mg of tissue. The tissue was then homogenised using 10-15 strokes of a micropestle (Sigma-Aldrich). Samples were then centrifuged at 1200 x g for 10 minutes at 4°C, the supernatant was transferred to a new tube, and the pellet was discarded. The supernatant was then centrifuged at 15,000 x g for 20 minutes at 4°C, and the resultant supernatant (the cytosolic fraction) was removed and stored at -20°C. The remaining synaptosome pellet was re-suspended in Syn-PER™/inhibitor cocktail mixture, at a volume of 1.5µL per mg of original tissue weight, and mixed by pipetting. 5µL of this synaptosomal sample was retained for use in a protein assay. The remaining synaptosomal sample was diluted with 3X sample buffer, in a 2:1 sample:buffer ratio, for Western blotting (see Table 2.2

for sample buffer details). Samples were then stored at -20°C until used in Western blot experiments.

Sample buffer ingredient	1X sample buffer	3X sample buffer
Tris base	0.38g	1.14g
SDS	2g	6g
Glycerol	5mL	15mL
B-mercaptoethanol	2.5mL	7.5mL
Bromophenol blue	0.5mL	1.5mL
dH_2O	To 50mL	To 50mL

Table 2.2. Sample buffer composition.

Protein quantification of samples was carried out for both ELISA and Western blot samples, using the Pierce[TM] bicinchoninic acid (BCA) Protein Assay Kit (Thermo Scientific[TM]). In brief, a linear range of protein standards (BSA 2mg/mL – 0.007mg/mL) were prepared via serial dilution using distilled H_2O. 25µL of standards or distilled H_2O standard blank and 1µL of samples (including a sample blank made of the relevant buffer solution) were pipetted, in duplicate, into a 96-well Sterilin[TM] Clear Microtiter[TM] plate (Thermo Scientific[TM]). 200µL of working reagent (BCA Reagent A with BCA Reagent B in a 50:1 ratio) were added to these wells, and the plate was incubated at 37°C for 20 minutes. The plate was then read at 540nm, and the averaged standard blank value was subtracted from the averaged standard values to produce a corrected standard curve. The averaged sample blank value was subtracted from the averaged sample values, and the protein concentration of the corrected sample values was calculated using GraphPad Prism software.

2.5 ELISA details

Invitrogen human Aβ40 and Aβ42 sandwich ELISA kits (ThermoFisher Scientific) were used to quantify soluble and insoluble Aβ40 and Aβ42 levels. Prior to Aβ quantification, plates were optimised to find the appropriate sample dilution factor, in line with the manufacturer's instructions. Aβ standards and samples were then prepared and/or diluted, pipetted into plate wells, and incubated with detection antibody for 3 hours, as per the manufacturer's instructions. Wells were then aspirated, washed 5 times with 1X wash buffer from the manufacturer's kit, and incubated for 30 minutes with anti-rabbit IgG HRP as directed. Wells were aspirated and washed again as previously described, then incubated with Stabilised Chromogen for 30 minutes in the dark, after which Stop Solution was added. The plate was read at 450nm, standard and sample values were

corrected via blank substitution, and a standard curve was generated using GraphPad Prism. Sample Aβ values were generated from the standard curve, corrected by the dilution factor used, and normalised to protein concentration to give pg/mg tissue values.

2.6 Western blot details

2.6.1 Gel preparation

All Western blotting experiments used a 5% sodium dodecyl sulphate polyacrylamide (SDS-PAGE) stacking gel, and a 10% SDS-PAGE separating gel (ingredients and volumes in Table 2.3), which were prepared in Invitrogen™ Novex™ 1.0mm cassettes (Fisher Scientific) with 12 or 15 well combs.

Ingredient	Manufacturer	10% separating gel	5% stacking gel
Distilled H$_2$O	n/a	52.7%	57%
Alfa Aesar™ Acrylamide/Bisacrylamide 37.5:1, 30% solution	Fisher Scientific	10% (acrylamide)	5% (acrylamide)
3M Tris.HCl	Fisher Scientific	12.5%	n/a
0.5M Tris.HCl/0.4% SDS (w/v)	Fisher Scientific	n/a	25%
10% SDS (w/v)	Fisher Scientific	0.1%	n/a
10% Ammonium persulfate (w/v)	Bio-Rad	0.05%	0.5%
TEMED	Sigma-Aldrich	0.05%	0.08%

Table 2.3. Components (stated in final concentrations) of separating and stacking gels.

2.6.2 Sample loading, electrophoresis and transfer

Samples were initially heated at 90°C for 40 minutes in a heating block, and on any subsequent use at 70°C for 5 minutes. Heated samples were briefly vortexed and centrifuged, then 20µg of sample was loaded into each well and gels were run in an Invitrogen™ Novex™ XCell SureLock system containing tank buffer (25mM Tris base, 190mM glycine, 0.05% SDS, pH 8.3). 10µL of a protein standard (Precision Plus Protein™, Bio-Rad) was also loaded, to aid in identifying the molecular mass of any bands. Samples were stacked at 45V for 25 minutes on ice and then run at 135V for a duration sufficient for protein separation. After separation, samples were transferred to a

0.45µm nitrocellulose membrane (Amersham Protran) with semi-dry transfer buffer (48mM Tris base, 39mM glycine, 0.038% SDS, 20% methanol) at 38mA for 1 hour.

After protein transfer to the nitrocellulose membrane was complete, the membrane was rinsed in Tris-buffered saline with Tween20 (TBST: 20mM Tris base, 150mM NaCl, 0.1% Tween20, pH 7.5) for 2 minutes to remove any remaining semi-dry transfer buffer. The membrane was cut into distinct sections if necessary (i.e. when investigating two different proteins of distant molecular weights from the same membrane), then blocked for 1 hour in 5% Blotto solution (fat-free dried milk 5% w/v, in TBST), except when probing for the $5HT_{1B}$ receptor protein when 5% BSA in TBST was used.

2.6.3 Antibody incubation, washing, and imaging

Following blocking, membranes were incubated overnight in a primary antibody solution at 5°C on a tube roller. After primary antibody incubation, membranes were washed with TBST (1x5min, 2x15min, discarded and replaced after each wash) on a rocker, then incubated with a secondary antibody. Secondary antibodies were incubated for 1 hour at room temperature, on a tube roller. Full details of primary and secondary antibodies are listed in Table 2.4; all antibodies were diluted in 1% Blotto except where otherwise noted. Following secondary antibody incubation, membranes were washed in TBST as previously described. Membranes were then briefly placed on tissue paper to remove any excess TBST, then incubated with an enhanced chemiluminescence (ECL) solution for 5 minutes in the dark. This imaging reagent comprised equal parts of solutions A and B from the Pierce™ ECL Western Blotting Substrate (Thermo Scientific™), or Luminol and Stable Peroxide Buffer from the SuperSignal™ West Dura Extended Duration Substrate (Thermo Scientific™), as per Table 2.4. After the 5 minute incubation time, membranes were imaged with a Syngene G:BOX Chemi XX6 using the program GeneSys G:BOX Chemi-XX6 (v1.6.1.0) to capture images, which were analysed as described below.

In certain cases the membranes were stripped of primary and secondary antibodies after imaging and reprobed, either for a second protein, or for β-actin. Where membrane stripping occurred, the membrane was washed in TBST for 2 minutes to remove any imaging reagent, then incubated in Restore™ PLUS Western Blot Stripping Buffer (Thermo Scientific™) for 7 minutes and the membrane was re-washed in TBST for 2 minutes. After this stripping procedure, membranes were re-incubated with a primary antibody solution for a new protein, or incubated with an anti-β-actin antibody directly conjugated to peroxidase (Table 2.4) for 40 minutes at room temperature and imaged as above.

Protein	Primary antibody	Dilution factor	Secondary antibody	Dilution factor	Imaging reagent
5HT$_{1B}$	Anti-5HT$_{1B}$ Receptor Antibody (ab13896, Abcam) Host: Rabbit Blocked with 5% BSA	1:1000 in 1% BSA	HRP Goat Anti-Rabbit IgG Antibody (Peroxidase) (PI-1000, Vector Laboratories)	1:15000 in 1% BSA	ECL
SERT	Anti-Serotonin Transporter (AB9726, Merck Millipore) Host: Rabbit	1:5000 in 5% Blotto	HRP Goat Anti-Rabbit IgG Antibody (Peroxidase) (PI-1000, Vector Laboratories)	1:10000 in 5% Blotto	ECL
5HT$_4$	Anti-5HT$_4$ Receptor (HTR4) (extracellular) Antibody (ASR-036, Alomone Labs) Host: Rabbit	1:500	HRP Goat Anti-Rabbit IgG Antibody (Peroxidase) (PI-1000, Vector Laboratories)	1:10000	West Dura
NR1	Anti-NMDAR1 (556308, BD Biosciences) Host: Mouse	1:500	HRP Horse Anti-Mouse IgG Antibody (Peroxidase) (PI-2000, Vector Laboratories)	1:10000	West Dura
NR2B	Anti-NMDAR2B Antibody (AB1557P, Merck Millipore) Host: Rabbit	1:500	HRP Goat Anti-Rabbit IgG Antibody (Peroxidase) (PI-1000, Vector Laboratories)	1:15000	West Dura
p-NR2B	Anti-NMDAR2B Antibody, phosphoTyr 1472 (AB5403, Merck Millipore) Host: Rabbit	1:750	HRP Goat Anti-Rabbit IgG Antibody (Peroxidase) (PI-1000, Vector Laboratories)	1:15000	West Dura
GluR1	Anti-Glutamate Receptor 1 (AMPA subtype) antibody (ab31232, Abcam) Host: Rabbit	1:2500	HRP Goat Anti-Rabbit IgG Antibody (Peroxidase) (PI-1000, Vector Laboratories)	1:10000	ECL
p-GluR1	Anti-Glutamate Receptor 1 (AMPA	1:1000	HRP Goat Anti-Rabbit IgG	1:10000	West Dura

	subtype) (phospho S845) antibody (ab3901, Abcam) Host: Rabbit		Antibody (Peroxidase) (PI-1000, Vector Laboratories)		
PSD95	Anti-PSD95 antibody (ab18258, Abcam) Host: Rabbit	1:1000	HRP Goat Anti-Rabbit IgG Antibody (Peroxidase) (PI-1000, Vector Laboratories)	1:15000	ECL
Mu	Anti-μ-Opioid Receptor (OPRM1) (extracellular) Antibody (AOR-011, Alomone Labs) Host: Rabbit	1:500	HRP Goat Anti-Rabbit IgG Antibody (Peroxidase) (PI-1000, Vector Laboratories)	1:15000	West Dura
Kappa	Anti-κ-Opioid Receptor (OPRK1) (extracellular) Antibody (AOR-012, Alomone Labs) Host: Rabbit	1:500	HRP Goat Anti-Rabbit IgG Antibody (Peroxidase) (PI-1000, Vector Laboratories)	1:12500	West Dura
Delta	Anti-δ-Opioid Receptor (extracellular) Antibody (AOR-014, Alomone Labs) Host: Rabbit	1:200	HRP Goat Anti-Rabbit IgG Antibody (Peroxidase) (PI-1000, Vector Laboratories)	1:10000	West Dura
β-actin	Anti-β-Actin–Peroxidase antibody (A3854, Sigma-Aldrich) Host: Mouse	1:20000	Included in primary antibody solution.	N/A	ECL

Table 2.4. Primary and secondary antibodies, dilution factors, and chemiluminescence reagents.

2.6.4 Image analysis

TIF images of membranes were analysed using the program ImageJ (v.1.52a, National Institutes of Health, USA). The intensity of sample bands was measured by framing each band in a box of equal dimensions (see Figure 2.4A for an exemplar image), which was then converted into a series of curves (see Figure 2.4B). The area under the curve was calculated using ImageJ (v.1.52a, National Institutes of Health, USA), and the values were imported into Microsoft Excel.
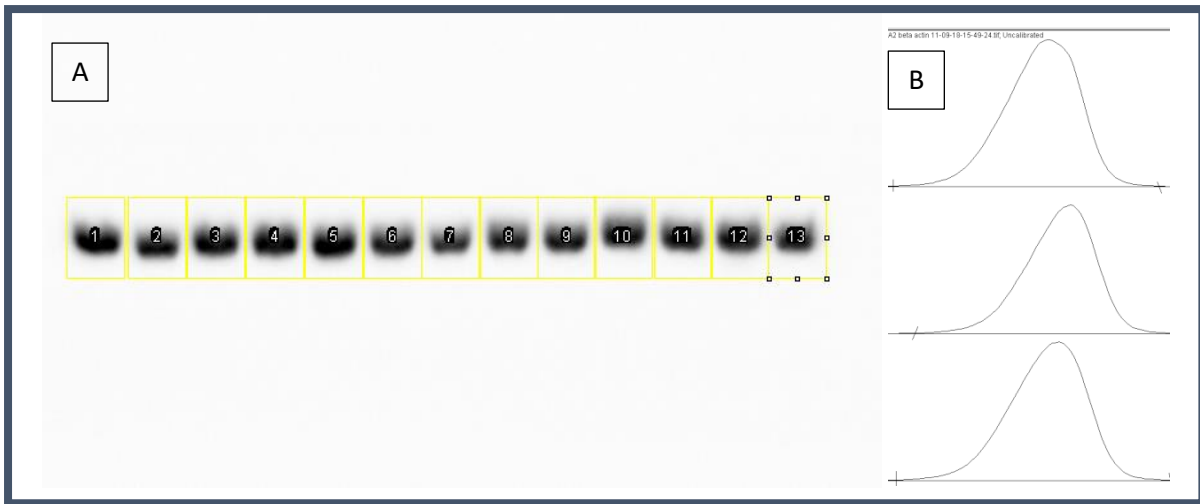
Figure 2.4. A: Framing of β-actin bands to produce a series of curves. B: Curves representing band intensity, derived from 2A.

Values for proteins were normalised to β-actin values for each sample (i.e. expressed as a proportion of the β-actin value). As the sample size for Western blot experiments was too large for all samples to be loaded onto one gel, multiple gels were used for each protein of interest. To account for inter-membrane variance, an internal control sample (generally a vehicle-treated wild type mouse in aged mice, and a wild-type mouse in young mice) was loaded across all gels for a given protein. β-actin-normalised sample values were then normalised to the internal control on each gel. This internal control sample was not included in the dataset for statistical analysis.

2.7 Statistical analysis

Two statistical approaches were taken in this thesis, for both of which JASP 0.8.0.0 was used. Conventional null hypothesis significance testing was used for all behavioural and biochemical data, as described in Chapters 3, 4 and 5. Where assumptions of sphericity were not met for ANOVAs, Greenhouse-Geisser corrections were used, and where assumptions of equal variance were not met for Student's t-tests, Welch's t-tests were used. In factorial ANOVAs simple effects analyses were carried out where results were of conceptual importance. An alpha level of .05 was used as the threshold of significance throughout the empirical work. In addition, a supplementary Bayesian analysis was conducted where null results were of material consequence, because conventional significance testing fails to distinguish between a genuine absence of effect or simply uninformative data (Dienes, 2014). Bayesian statistics provides a ratio of the probability of observed data under various models, e.g. a model of the null hypothesis relative to a particular alternative model. The output of this analysis comes in the form of Bayes factors, which can be interpreted using a

convention suggested by Harold Jeffreys (Jeffreys, 1998). A Bayes factor of 1 indicates that the data are equally consistent with the null and alternative hypotheses (i.e. are uninformative), whereas Bayes factors greater than 1 indicate that the data favour the alternative over the null hypothesis, and Bayes factors lower than 1 indicate that the data favour the null over the alternative hypothesis (Dienes, 2014). The convention of interpreting Bayes factors for null results is that a Bayes factor between 1 and 1/3 represents anecdotal evidence for a true null effect, between 1/3 and 1/10 suggests some degree of support for the null hypothesis, and a Bayes factor lower than 1/10 can be taken as strong evidence for a null effect, though these are not rigid cut-off values. Bayesian ANOVAs and independent samples t-tests were used in Chapters 3, 4 and 5, as described by others (Rouder, Morey, Speckman, & Province, 2012; Rouder, Speckman, Sun, Morey, & Iverson, 2009). For Bayesian interactions, Bayes factors were created by comparing the simplest model with the interaction to the best model without that interaction including only main effects that were within the interaction model.

Chapter 3: Hedonic profile of Tg2576 mice

3.1. Introduction

Chapter 1 discussed how depression is a common occurrence in Alzheimer's disease, and may in fact be an early or prodromal neuropsychiatric symptom. It is also unclear whether standard antidepressants offer any tangible benefit in depression in Alzheimer's disease. Anhedonia is a core and pernicious component of depression, which may not be sufficiently addressed by conventional antidepressants. It is also present in Alzheimer's disease, and may be more common as the disease progresses. Mouse models of Alzheimer's disease provide an opportunity for investigating the presence of a depressive behaviour such as anhedonia, its biochemical basis, and the possibility of its treatment.

A major issue in studying anhedonia in Alzheimer's disease is determining which aspect or aspects of the pathological process (broadly: Aβ pathology, tau pathology, and overt neurodegeneration) may give rise to this depression symptom. A broad investigation of these possibilities is not the purpose of this thesis. However, given that depression generally, and anhedonia specifically, are present in both early Alzheimer's disease and mild cognitive impairment (Gabryelewicz et al., 2004; Lopez et al., 2003; Starkstein et al., 2005), the latter being potentially a precursor to Alzheimer's disease, a sensible place to begin the investigation is with Aβ, the earliest pathological event. In addition to this being a logical starting point, there are suggestions from biomarker studies of a relationship between Aβ levels and depression symptoms. For example, greater cortical Aβ load was associated with a greater apathy-anhedonia factor score in cognitively normal older adults with subthreshold depression symptoms (Donovan et al., 2015). In a follow-up study with a similar cohort, participants with current subthreshold depression had greater cortical Aβ load than those with no history of depression (Donovan et al., 2018). However, a longitudinal analysis in the latter study found that greater cortical Aβ load was associated with a steeper increase in depression scale score over time, and with increasing anxious-depressive but not apathy-anhedonia symptoms (Donovan et al., 2018). Similar studies have also reported an association over time between greater Aβ burden and both lower mood (Babulal et al., 2016), and clinically significant depressive symptoms (Harrington et al., 2017). While these studies point to a potential relationship between Aβ and depression, if not specifically anhedonia, they measured fibrillar Aβ, whereas it is soluble Aβ species which are thought to have the most pathological relevance. Soluble

CSF Aβ42 has also been investigated in relation to depression in various populations, with both some type of relationship between the two (Gudmundsson et al., 2007; Pomara et al., 2012), and no relationship (Kramberger et al., 2012; Ramakers et al., 2013), being reported. However, CSF Aβ42 is essentially an inverse measure of plaque or fibrillar Aβ load (Blennow, Mattsson, Schöll, Hansson, & Zetterberg, 2015), and as such these studies are not necessarily providing a measure of biologically active soluble Aβ in the brain. Despite not being greatly informative as to the exact relationship between soluble Aβ and symptoms of depression such as anhedonia, considered as a whole these two sets of studies suggest there may be an association worth investigating.

If there is indeed a relationship between Aβ and anhedonia, a logical explanation could be that Aβ acts within the brain's reward system in a manner detrimental to its function, resulting in a diminished hedonic response to pleasurable events. A general reward system comprising a number of cortical and limbic structures undergirds the response to a broad range of pleasurable experiences (Berridge & Kringelbach, 2013). The direct experience of hedonic reward appears to involve at least the orbitofrontal cortex (Kringelbach, O'Doherty, Rolls, & Andrews, 2003), nucleus accumbens (Roitman, Wheeler, Tiesinga, Roitman, & Carelli, 2010), and ventral pallidum (Tindell, Smith, Peciña, Berridge, & Aldridge, 2006). Finer-grained analyses have revealed that there exist in these reward sites and elsewhere a number of localised sub-regions responsible for generating 'normal' pleasure sensations, amplifying hedonic reactions, or dampening down pleasure (so-called hedonic 'hot spots' and 'cold spots') (Berridge & Kringelbach, 2015). These sub-regions are present in the nucleus accumbens, ventral pallidum, orbitofrontal cortex, parabrachial nucleus, and possibly insula (Berridge & Kringelbach, 2015; Castro & Berridge, 2017; Peciña, Smith, & Berridge, 2006). These reward sites, either at the level of their broad functionality, or in discrete but important sub-regions, could underlie the experience of anhedonia.

Imaging studies of depressed and non-depressed patients have revealed that reward circuitry dysfunction coincides with anhedonia. For example, anhedonia correlates with reduced nucleus accumbens reward response in non-depressed humans in relation to monetary reward, and also correlates with reduced nucleus accumbens volume (Wacker, Dillon, & Pizzagalli, 2009). Depressed patients show a lack of response to positive stimuli in the ventral striatum, the region containing the nucleus accumbens (Epstein et al., 2006), and an earlier fMRI-based study found that depressed female adults possessing a high degree of anhedonia showed, among other changes, increased activation of the insula (Mitterschiffthaler et al., 2003). In depressed individuals presented with 'happy' stimuli, anhedonia severity showed, among other correlations, a negative correlation with ventral striatum activity (Keedwell, Andrew, Williams, Brammer, & Phillips, 2005). Collectively, studies such as these suggest that anhedonia may be a consequence of dysfunctional components of

the brain's reward system. As noted in Chapter 1, reward sites such as the nucleus accumbens (Suenaga et al., 1990), orbitofrontal cortex (Van Hoesen et al., 2000), and parabrachial nucleus (German et al., 1987; Parvizi et al., 1998), are pathologically altered in Alzheimer's disease. These alterations include the appearance of Aβ plaques, suggesting the possibility of soluble Aβ perturbing the reward system and producing anhedonia.

One way of investigating the potential association between soluble Aβ and anhedonia, and whether such an anhedonia can be treated, is by using a mouse model of Alzheimer's disease. Mouse models such as Tg2576 mice, which accumulate soluble Aβ in the brain, but do not display the other pathological hallmarks of NFTs and overt neurodegeneration, are an ideal type of model to use for this. Tg2576 mice accumulate both soluble and insoluble Aβ40 and Aβ42 as they age, a process which begins at around 8 months of age for soluble Aβ (Kawarabayashi et al., 2001). Mouse models of Alzheimer's disease which principally accumulate Aβ, such as Tg2576 mice, exhibit regional Aβ plaque deposition similar to human Alzheimer's disease cases (Hsiao et al., 1996), and as such it could reasonably be assumed that Aβ species will be present in nodes of the Tg2576 mouse reward system much as they are in humans. If there is an association between soluble Aβ and anhedonia, and Aβ is present and biologically active in reward sites of Tg2576 mice, then this regional activity could account for the emergence of anhedonia. This finer-grained regional specificity is not a topic of investigation in this thesis, but will form a working assumption moving forwards.

This chapter also aims to investigate the utility of an anti-depressant agent in improving hedonic behaviour. As mentioned in Chapter 1, standard antidepressant agents, such as SSRIs and SNRIs, have no clear therapeutic benefit in Alzheimer's disease (Orgeta et al., 2017). This, coupled with the fact that the serotonergic and noradrenergic neurotransmitter systems are pathologically altered in Alzheimer's disease (Garcia-Alloza et al., 2005; Iversen et al., 1983; Palmer et al., 1987; Reinikainen et al., 1988), suggests that trialling an antidepressant agent acting on other neurotransmitter systems may be worthwhile. In addition, an antidepressant with a demonstrable anti-anhedonic effect would be desirable, given that anhedonia is the specific component of depression under investigation in this thesis. Lastly, an antidepressant agent that could potentially decrease Aβ levels would be an attractive prospect, as it would provide an opportunity for further examining the relationship between Aβ and anhedonia. One compound which appears to fulfil these criteria is the novel antidepressant agent, ketamine.

Ketamine is an NMDA receptor antagonist, which in recent years has come under investigation as a novel antidepressant agent at sub-anaesthetic doses, with a rapid onset of action

and utility in treatment-resistant cases (Berman et al., 2000; Murrough, Iosifescu, et al., 2013; Murrough, Perez, et al., 2013). Unlike conventional antidepressants, ketamine interacts with or influences receptors within a wide range of neurotransmitter systems, including NMDA and AMPA receptors (Browne & Lucki, 2013; Kavalali & Monteggia, 2015), 5HT receptors (Gigliucci et al., 2013; Yamanaka et al., 2014), and opioid receptors (Gupta, Devi, & Gomes, 2011; Nemeth et al., 2010). Ketamine also appears, at least in bipolar disorder patients, to have a specific anti-anhedonic effect (Lally et al., 2014), marking it as a drug that may be able to address this core, common, and difficult to treat depression symptom. There is also some evidence that ketamine, at least at anaesthetic doses, can lower Aβ levels in Tg2576 mice (Quiroga et al., 2014). Due to its broad pharmacological profile, and its potential as both an anti-anhedonic and Aβ-lowering agent, ketamine would appear to be a good candidate drug in attempting to treat anhedonia in Tg2576 mice.

While depressive behaviour has been investigated previously in Alzheimer's disease mouse models, the behavioural tests used may not be particularly sensitive to anhedonia (see discussion in Section 3.4). It is therefore currently unclear whether this core symptom of depression is present in Alzheimer's disease model mice, and whether it is responsive to treatment. In addition, if anhedonia does arise in Tg2576 mice as a result of Aβ, then given the accumulation of Aβ in these mice over time, anhedonia should be of an age-dependent nature. That is, it should emerge and potentially worsen with greater Aβ accumulation over time. The first major aim of this chapter, therefore, is to use lick cluster analysis (an approach thought to be sensitive to anhedonia) in a longitudinal fashion, to investigate whether anhedonia is present in the Tg2576 mouse model of Alzheimer's disease. The second major aim is to investigate whether any hedonic deficit that does occur is treatable with a non-standard antidepressant agent. Experiment 1 of this chapter will gauge the presence and extent of anhedonia in Tg2576 mice over time, and Experiment 2 will examine whether anhedonia in these mice responds to low-dose ketamine therapy.

3.2. Experiment 1

3.2.1 Subjects & apparatus

Experiment 1 is a longitudinal investigation of the licking behaviour of two mouse cohorts, whose data were combined for analysis. These two cohorts consisted of male Tg2576 mice and age-matched wild-type male littermates, housed as previously described (Chapter 2) and tested at 4-5, 8, 12 and 16 months of age. Mice were initially group-housed, but due to aggressive behaviour many were eventually separated, beginning around 3 months of age, and single-housed. To ensure separation did not disproportionately affect one genotype, roughly equivalent percentages of each

genotype were single-housed prior to the start of behavioural testing. The combined numbers of each genotype, and percentage single-housed, at each time point were as follows (and declined largely due to attrition from animal death): 4-5 months – 42 Tg2576 (76.2%) and 41 wild-type (75.6%); 8 months – 42 Tg2576 (85.7%) and 39 wild-type (84.6%); 12 months – 40 Tg2576 (90%) and 38 wild-type (89.5%); 16 months – 34 Tg2576 (97%) and 37 wild-type (97.3%). Mice were placed on a mild food deprivation regime, as described in Chapter 2. At 4-5, 8 and 12 months of age, food deprivation was present throughout pre-training and testing. At 16 months of age the effect of food deprivation versus free-feeding on lick cluster size was investigated; at this age mice alternated between food-deprived and free-feeding days throughout pre-training and testing, with both genotypes counterbalanced by day and concentration order. At 4-5, 8 and 12 months of age, both genotypes were counterbalanced with respect to sucrose concentration order. Baseline weights and deprivation-induced weight change are presented in Section 3.2.4. Testing apparatus was as described in Chapter 2.

The rationale for investigating the effect of food deprivation versus a free-feeding state on lick cluster size at 16 months was as follows: The previous lick cluster experiments within this thesis had taken place exclusively under the condition of food deprivation, therefore there was a question as to whether any observed lick cluster size deficit was state-dependent, that is, would only manifest when mice were food deprived and not when mice were free-feeding. To explicitly examine this at 16 months of age, a manipulation was introduced such that mice were tested under both food deprived and free-feeding conditions on alternate days, and in a counterbalanced manner. There was no specific prediction as to whether a lick cluster size deficit would be dependent on food deprivation or would be a generalised deficit present in both conditions.

3.2.2 Procedure

Pre-training was conducted as described in Chapter 2, using an 8% sucrose solution made daily with deionised water. Drinking spouts were initially extended inside the drinking chambers to facilitate their detection, generally for 1-2 days, then shortened so mice could only lick the tip of the spout. The pre-training period needed to produce a consistent level of licking behaviour shortened with repeated testing, and was of the following ranges at each time point: 7-10 days at 4-5 months; 5-6 days at 8 months; 4-5 days at 12 months; 6 days at 16 months. Testing occurred in two phases using 4% and 16% sucrose solutions, as described in Section 2.2.2. At 4-5, 8 and 12 months of age, phase 1 and 2 each lasted 5 days. At 16 months of age, phase 1 and 2 each lasted 6 days (each phase consisting of 3 days food-deprived and 3 days free-feeding, alternated).

3.2.3 Data analysis

Data were initially collected as described in Chapter 2. Inter-lick intervals were calculated within lick clusters, and were recorded onset to onset. Licking data were cleaned to remove low or unreliable drinking measurements, as described in Section 2.2.2. Data cleaning resulted in excluded licking data of 8 mice at 4-5 months and 1 mouse at 8 months (plus 1 mouse culled during the experiments at 4-5, 8 and 16 months). Only the subset of mice which were present at every time point until (and including) 16 months of age informed the aging analysis. For baseline weight and weight change data over time, 34 Tg2576 and 36 wild-type mice were analysed. After drinking data were cleaned, the consumption and licking behaviour over time of 31 Tg2576 and 31 wild-type mice was analysed. The number of animals in the aging analysis differs from the numbers analysed at each individual time point, the latter of which are noted in the relevant figures and tables. The analyses of each individual time point are presented after the aging data.

For the aging analysis, aggregated licking data were analysed using mixed ANOVA, with a between-subjects factor of genotype, and a within-subjects factors of age and concentration. As mice at the first 3 time points were tested under food-deprived conditions, the 16-month data were taken only from the food-deprived condition, which still provided a reasonable sampling period of drinking behaviour. At each individual time point aggregated licking data were analysed using mixed ANOVA, with a between-subjects factor of genotype, and a within-subjects factor of concentration. Follow-up tests between groups or conditions were conducted using t-tests, as described in Section 2.7.

3.2.4 Results

3.2.4.1 Aging study

Table 3.1 presents the mean baseline weights and deprivation-induced weight change of all Tg2576 and wild-type mice in the aging analysis. Inspection of the baseline weight data indicates that mice gain weight over their lifespan irrespective of genotype, but that Tg2576 mice have a lower body weight in general, and this genotype difference becomes more pronounced over time. The precise sucrose concentration being consumed in the test sessions seems to have little material effect on baseline body weight for either genotype. ANOVA revealed a significant effect of age $(F(1.734,117.913) = 93.877, p<.001, MSE = 1014.854, \eta^2_p=0.580)$, genotype $(F(1,68) = 19.09, p<.001, MSE = 1868.55, \eta^2_p=0.219)$, and a significant age × genotype interaction $(F(1.734,117.913) = 5.655, p = .007, MSE = 61.134, \eta^2_p= 0.077)$. There was no significant main effect of concentration $(F(1,68) =$

0.911, p = .343, MSE = 0.157, η^2_p= 0.013), nor any significant interaction featuring concentration (highest F for age × concentration × genotype (3,204) = 0.378, p = .769, MSE = 0.075, η^2_p= 0.006).

Inspection of the weight loss data indicates that absolute weight loss as a result of food deprivation generally increased with age for both genotypes, but that Tg2576 mice were affected more at each time point than were wild-type mice. No consistent appreciable effect of sucrose concentration on weight loss appears to be present. ANOVA revealed a significant effect of age (F(2.716,184.701) = 10.622, p<.001, MSE = 1.859, η^2_p= 0.135) and genotype (F(1,68) = 29.60, p<.001, MSE = 11.033, η^2_p= 0.303), but no significant age × genotype interaction (F(2.716,184.701) = 0.537, p = .640, MSE = 0.094 , η^2_p= 0.008). There was no significant main effect of concentration (F(1,68) = 0.639, p = .427, MSE = 0.048, η2p= 0.009), nor any significant interaction featuring concentration (highest F for age × concentration × genotype (3,204) = 1.798, p = .149, MSE = 0.164, η^2p= 0.026).

Age	Weight variable	TG 4%	TG 16%	WT 4%	WT 16%
4 – 5 months	Baseline weight (g)	29.58 (±0.506)	29.54 (±0.490)	32.07 (±0.458)	32.11 (±0.448)
	Weight change (g)	0.806 (±0.065)	0.847 (±0.072)	0.544 (±0.062)	0.611 (±0.055)
8 months	Baseline weight (g)	32.02 (±0.552)	32.00 (±0.562)	35.28 (±0.588)	35.18 (±0.580)
	Weight change (g)	1.071 (±0.062)	1.006 (±0.056)	0.756 (±0.049)	0.683 (±0.051)
12 months	Baseline weight (g)	32.94 (±0.643)	32.96 (±0.626)	36.99 (±0.792)	36.93 (±0.806)
	Weight change (g)	1.012 (±0.075)	1.059 (±0.082)	0.772 (±0.071)	0.644 (±0.058)
16 months	Baseline weight (g)	32.97 (±0.599)	32.87 (±0.581)	37.79 (±0.914)	37.77 (±0.888)
	Weight change (g)	1.127 (±0.070)	1.029 (±0.080)	0.819 (±0.057)	0.880 (±0.067)

Table 3.1. Mean data (±SEM) for baseline weights and food deprivation-induced weight change (g) of Tg2576 (TG) and wild-type (WT) mice at each sucrose concentration and time point. N=34 TGs and 36 WTs. The 16 month weight data are from the food deprived phase of the experiment. All weights were taken prior to food deprivation.

Figure 3.1 shows the consumption and lick cluster data for aging Tg2576 and wild-type mice at each sucrose concentration. Inspection of panel A of Figure 3.1 indicates that consumption of sucrose initially increases with age for both genotypes, plateauing somewhere around 8 to 12 months of age. Within this general aging profile, Tg2576 mice consistently consume greater amounts of sucrose than wild-type mice, and this may be more pronounced over time for 16% sucrose. In general, though not consistent at all time points, both genotypes appear to consume slightly more 4% than 16% sucrose. ANOVA results were broadly consistent with these observations, revealing significant effects of age ($F_{(2.549,152.937)} = 11.891$, $p<.001$, MSE = 4.678, $\eta^2_p = 0.165$), and genotype ($F_{(1,60)} = 16.07$, $p<.001$, MSE = 15.408, $\eta^2_p = 0.211$), with no significant age × genotype interaction ($F_{(2.549,152.937)} = 1.686$, $p = .180$, MSE = 0.663, $\eta^2_p = 0.027$). There was a significant effect of concentration ($F_{(1,60)} = 5.47$, $p = .023$, MSE = 0.747, $\eta^2_p = 0.084$), and a non-significant age × concentration × genotype interaction ($F_{(3,180)} = 2.464$, $p = .064$, MSE = 0.194, $\eta^2_p = 0.039$).

A **Consumption**

B **Lick Cluster Size**

Figure 3.1. Mean (±SEM) consumption (g) (A) and lick cluster size data (B), in response to 4% and 16% sucrose solutions, of Tg2576 and wild-type mice in 10 minute test sessions, over time. N = 31 Tg2576 and 31 wild-type mice. The 16 month data are from the food-deprived phase of the experiment.

Inspection of panel B of Figure 3.1 suggests that both genotypes generally display the expected lick cluster size increase at a higher sucrose concentration. However, after the first time point Tg2576 mice display a noticeably lower lick cluster size than wild-type mice. This begins at 8 months of age, and is numerically apparent at both 4% and 16% sucrose (especially at later timepoints), though is most prominent at 16% sucrose. This genotypic difference occurs within the context of an aging profile displayed by both genotypes; lick cluster size tends to initially increase with age, then gradually declines, and this is most pronounced at 16% sucrose, especially between 12 and 16 months of age. ANOVA results were broadly consistent with these observations, revealing

a significant main effect of concentration ($F(1,60)$ = 69.382, p<.001, MSE = 9340.59, η^2_p= 0.536), a significant main effect of genotype ($F(1,60)$ = 4.411, p = .040, MSE = 2550.3, η^2_p= 0.068), and a significant concentration × genotype interaction ($F(1,60)$ = 6.214, p = .015, MSE = 836.59, η^2_p= 0.094). The aging profile impression was confirmed by a significant main effect of age ($F(3,180)$ = 8.34, p<.001, MSE = 1353.80, η^2_p= 0.122) and, although the genotype lick cluster size difference was numerically larger from 8-16 months than at 4-5 months of age, especially at 16% sucrose, neither the age × genotype interaction ($F(3,180)$ = 2.144, p = .096, MSE = 348, η^2_p= 0.034) nor the age × concentration × genotype interaction ($F(2.678,160.662)$ = 1.17, p = .321, MSE = 100.48, η^2_p= 0.019) reached conventional significance levels. There was also a non-significant age × concentration interaction ($F(2.678,160.662)$ = 2.57, p = .063, MSE = 220.70, η^2_p= 0.041).

Table 3.2 presents the amount consumed per lick (µg) and inter-lick interval data for aging Tg2576 and wild-type mice at each sucrose concentration. Inspection of the amount consumed per lick indicates that, generally, amount consumed per lick increases with age for Tg2576 mice, while a consistently changing age profile is less obvious for wild-type mice. Wild-type mice show a numerically higher amount consumed per lick than Tg2576 mice until 16 months of age, at which point it is numerically lower than that of Tg2576 mice. There appears to be a fairly consistent effect of concentration, in that amount consumed per lick is generally lower at 16% sucrose compared with 4% sucrose. ANOVA results were broadly consistent with these impressions, revealing a significant effect of age ($F(2.024,121.440)$ = 3.584, p = .030, MSE = 3.148, η^2_p= 0.056), no significant effect of genotype ($F(1,60)$ = 1.276, p = .263, MSE = 1.27, η^2_p= 0.021), and a significant age × genotype interaction ($F(2.024,121.440)$ = 3.363, p = .037, MSE = 2.953, η^2_p= 0.053), consistent with the aging change being largely driven by Tg2576 mice. In keeping with the generally observed concentration change in both genotypes, there was a significant effect of concentration ($F(1,60)$ = 7.334, p = .009, MSE = 1.942, η^2_p= 0.109), with no significant concentration × genotype interaction ($F(1,60)$ = 1.481, p = .228, MSE = 0.392, η^2_p= 0.024).

Inspection of the inter-lick interval data indicates a general increase in the inter-lick interval with age, present in both Tg2576 and wild-type mice, albeit with different trajectories (smaller incremental increases in wild-type mice until 16 months of age). No clear and consistent difference between Tg2576 and wild-type mice is apparent. Inter-lick interval of Tg2576 mice looks to decrease slightly as concentration increases; no clear relationship with concentration is apparent in wild-type mice. ANOVA results were consistent with these impressions, revealing a significant effect of age ($F(2.571,154.249)$ = 29.756, p<.001, MSE = 2115.83, η^2_p= 0.332), no significant effect of genotype ($F(1,60)$ = 0.367, p = .547, MSE = 198.4, η^2_p= 0.006), and a significant age × genotype interaction ($F(2.571,154.249)$ = 4.59, p = .006, MSE = 326.35, η^2_p= 0.071). A non-significant effect of

concentration (F(1,60) = 1.782, p = .187, MSE = 45.07, η^2_p= 0.029) was consistent with the lack of a clear overall concentration relationship, and a significant concentration × genotype interaction (F(1,60) = 8.591, p = .005, MSE = 217.28, η^2_p= 0.125) was in keeping with the decrease seen in Tg2576 mice as concentration increased.

Age	Control variable	TG 4%	TG 16%	WT 4%	WT 16%
4 – 5 months	Amount consumed per lick (µg)	1.142 (±0.068)	1.128 (±0.09)	1.631 (±0.288)	1.328 (±0.082)
	Inter-lick interval (ms)	118.4 (±1.875)	116.8 (±1.45)	119.8 (±1.79)	121 (±1.899)
8 months	Amount consumed per lick (µg)	1.09 (±0.061)	1.115 (±0.063)	1.383 (±0.066)	1.286 (±0.037)
	Inter-lick interval (ms)	124.3 (±1.752)	122.2 (±1.716)	121.2 (±2.359)	120.2 (±1.722)
12 months	Amount consumed per lick (µg)	1.408 (±0.103)	1.296 (±0.076)	1.473 (±0.066)	1.365 (±0.073)
	Inter-lick interval (ms)	127.1 (±1.629)	123.7 (±1.292)	120.4 (±2.023)	121.9 (±1.65)
16 months	Amount consumed per lick (µg)	1.734 (±0.224)	1.558 (±0.192)	1.516 (±0.08)	1.300 (±0.113)
	Inter-lick interval (ms)	129 (±1.53)	128.4 (±1.512)	127 (±2.144)	128.2 (±1.933)

Table 3.2. Mean data (±SEM) for amount consumed per lick (µg) and inter-lick interval (ms) of Tg2576 (TG) and wild-type (WT) mice at each sucrose concentration and time point. N=31 Tg2576 and 31 wild-type mice. The 16 month data are from the food-deprived phase of the experiment.

The results of these control variables suggest that the lower lick cluster sizes present in Tg2576 mice are not an artefact of motor differences in licking behaviour. For example, the aging analysis revealed an overall lick cluster size decrement in Tg2576 mice, while there was no overall genotype difference in amount consumed per lick or inter-lick interval. Further, the lick cluster size deficit was numerically most apparent at 16% sucrose from 8 months of age onward, at which points the control variables do not appear appreciably different. Inter-lick interval, in particular, shows greater numerical differences at 4% sucrose than 16% sucrose from 8 months onwards, while the Tg2576 lick cluster size deficit shows the opposite pattern. Taken together, these observations

suggest that the lick cluster size decrease shown by Tg2576 mice in the aging analysis can be interpreted as a hedonic deficit.

Although the aging lick cluster size data shown in Figure 3.1 suggested a Tg2576 hedonic deficit that emerged with age after 4-5 months, the analysis revealed only a main effect of genotype, with no significant age × genotype or age × concentration × genotype interactions. The lack of a significant age × genotype or age × concentration × genotype interaction could be due to 3 of the 4 tests occurring after this genotype difference was numerically apparent, limiting the power of the interaction test. An exploratory re-analysis was thus conducted, comparing lick cluster sizes averaged across 8-16 months against lick cluster sizes at 4-5 months. Results of this ANOVA revealed a significant effect of concentration ($F(1,60) = 52.298$, $p<.001$, MSE = 4053.20, $\eta^2_p = 0.466$), no significant effects of age ($F(1,60) = 2.417$, $p = .125$, MSE = 270.90, $\eta^2_p = 0.039$) or genotype ($F(1,60) = 2.370$, $p = .129$, MSE = 591.1, $\eta^2_p = 0.038$), but a significant age × genotype interaction ($F(1,60) = 4.636$, $p = .035$, MSE = 519.48, $\eta^2_p = 0.072$). None of the other interactions were significant (highest F for concentration × genotype $(1,60) = 2.787$, $p = .100$, MSE = 216.02, $\eta^2_p = 0.044$). The significant age × genotype interaction is consistent with the Tg2576 lick cluster size deficit appearing after 4-5 months, supporting the impression that the hedonic capacity of Tg2576 mice does materially change with age. Each individual time point will be analysed in isolation next, to determine both the time at which anhedonia first presents in Tg2576 mice, and its concentration dependency.

3.2.4.2 4-5 month licking results

Inspection of panel A of Figure 3.2 indicates that at 4-5 months of age, Tg2576 mice consume more sucrose than wild-type mice overall, with generally (across genotypes) greater consumption of 4% than 16% sucrose. ANOVA results were consistent with these observations, revealing significant effects of genotype ($F(1,72) = 9.675$, $p = .003$, MSE = 4.811, $\eta^2_p = 0.118$), and concentration ($F(1,72) = 4.605$, $p = .035$, MSE = 0.358, $\eta^2_p = 0.06$), with no significant concentration × genotype interaction ($F(1,72) = 0.001$, $p = .975$, MSE = $7.889e^{-5}$, $\eta^2_p < 0.001$).
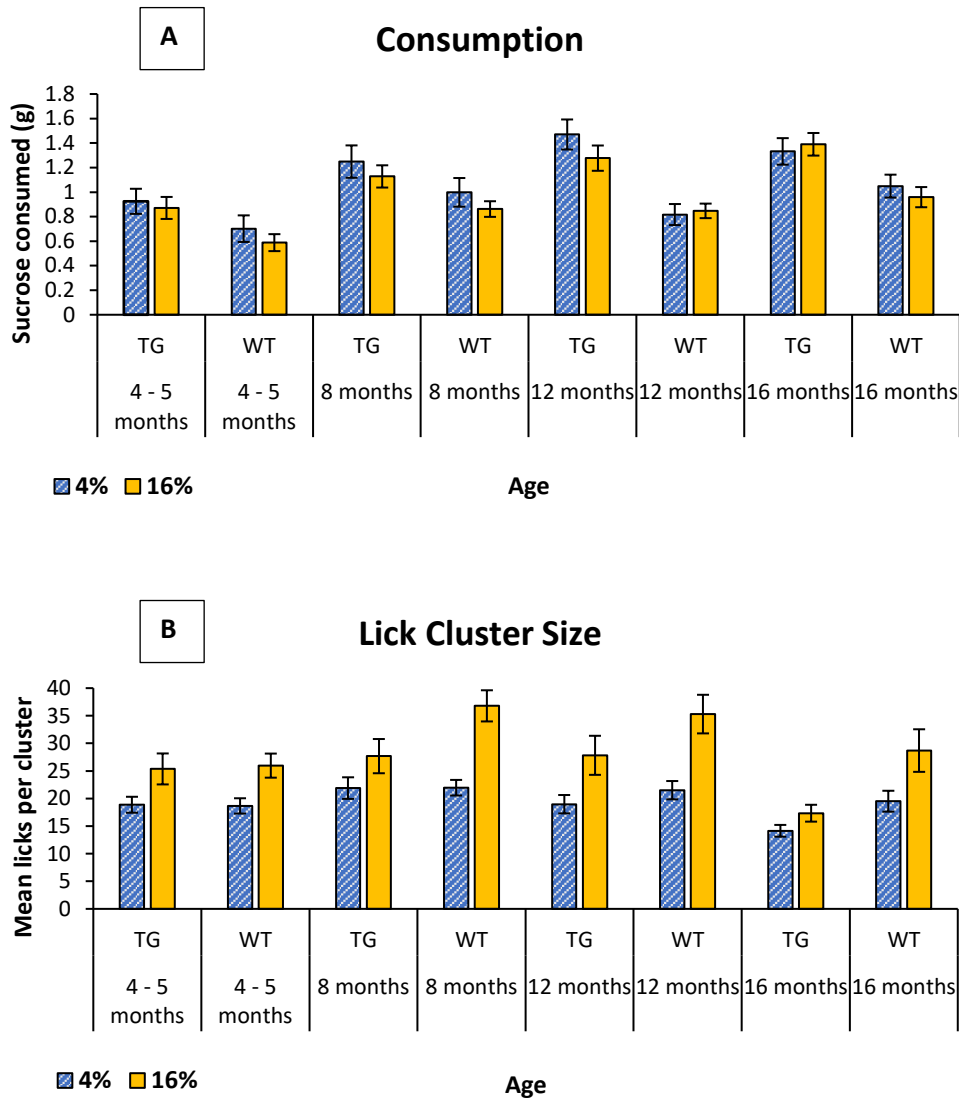
Figure 3.2. Mean (±SEM) consumption (g) (A) and lick cluster size data (B), in response to 4% and 16% sucrose solutions, of Tg2576 (TG) and wild-type (WT) mice in 10 minute test sessions, at 4-5 months of age. N = 38 Tg2576 and 36 wild-type mice.

Inspection of panel B of Figure 3.2 indicates that both Tg2576 and wild-type mice display the typical lick cluster size increase with increasing sucrose concentration. Lick cluster size values for both genotypes at each concentration appear similar, albeit those of wild-type mice are numerically lower than in Tg2576 mice. ANOVA results were consistent with these observations, revealing a significant effect of concentration ($F(1,72) = 21.429$, $p<.001$, MSE = 1682.709, $\eta^2_p= 0.229$), no significant effect of genotype ($F(1,72) = 0.177$, $p = .675$, MSE = 28.72, $\eta^2_p= 0.002$), and no significant concentration × genotype interaction ($F(1,72) = 0.005$, $p = .942$, MSE = 0.418, $\eta^2_p<0.001$). The absence of a Tg2576 lick cluster size deficit at this initial time point is clearly of material importance.

Importantly, a Bayesian mixed ANOVA provided evidence suggesting a genuine absence of genotype effect regarding lick cluster size (BF_{10} = 0.273). Lick cluster sizes within each genotype were compared at 4% vs 16% to investigate hedonic sensitivity to concentration change: both Tg2576 and wild-type mice showed a significant difference between concentrations (Student's t(37) = 2.742, p = .009, d = 0.445) and (t(35) = 4.394, p<.001, d = 0.732) respectively.

Inspection of Table 3.3 indicates that amount consumed per lick data is in keeping with the general description in the aging results in Section 3.2.4.1. That is, amount consumed per lick is higher in wild-type mice, and numerically decreases with concentration across both genotypes. ANOVA results were consistent with this impression, revealing a significant effect of genotype (F(1,72) = 4.304, p = .042, MSE = 3.117, η^2_p= 0.056), no significant effect of concentration (F(1,72) = 1.543, p = .218, MSE = 1.017, η^2_p= 0.021), and no significant concentration × genotype interaction (F(1,72) = 0.63, p = .430, MSE = 0.415, η^2_p= 0.009).

Control variable	TG 4%	TG 16%	WT 4%	WT 16%
Amount consumed per lick (μg)	1.172 (±0.06)	1.112 (±0.075)	1.568 (±0.25)	1.296 (±0.075)
Inter-lick interval (ms)	120 (±1.693)	118 (±1.339)	120.1 (±1.59)	121.6 (±1.695)

Table 3.3. Mean data (±SEM) for amount consumed per lick (μg) and inter-lick interval (ms) of Tg2576 (TG) and wild-type (WT) mice at each sucrose concentration, at 4-5 months of age. N=38 Tg2576 and 36 wild-type mice.

Little material difference in inter-lick interval between genotypes is visually apparent in Table 3.3, though concentration increase appears to produce diverging effects in Tg2576 and wild-type mice. ANOVA results were consistent with this impression, revealing no significant effects of genotype (F(1,72) = 0.701, p = .405, MSE = 118.8, η^2_p= 0.010) or concentration (F(1,72) = 0.119, p = .731, MSE = 1.914, η^2_p= 0.002), but a significant concentration × genotype interaction (F(1,72) = 6.862, p = .011, MSE = 109.989, η^2_p= 0.087). Motoric differences between the two genotypes do not appear to be complicating lick cluster size interpretation. Firstly, while inter-lick interval responds differently to concentration increase in Tg2576 and wild-type mice, the numeric differences at each concentration are negligible. Secondly, while wild-type mice do consume a greater amount of sucrose per lick than Tg2576 mice, the numeric difference is greatest at 4% sucrose, where it is largely driven by the data from one mouse. Thus motor effects are unlikely to meaningfully impact

the licking of one genotype over another, and the lick cluster size results can be interpreted as a measure of hedonic capacity.

3.2.4.3 8 month licking results

Inspection of panel A of Figure 3.3 indicates that at 8 months of age, the same genotype and concentration consumption pattern occurs as at 4-5 months of age. ANOVA results were consistent with this observation, revealing a significant effect of genotype ($F_{(1,77)} = 6.257$, p = .014, MSE = 3.568, $\eta^2_p = 0.075$), a non-significant effect of concentration ($F_{(1,77)} = 3.649$, p = .060, MSE = 0.424, $\eta^2_p = 0.045$), and a non-significant concentration × genotype interaction ($F_{(1,77)} = 0.182$, p = .671, MSE = 0.021, $\eta^2_p = 0.002$). Inspection of panel B of Figure 3.3 indicates that while both Tg2576 and wild-type mice show the usual lick cluster size increase with concentration, at 8 months of age Tg2576 mice have a numerically lower lick cluster size at both concentrations, particularly so at 16% sucrose. This impression was confirmed by ANOVA results, which revealed significant effects of both concentration ($F_{(1,77)} = 65.97$, p<.001, MSE = 4084.85, $\eta^2_p = 0.461$), and genotype ($F_{(1,77)} = 4.117$, p = .046, MSE = 1102.2, $\eta^2_p = 0.051$). The blunted lick cluster response to an increase in sucrose concentration shown by Tg2576 mice was confirmed by a significant concentration × genotype interaction ($F_{(1,77)} = 10.31$, p = .002, MSE = 638.41, $\eta^2_p = 0.118$). Follow-up unpaired t-tests comparing Tg2576 and wild-type lick cluster size at 4% and 16% sucrose revealed no significant genotype difference at 4% sucrose (Student's $t_{(77)} = 0.59$, p = .557, $d = 0.133$), but a significant genotype difference at 16% sucrose (Student's $t_{(77)} = 2.673$, p = .009, $d = 0.603$). Despite this Tg2576 lick cluster size reduction, paired samples t-tests comparing lick cluster size at 4% and 16% sucrose for each genotype revealed a significant difference for both Tg2576 (Student's $t_{(41)} = 3.979$, p<.001, $d = 0.614$) and wild-type (Student's $t_{(36)} = 7.057$, p<.001, $d = 1.160$) mice.

Figure 3.3. Mean (±SEM) consumption (g) (A) and lick cluster size data (B), in response to 4% and 16% sucrose solutions, of Tg2576 (TG) and wild-type (WT) mice in 10 minute test sessions, at 8 months of age. N = 42 Tg2576 and 37 wild-type mice.

Inspection of Table 3.4 indicates that, at 8 months of age, the amount of sucrose consumed per lick data is similar to that at the 4-5 month time point. ANOVA results were consistent with this impression, revealing a significant effect of genotype ($F_{(1,77)} = 7.365$, $p = .008$, MSE = 1.32, $\eta^2_p = 0.087$), no significant effect of concentration ($F_{(1,77)} = 1.763$, $p = .188$, MSE = 0.085, $\eta^2_p = 0.022$) and no significant concentration × genotype interaction ($F_{(1,77)} = 2.264$, $p = .137$, MSE = 0.11, $\eta^2_p = 0.029$). Inspection of the inter-lick interval data in Table 3.4 indicates a similar pattern of results as seen at 4-5 months, though without concentration appearing to interact with genotype. ANOVA results were consistent with this impression, revealing non-significant effects of both genotype

(F(1,77) = 3.689, p = .058, MSE = 667.9, η^2_p= 0.046) and concentration (F(1,77) = 3.27, p = .074, MSE = 72.57, η^2_p= 0.041), and a non-significant concentration × genotype interaction (F(1,77) = 2.082, p = .153, MSE = 46.21, η^2_p= 0.026). The lick cluster size difference observed does not appear to be an artefact of these control variables, as the lick cluster size deficit was statistically confirmed at 16% sucrose, while amount consumed per lick and inter-lick interval numerically differ most at 4% sucrose and least at 16% sucrose. That these control measures show the opposite pattern to the lick cluster size results suggests that the Tg2576 lick cluster size reduction at 8 months can be interpreted as a hedonic deficit.

Control variable	TG 4%	TG 16%	WT 4%	WT 16%
Amount consumed per lick (μg)	1.123 (±0.062)	1.130 (±0.05)	1.359 (±0.056)	1.260 (±0.04)
Inter-lick interval (ms)	125.3 (±1.487)	122.9 (±1.334)	120.1 (±2.051)	119.9 (±1.54)

Table 3.4. Mean data (±SEM) for amount consumed per lick (μg) and inter-lick interval (ms) of Tg2576 (TG) and wild-type (WT) mice at each sucrose concentration, at 8 months of age. N=42 Tg2576 and 37 wild-type mice.

3.2.4.4 12 month licking results

Inspection of panel A of Figure 3.4 indicates that, at 12 months of age, consumption follows a pattern similar to that reported at previous time points, although wild-type mice now display a response to concentration increase in opposition to that of Tg2576 mice. ANOVA results were consistent with these observations, revealing a significant effect of genotype (F(1,76) = 22.61, p<.001, MSE = 13.898, η^2_p= 0.229), no significant effect of concentration (F(1,76) = 0.552, p = .460, MSE = 0.045, η^2_p= 0.007), but a significant concentration × genotype interaction (F(1,76) = 5.525, p = .021, MSE = 0.447, η^2_p= 0.068). Inspection of panel B of Figure 3.4 indicates a similar pattern of lick cluster size results as described at 8 months of age, albeit the genotype lick cluster size difference at 16% sucrose is numerically smaller than at 8 months. ANOVA results were partially consistent with this impression, revealing a significant effect of concentration (F(1,76) = 43.949, p<.001, MSE = 5788.6, η^2_p= 0.366), a non-significant effect of genotype (F(1,76) = 2.87, p = .094, MSE = 1083.1, η^2_p= 0.036), and a non-significant concentration × genotype interaction (F(1,76) = 2.115, p = .150, MSE = 278.5, η^2_p= 0.027). A Bayesian mixed ANOVA provided uninformative evidence regarding the null genotype effect as regards lick cluster size (BF_{10} = 0.791). Despite a numerical Tg2576 lick cluster size reduction, paired samples t-tests comparing lick cluster size at 4% and 16% sucrose for each

genotype revealed a significant difference for both Tg2576 (Student's t(39) = 3.285, p=.002, *d* = 0.519) and wild-type (Student's t(37) = 6.692, p<.001, *d* = 1.086) mice.
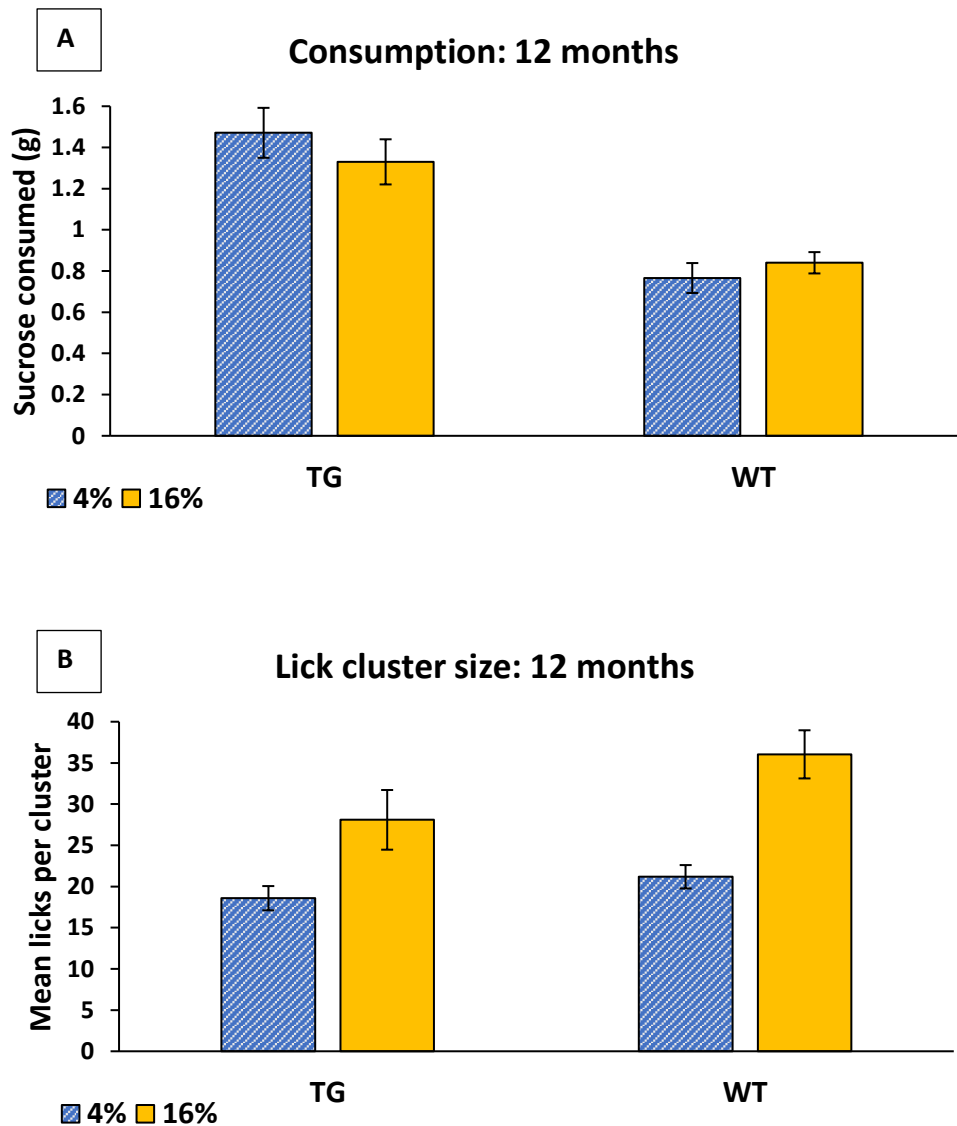
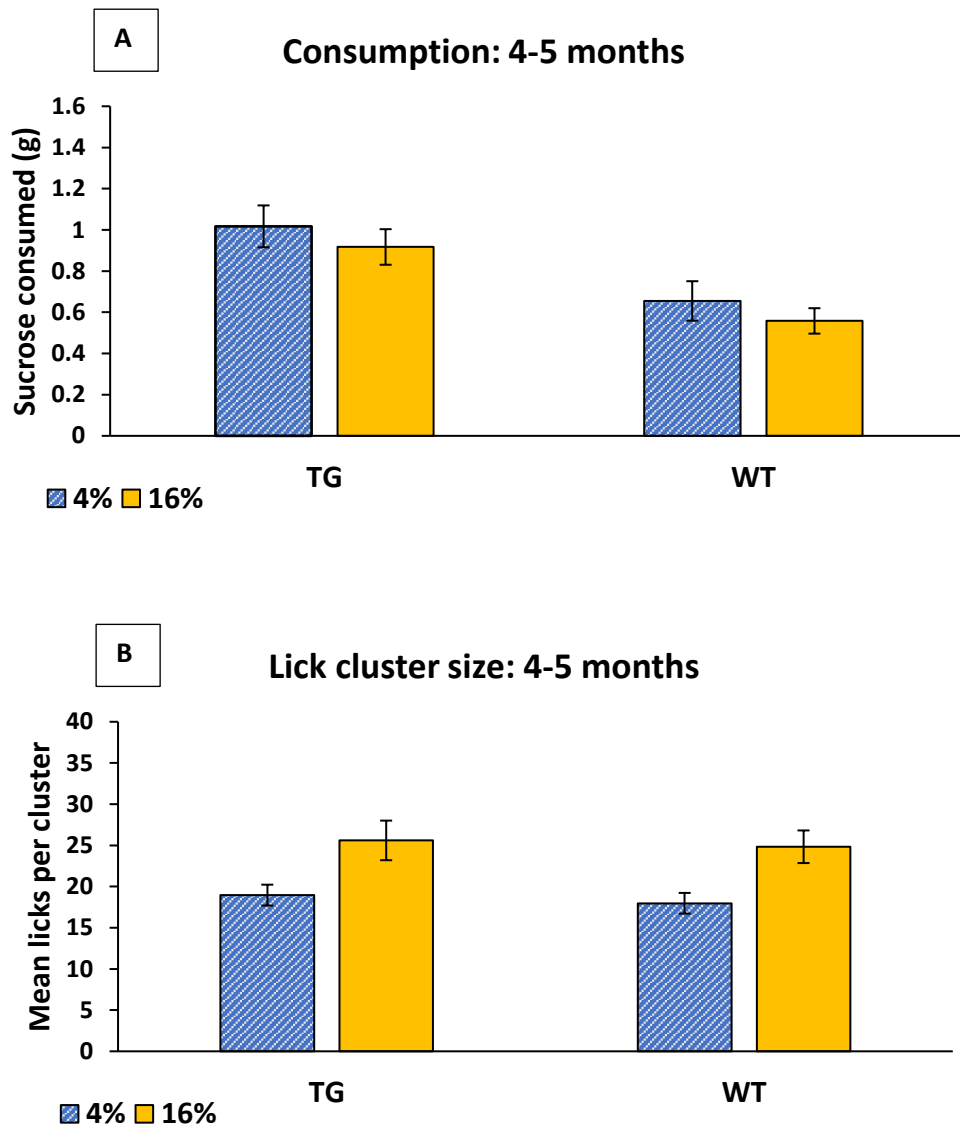Figure 3.4. Mean (±SEM) consumption (g) (A) and lick cluster size data (B), in response to 4% and 16% sucrose solutions, of Tg2576 (TG) and wild-type (WT) mice in 10 minute test sessions, at 12 months of age. N = 40 Tg2576 and 38 wild-type mice.

Inspection of Table 3.5 indicates a similar pattern of amount consumed per lick results as described at 4-5 months, only with a more apparent effect of concentration increase. ANOVA results were consistent with this impression, revealing no significant effect of genotype (F(1,76) = 0.935, p =

.337, MSE = 0.29, η^2_p= 0.012), a significant effect of concentration (F(1,76) = 8.504, p = .005, MSE = 0.546, η^2_p= 0.101), and no significant concentration × genotype interaction (F(1,76) = 0.004, p = .949, MSE = 2.625e^{-4}, η^2_p<0.001). The inter-lick interval data in Table 3.5 also show a similar pattern to that observed at 4-5 months, although at 12 months longer inter-lick intervals are seen in Tg2576 mice. ANOVA results were consistent with these observations, revealing a significant effect of genotype (F(1,76) = 5.116, p = .027, MSE = 814.6, η^2_p= 0.063), no significant effect of concentration (F(1,76) = 2.667, p = .107, MSE = 36.52, η^2_p= 0.034), and a significant concentration × genotype interaction (F(1,76) = 14.164, p<.001, MSE = 193.92, η^2_p= 0.157). Though a clear Tg2576 lick cluster size decrease was not statistically confirmed at 12 months of age, the numerically greater genotype lick cluster size difference seen at 16% sucrose would not appear to be the result of control measure differences. No significant differences were found in amount consumed per lick, and any notable numeric differences in inter-lick interval are apparent at 4%, not 16%, sucrose.

Control variable	TG 4%	TG 16%	WT 4%	WT 16%
Amount consumed per lick (µg)	1.392 (±0.086)	1.271 (±0.067)	1.475 (±0.057)	1.36 (±0.06)
Inter-lick interval (ms)	126.8 (±.1.389)	123.6 (±1.13)	120 (±1.825)	121.3 (±1.565)

Table 3.5. Mean data (±SEM) for amount consumed per lick (µg) and inter-lick interval (ms) of Tg2576 (TG) and wild-type (WT) mice at each sucrose concentration, at 12 months of age. N=40 Tg2576 and 38 wild-type mice.

3.2.4.5 16 month licking results

Inspection of panel A of Figure 3.5 indicates that consumption was lower with ad-lib feeding than under food restriction, and that the relationship between consumption and concentration was reversed between feeding conditions, while the overall higher consumption by Tg2576 mice was maintained. ANOVA results were consistent with this impression, revealing significant effects of genotype (F(1,67) = 13.58, p<.001, MSE = 11.78, η^2_p= 0.169) and deprivation (F(1,67) = 125.691, p<.001 , MSE = 6.887, η^2_p= 0.652), no significant effect of concentration (F(1,67) = 0.233, p = .631, MSE = 0.035, η^2_p= 0.003), with a significant deprivation × concentration interaction (F(1,67) = 9.48, p = .003, MSE = 0.301, η^2_p= 0.124). No other interaction terms were significant (highest F for deprivation × concentration × genotype (1,67) = 3.186, p = .079, MSE = 0.101, η^2_p= 0.045).

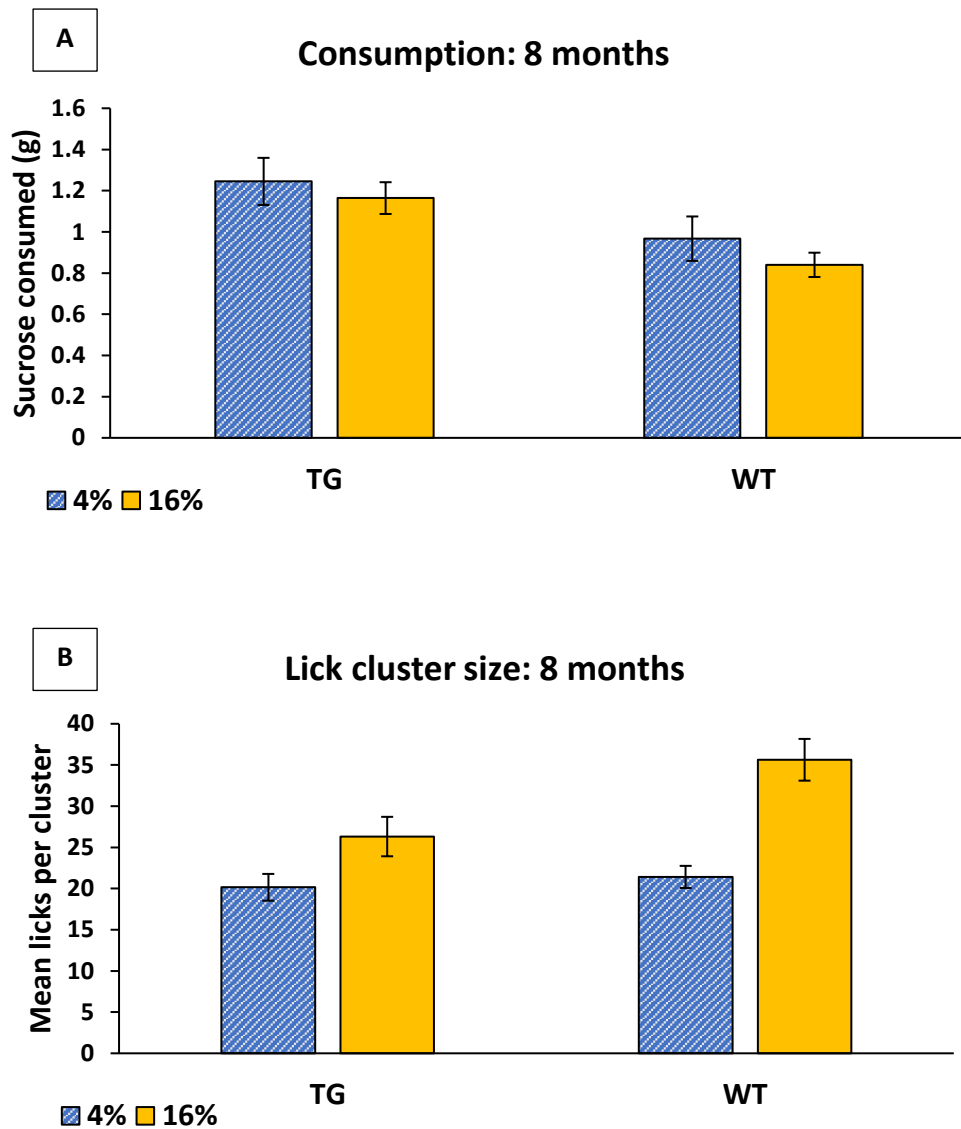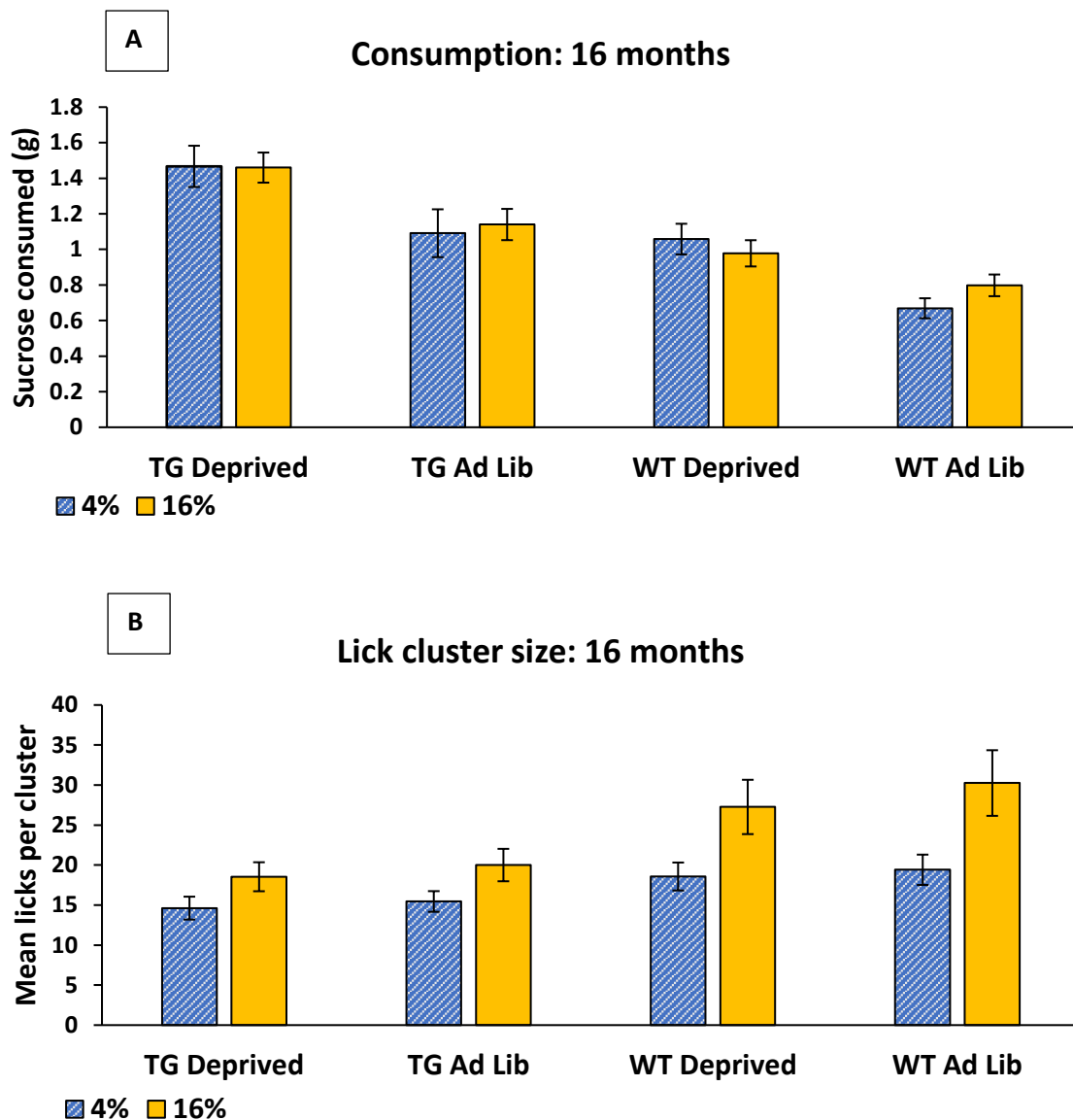**Figure 3.5.** Mean (±SEM) consumption (g) (A) and lick cluster size data (B), in response to 4% and 16% sucrose solutions, of Tg2576 (TG) and wild-type (WT) mice in 10 minute test sessions, at 16 months of age, under food deprived and ad lib conditions. N = 33 Tg2576 and 36 wild-type mice.

Inspection of panel B of Figure 3.5 indicates a pattern of lick cluster size results similar to those of 8 and 12 months, with a more pronounced overall lick cluster size reduction in Tg2576 mice, though still largest at 16% sucrose. In addition, lick cluster sizes are overall numerically higher in the free-feeding condition, regardless of genotype. ANOVA results were broadly consistent with this impression, revealing significant effects of both concentration ($F_{(1,67)}$ = 22.474, p<.001, MSE = 3374.681, η^2_p= 0.251) and genotype ($F_{(1,67)}$ = 5.543, p = .021, MSE = 3112.4, η^2_p= 0.076), with no significant concentration × genotype interaction ($F_{(1,67)}$ = 3.507, p = .065, MSE = 526.568, η^2_p= 0.05). There was no significant effect of deprivation ($F_{(1,67)}$ = 3.305, p = .074, MSE = 162.330, η^2_p=

0.047), and no other significant interaction (highest F for deprivation × concentration (1,67) = .465, p = .498, MSE = 33.342, η^2_p= 0.007). Follow-up unpaired t-tests comparing Tg2576 and wild-type lick cluster size at 4% and 16% sucrose, averaged across deprivation condition, revealed no significant genotype difference at 4% sucrose (Student's t(68) = 1.802, p = .076, *d* = 0.431), but a significant genotype difference at 16% sucrose (Student's t(68) = 2.324, p = .023, *d* = 0.556). Paired samples t-tests comparing lick cluster size at 4% and 16% sucrose, averaged across deprivation condition, within each genotype revealed a significant difference for both Tg2576 (Student's t(33) = 4.106, p<.001, *d* = 0.704) and wild-type (Student's t(35) = 3.571, p = .001, *d* = 0.595) mice.

Inspection of Table 3.6 indicates that the amount consumed per lick, unlike at earlier time points, was generally numerically higher in Tg2576 mice, while the decrease with concentration seen at previous time points remained in place. There was no clear effect of deprivation on amount consumed per lick, nor did deprivation clearly interact with genotype or concentration. ANOVA results were generally consistent with this impression, revealing a significant effect of concentration (F(1,67) = 5.166, p = .026, MSE = 1.103, η^2_p= 0.072), no significant effect of genotype (F(1,67) = 0.224, p = .637, MSE = 0.648, η^2_p= 0.003), and no significant effect of deprivation (F(1,67) = 1.506, p = .224, MSE = 0.411, η^2_p= 0.022). No interaction terms were significant (highest F for deprivation × concentration (1,67) = 2.196, p = .143, MSE =0.751 , η^2_p= 0.032).

Control variable		TG 4%	TG 16%	WT 4%	WT 16%
Amount consumed per lick (µg)	Deprived	1.724 (±0.211)	1.564 (±0.180)	1.677 (±0.160)	1.375 (±0.113)
	Non-deprived	1.518 (±0.156)	1.573 (±0.235)	1.519 (±0.121)	1.420 (±0.111)
Inter-lick interval (ms)	Deprived	129.3 (±.1.287)	127.9 (±1.474)	127.1 (±1.904)	127.9 (±1.687)
	Non-deprived	127.7 (±1.298)	125.5 (±1.321)	125.2 (±2.009)	126.3 (±1.831)

Table 3.6. Mean data (±SEM) for amount consumed per lick (µg) and inter-lick interval (ms) of food-deprived and non-deprived Tg2576 (TG) and wild-type (WT) mice at 4% and 16% sucrose, at 16 months of age. N=33 Tg2576 and 36 wild-type mice.

The inter-lick interval data presented in Table 3.6 show that, consistent with some previous time points, concentration increase has opposing effects in Tg2576 and wild-type mice, while across concentration there is little material difference between genotypes. In addition, food deprivation

appears to increase inter-lick interval in both genotypes and at both concentrations. ANOVA results were consistent with this impression, revealing no significant effects of genotype ($F(1,67) = 0.188$, $p = .666$, MSE = 60.79, $\eta^2_p = 0.003$) or concentration ($F(1,67) = 0.410$, $p = .524$, MSE = 12.603, $\eta^2_p = 0.006$), and a significant concentration × genotype interaction ($F(1,67) = 4.480$, $p = .038$, MSE = 137.850, $\eta^2_p = 0.063$). There was a significant effect of deprivation ($F(1,67) = 20.653$, $p<.001$, MSE = 236.136, $\eta^2_p = 0.236$), and no other significant interaction (highest F for deprivation × concentration × genotype $(1,67) = 0.554$, $p = .459$, MSE = 4.919, $\eta^2_p = 0.008$). As at prior time points, these control measures do not appear to account for the Tg2576 lick cluster size decrease, as there were no statistically detectable genotype differences, and inter-lick interval numeric differences were smallest at 16% sucrose, where the genotype lick cluster size difference is numerically largest. These facts suggest that the lick cluster size decrease can be interpreted as a hedonic deficit.

3.2.5 Summary

Experiment 1 investigated the lick cluster size responses of Tg2576 mice to two sucrose concentrations longitudinally, from 4-5 months to 16 months of age. The critical finding, suggested visually by the complete aging study and supported by further analysis, was that Tg2576 mice develop a lick cluster size deficit over time. The follow-up aging analysis was consistent with this deficit emerging at some point after 4-5 months of age. Investigation of each time point in isolation demonstrated that the lick cluster size reactions of Tg2576 mice were comparable to those of wild-type mice at 4-5 months, where a Bayesian analysis suggested this was a genuine absence of genotype difference. However, at 8 months of age a lick cluster size deficit had manifested, specifically at 16% sucrose. At 12 months of age, despite the lick cluster size patterns being numerically similar to those of the 8-month time point, there was no significant difference between Tg2576 and wild-type mice; here a Bayesian analysis suggested the evidence was uninformative rather than indicating a true null genotype effect. At the final aging time point of 16 months of age, Tg2576 mice displayed lower lick cluster sizes, particularly in response to 16% sucrose. An added manipulation at 16 months of age, comparing food-deprived with free-feeding mice, revealed no statistically significant effect of food deprivation on lick cluster size, though lick cluster sizes of both genotypes were numerically higher when free-feeding. Taken together, these results suggest that Tg2576 mice develop a hedonic deficit with age, most obviously at 16% sucrose, which manifests at some point between 4-5 and 8 months of age.

A notable feature of the drinking behaviour of Tg2576 mice seen in Experiment 1 is that their hedonic reactions dissociate from their consumption levels. That is, Tg2576 mice consistently

consume more sucrose than wild-type mice regardless of sucrose concentration, and this elevated consumption still occurs when a lick cluster size deficit is present. Having demonstrated a hedonic deficit in Tg2576 mice that appears to be age-dependent, a second experiment was conducted to determine whether this deficit could be alleviated by the novel antidepressant agent ketamine. Section 3.3 details this experiment and its results.

3.3. Experiment 2

3.3.1 Subjects & apparatus

Experiment 2 is a single study of the licking behaviour of Tg2576 and wild-type mice featuring a between-subjects drug condition. The mice used were the same mice that had gone through the aging study presented in Experiment 1. 31 Tg2576 and 31 wild-type mice were used in the experiment, at 19 months of age; 16 mice of each genotype were assigned to the ketamine treatment group, and 15 mice of each genotype were assigned to the vehicle group. At this time point, the percentage of mice in each genotype being single housed was 96.7%. Both genotypes were counterbalanced with respect to concentration order and treatment group. Testing apparatus used was as described in Chapter 2.

3.3.2 Procedure

Pre-training was conducted as described in Chapter 2. The pre-training period needed to produce a consistent level of licking behaviour was 4 days. Testing occurred as described in Chapter 2, with each test phase lasting 4 days. To ensure the ketamine and vehicle groups were equivalent prior to ketamine administration, the treatment groups within each genotype were matched on their baseline weight, consumption and lick cluster size. Ketamine hydrochloride (Sigma) and vehicle (0.9% sodium chloride) stock solutions were made up during the pre-training period using deionised water and stored at 2-5°C. Ketamine was administered via intraperitoneal injection at a dose of 30mg/kg, and both ketamine and vehicle were injected at a volume of 10mL/kg. Each mouse received one ketamine or vehicle injection per test phase of the experiment: the first on the day after pre-training, 24 hours before the start of test phase 1, and the second on the day between phases 1 and 2, 24 hours before the start of test phase 2.

3.3.3 Data analysis

Data were collected and cleaned as described in Chapter 2. No mice had their drinking data excluded from the analysis. Data were analysed using mixed ANOVA, with between-subjects factors of genotype and drug, and a within-subjects factor of concentration. Pairwise comparisons between genotypes within a concentration were conducted using unpaired Student's t-tests.

3.3.4 Results

Table 3.7 presents the mean baseline weight and deprivation-induced weight change of ketamine- and vehicle-treated Tg2576 and wild-type mice. Inspection of the baseline weight data indicates that Tg2576 display the typical lower weight seen at previous ages, with no clear differences seen due to ketamine treatment or concentration change. ANOVA results were consistent with this impression, revealing a significant effect of genotype ($F(1,58) = 21.387$, $p<.001$, $MSE = 803.334$, $\eta^2_p = 0.269$), and no significant effects of concentration ($F(1,58) = 2.057$, $p = .157$, $MSE = 1.291$, $\eta^2_p = 0.034$) or drug ($F(1,58) = 0.157$, $p = .693$, $MSE = 5.899$, $\eta^2_p = 0.003$). No interaction terms were significant (highest F for concentration × genotype × drug $(1,58) = 1.103$, $p = .298$, $MSE = 0.692$, $\eta^2_p = 0.019$).

	Treatment	TG 4%	TG 16%	WT 4%	WT 16%
Baseline weight (g)	Ketamine	32.25 (±0.702)	32.39 (±0.650)	37.22 (±1.362)	37.33 (±1.488)
	Vehicle	32.62 (±1.018)	32.62 (±1.081)	37.57 (±1.178)	38.13 (±1.118)
Weight change (g)	Ketamine	1.188 (±0.100)	0.953 (±0.086)	0.859 (±0.079)	1.000 (±0.116)
	Vehicle	1.017 (±0.082)	0.950 (±0.095)	0.783 (±0.084)	0.800 (±0.124)

Table 3.7. Mean data (±SEM) for baseline weight and deprivation-induced weight change (g) of ketamine- and vehicle-treated Tg2576 (TG) and wild-type (WT) mice at each sucrose concentration. N=31 TGs and 31 WTs, 16 receiving ketamine and 15 receiving vehicle in each genotype.

Inspection of the weight change data indicates that Tg2576 mice generally display the previously observed greater weight loss, with concentration change having opposing effects in both genotypes regardless of treatment. Ketamine-treated mice of both genotypes display a numerically greater weight loss. ANOVA results were generally consistent with this impression, revealing a

significant effect of genotype (F(1,58) = 4.100, p = .047, MSE = 0.855, η^2_p= 0.066), no significant effect of concentration (F(1,58) = 0.468, p = .497, MSE = 0.040, η^2_p= 0.008), and a significant concentration × genotype interaction (F(1,58) = 4.754, p = .033, MSE = 0.407, η^2_p= 0.076). There was no significant effect of drug (F(1,58) = 1.880, p = .176, MSE = 0.392, η^2_p= 0.031), and no other interaction was significant (highest F for concentration × genotype × drug (1,58) = 1.925, p = .171, MSE = 0.165, η^2_p= 0.032).

Inspection of panel A of Figure 3.6 indicates that Tg2576 mice continue to display greater consumption than wild-type mice regardless of concentration or drug treatment, albeit at this age there is generally numerically greater consumption at 16%, rather than 4%, sucrose. No consistent effect of ketamine treatment on consumption is apparent, though ketamine-treated Tg2576 mice display numerically higher consumption than their vehicle-treated counterparts at both sucrose concentrations. ANOVA results were consistent with this impression, revealing a significant effect of genotype (F(1,58) = 28.046, p<.001 , MSE = 11.456, η^2_p= 0.326), and no significant effects of concentration (F(1,58) = 0.016, p = .900, MSE = 0.002, η^2_p<0.001) or drug (F(1,58) = 2.458, p = .122, MSE = 1.004, η^2_p= 0.041). No interaction term was significant (highest F for concentration × genotype (1,58) = 1.649, p = .204, MSE = 0.159, η^2_p= 0.028).

Inspection of panel B of Figure 3.6 indicates that the general lick cluster size pattern observed at 16 months of age is still present, regardless of drug treatment. There is no appearance of a pronounced effect of ketamine treatment, though in ketamine-treated Tg2576 mice lick cluster size numerically decreased, while in ketamine-treated wild-type mice it numerically increased. ANOVA results were consistent with this impression, revealing significant effects of concentration (F(1,58) = 42.313, p<.001 , MSE = 1515.008, η^2_p= 0.422), genotype (F(1,58) = 8.828, p = .004, MSE = 1842.55, η^2_p= 0.132), and a significant concentration × genotype interaction (F(1,58) = 15.512, p<.001 , MSE = 555.393, η^2_p= 0.211). There was no significant effect of drug (F(1,58) = 0.205, p = .652, MSE = 42.88, η^2_p= 0.004), and no other interaction term was significant (highest F for genotype × drug (1,58) = 0.647, p = .424, MSE = 135.05, η^2_p= 0.011). Bayesian mixed ANOVA provided uninformative evidence regarding the null effect of ketamine on lick cluster size (BF$_{10}$ = 0.567), and the null genotype × drug interaction (BF$_{10}$ = 0.580). Follow-up unpaired t-tests comparing Tg2576 and wild-type lick cluster size at 4% and 16% sucrose, across treatment groups, revealed a significant difference at 16% (Student's t(60) = 3.780, p<.001, d = 0.960) but not 4% (Student's t(60) = 1.528, p = .132, d = 0.388) sucrose. Despite this Tg2576 lick cluster size reduction, paired samples t-tests comparing lick cluster size at 4% and 16% sucrose within each genotype, across treatment groups, revealed a significant difference for both Tg2576 (Student's t(30) = 2.263, p = .031, d = 0.406) and wild-type (Student's t(30) = 6.445, p<.001, d = 1.158) mice.

Consumption: ketamine study

Lick cluster size: ketamine study
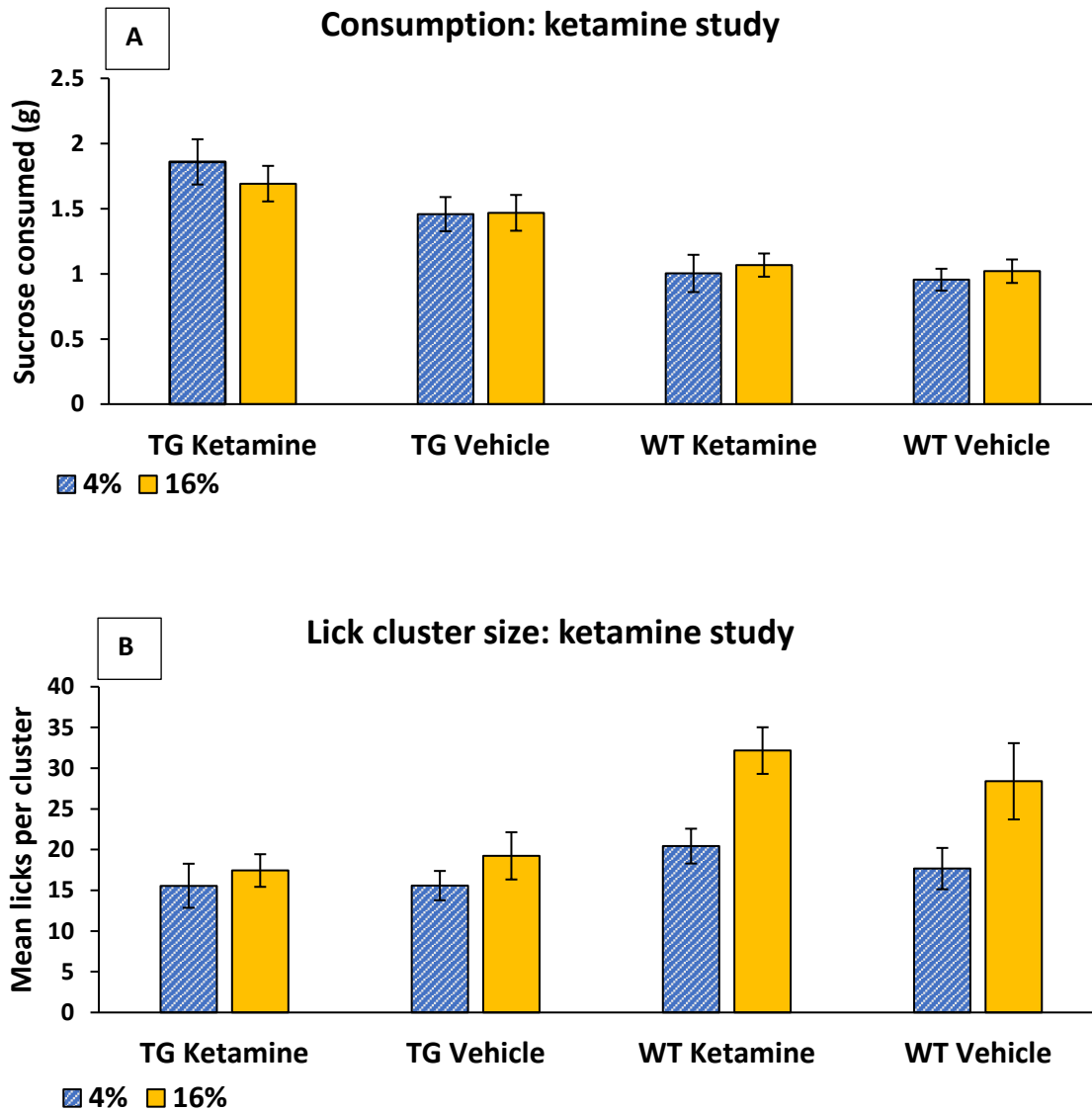
Figure 3.6. Mean (±SEM) sucrose consumption (g) (A) and lick cluster size (B) data of ketamine- and vehicle-treated Tg2576 (TG) and wild-type (WT) mice, in response to 4% and 16% sucrose solutions and in 10 minute test sessions, at 19 months of age. N = 31 Tg2576 and 31 wild-type mice, 16 receiving ketamine and 15 receiving vehicle in each genotype.

Inspection of Table 3.8 indicates that the amount consumed per lick data show the same general pattern as described in Experiment 1, Sections 3.2.4.4 and 3.2.4.5, regardless of treatment group. Ketamine treatment appeared to have numerically opposing effects in the two genotypes. ANOVA results were consistent with this impression, revealing a significant effect of concentration ($F_{(1,58)} = 6.438$, $p = .014$, MSE = 0.467, $\eta^2_p = 0.100$), and no significant effects of genotype ($F_{(1,58)} = 0.114$, $p = .736$, MSE = 0.054, $\eta^2_p = 0.002$) or drug ($F_{(1,58)} = 0.756$, $p = .388$, MSE = 0.355, $\eta^2_p = 0.013$). None of the interaction terms were significant (largest F for genotype × drug $(1,58) = 2.343$, $p = .131$,

MSE = 1.102, η^2_p= 0.039). Inspection of the inter-lick interval data in Table 3.8 indicates a similar pattern of results as seen in Experiment 1, Section 3.2.4.5, regardless of treatment group. ANOVA results were consistent with this impression, revealing no significant effects of genotype (F(1,58) = 2.769, p = .101, MSE = 408.51, η^2_p= 0.046) or drug (F(1,58) = 0.108, p = .743, MSE = 15.95, η^2_p= 0.002). There was a significant effect of concentration (F(1,58) = 11.144, p = .001, MSE = 133.521, η^2_p= 0.161), and significant concentration × genotype (F(1,58) = 25.036, p<.001, MSE = 299.961, η^2_p= 0.302) and concentration × genotype × drug (F(1,58) = 7.023, p = .010, MSE = 84.142, η^2_p= 0.108) interactions. No other interaction terms were significant (highest F for genotype × drug (1,58) = 0.306, p = .582, MSE = 45.15, η^2_p= 0.005). As in Experiment 1, the lack of overall genotype difference in these measures, and larger numeric differences in inter-lick interval occurring at 4% sucrose, where lick cluster size differences are numerically smaller, would suggest that the genotype lick cluster size difference is not artefactual, and can be interpreted as a hedonic impairment.

	Treatment	TG 4%	TG 16%	WT 4%	WT 16%
Amount consumed per lick (µg)	Ketamine	1.471 (±0.144)	1.418 (±0.141)	1.283 (±0.081)	1.146 (±0.059)
	Vehicle	1.387 (±0.178)	1.340 (±0.100)	1.637 (±0.167)	1.384 (±0.147)
Inter-lick interval (ms)	Ketamine	133.7 (±2.032)	126.6 (±1.292)	126.6 (±1.897)	129.0 (±1.964)
	Vehicle	132.3 (±2.223)	129.0 (±2.301)	126.0 (±3.133)	125.7 (±2.972)

Table 3.8. Mean data (±SEM) for amount consumed per lick (µg) and inter-lick interval (ms) of ketamine- and vehicle-treated Tg2576 (TG) and wild-type (WT) mice at 4% and 16% sucrose. N=31 TG and 31 WT mice, 16 receiving ketamine and 15 receiving vehicle in each genotype.

3.3.5 Summary

Experiment 2 investigated whether the lick cluster size deficit exhibited by Tg2576 mice, established in Experiment 1, could be alleviated by treatment with the novel antidepressant agent ketamine. The results showed that, despite receiving one conventional dose of ketamine per test phase, drug-treated Tg2576 mice showed no improvement in their lick cluster size response. In fact, lick cluster size of ketamine-treated Tg2576 mice was numerically lower than that of their vehicle-treated counterparts. In contrast to this, ketamine-treated wild-type mice displayed numerically higher lick cluster sizes than their vehicle-treated counterparts. As at the final time point (16

months) tested in Experiment 1, the lick cluster size deficit shown here at 19 months of age, regardless of treatment group, was numerically present at both 4% and 16% sucrose, though statistically demonstrable only at 16% sucrose. This lick cluster size deficit appears to represent an anhedonia which is present only at more palatable solutions, and which is not responsive to ketamine treatment under the dosage regime implemented. As in Experiment 1, Tg2576 mice still retained a hedonic sensitivity to an increase in concentration, suggesting their hedonic reaction to sucrose was attenuated rather than entirely blunted.

Similar to the results seen in Experiment 1, lowered hedonic responsiveness in Tg2576 mice occurred alongside a high level of sucrose consumption, in both treatment groups. Though ketamine-treated mice displayed numerically greater amounts of sucrose consumption, most notably with Tg2576 mice, there was no statistically demonstrable effect of ketamine on sucrose consumption.

3.4. Discussion

Experiment 1 demonstrated that Tg2576 mice develop a hedonic deficit which is not present at 4-5 months of age. The complete aging study visually suggested that this was an age-related deficit, and this suggestion was supported by an additional aging analysis. Examination of the hedonic behaviour at each individual time point revealed that the hedonic deficit manifests at some point between 4-5 and 8 months of age, and is statistically demonstrable only at 16% sucrose. This appears to demonstrate a selective reduction in the hedonic response to more rewarding solutions, rather than a generalised anhedonia (although the possibility of a floor effect at 4% sucrose masking a generalised hedonic deficit cannot be discounted). Moreover, at all ages Tg2576 mice retained the capacity to distinguish between 4% and 16% sucrose, suggesting diminished but not totally insensitive hedonic responsiveness over this age range. While it is possible that the Tg2576 mouse hedonic deficit is to some extent attributable to their increased single housing with age, this seems unlikely for two reasons. Firstly, the majority of Tg2576 mice were already single housed before testing occurred at 4-5 months of age, at which time point Tg2576 mice displayed lick cluster sizes numerically greater than wild-type mice. Secondly, roughly equivalent percentages of single- and group-housed Tg2576 and wild-type mice were tested at every time point; there was no time point at which Tg2576 mice experienced single-housing to a greater extent than wild-type mice. This is not only the first study in which lick cluster analysis has been used to profile the hedonic capacity of a mouse model of Alzheimer's disease, but also the first study in which hedonic responses to sucrose in mice have been examined longitudinally.

The results of this chapter, which reveal a depressive phenotype in Tg2576 mice, are consistent with the wider literature, in which depressive behaviour has been observed in various Alzheimer's disease mouse models using conventional behavioural tests. Studies conducted at single time points have revealed that both APP/PS1 and tgDimer mice show some degree of 'depressive' behaviour using the forced swim test, at 6-9 and 7 months of age, respectively (Abdel-Hafiz et al., 2018; Filali et al., 2009), and that 18 month-old 3xTgAD mice display a depressive phenotype in forced swim, tail suspension and sucrose preference tests (Romano et al., 2015). While the procedural details of the forced swim test were not identical in these three studies, the results demonstrate that genetically altered rodents harbouring Alzheimer's disease mutations display greater immobility or lesser swimming time in the forced swim test. These are behavioural changes typically interpreted as evidence of depressive behaviour. In addition, the reduced sucrose preference shown by 3xTgAD mice is commonly interpreted as evidence of anhedonia (Willner et al., 1987). However, as these studies only examined one time point, a developmental profile of depressive behaviour in those particular mouse models has not been obtained. As a result, the age at which depressive behaviour emerges in APP/PS1, tgDimer and 3xTgAD mice has not be established. Unlike these single time point studies, the results presented in this chapter have revealed a depressive behaviour, consistent with anhedonia, which manifests and continues with age.

Other studies of depressive behaviour in Alzheimer's disease model mice have utilised multiple time points, albeit using what appears to be a cross-sectional rather than longitudinal study design. For example, J20 mice were considered to display a subtle depressive phenotype, as they spent less time immobile than wild-type mice in the tail suspension test at 5-7 months, but more time immobile at 13-15 months, when the first 3 minutes of the test sessions were analysed (Iascone et al., 2013). Tg2576 mice display impaired chocolate-induced conditioned place preference, and a reduction in chocolate consumption during conditioning, at 6 months of age, while these behaviours appear normal at 2 months of age (Nobili et al., 2017). These latter changes were interpreted by the authors as showing a deficit in reward-associated cognition and depressive-like symptomatology. While behavioural changes that are absent in younger but present in older mice, such as in these two studies, are certainly consistent with an age-related depressive phenotype, these studies did not explicitly examine the effect of age. One study reporting a null result when investigating depressive behaviour in an Alzheimer's disease mouse model has also been published (Vloeberghs, Van Dam, Franck, Staufenbiel, & De Deyn, 2007). In this study, APP23 mice were examined for depressive behaviour at 3, 6 and 12 months of age, again apparently cross-sectionally, using the forced swim, tail suspension and sucrose preference tests. Interestingly, transgenic APP23 mice spent significantly

less time immobile than wild-type mice in the forced swim test at all ages, significantly less time immobile in the tail suspension test at 6 months, and displayed no significant diminishment in sucrose preference at any age. It would seem counter-intuitive to interpret the forced swim or tail suspension test results as evidence that APP23 mice are less depressed than their wild-type counterparts, and indeed the authors suggest that agitation could explain the genotype differences seen in these two tests. This questions how useful the forced swim and tail suspension tests may be for investigating depression. The results of the sucrose preference test are interesting; although no deficit in APP23 mice was apparent, there was an overall effect of age, in that sucrose preference declined in both genotypes as age increased. This pattern is somewhat similar to the lick cluster size aging profile presented in Experiment 1, in which lick cluster size initially increased then declined, in both genotypes and at both sucrose concentrations. This convergence upon a similar decline with age could be taken as evidence that the sucrose preference test results are providing at least in part a measure of hedonic response. While the results of Experiment 1 are largely consistent with the wider literature, the lick microstructure technique used has some distinct differences from the more traditional behavioural tests for depression used in other studies. These differences and their implications will be discussed shortly. Before that discussion, the question of how attributable the results of this chapter, and of other studies, are to the pathological hallmarks of Alzheimer's disease will be addressed.

A possibility that has been explored by this chapter is that Aβ, in particular soluble Aβ, could account for a depression symptom such as anhedonia. Were this causal relationship to exist, then whatever its precise nature, an age-related hedonic deficit should be expected to arise in Tg2576 mice. That this exact phenomenon was observed suggests that Aβ may contribute to hedonic dysfunction. An important point is that, in principle, APP overexpression in its own right can produce phenotypic differences in transgenic mice (Hsiao et al., 1995; Saito, Matsuba, Yamazaki, Hashimoto, & Saido, 2016). However, the simple presence of APP overexpression seems unlikely to be a cause of the hedonic deficit observed here in Tg2576 mice; in the Tg2576 mouse brain, human APP is detectable from at least 2 months of age (Kawarabayashi et al., 2001), and does not show an increased expression in older mice (Hsiao et al., 1996; Kawarabayashi et al., 2001). That elevated APP levels are present from an early age and stable over time, while the hedonic deficit was not present at 4-5 months of age and appeared as Tg2576 mice aged, would suggest that APP overexpression per se is unlikely to be the cause of the deficit. Soluble Aβ species, however, are known to accumulate over time in the Tg2576 mouse brain (Kawarabayashi et al., 2001), and would appear to be a more likely candidate to explain the observed hedonic deficit. Existing studies of depressive behaviour in Alzheimer's disease model mice have either only studied one time point in

isolation, or have not explicitly examined the effect of age on depressive behaviour. As a result, prior investigations cannot exclude the possibility of APP overexpression in its own right causing a depressive phenotype. The current results in Tg2576 mice presented herein are thus the first suggestion that a depressive phenotype in an Alzheimer's disease mouse model can reasonably be attributed to Aβ accumulation. This is in keeping with a study in rats which found that soluble Aβ42 administration led to a depressive phenotype, as evidenced by greater immobility time and reduced swimming time in the forced swim test (Colaianna et al., 2010). Administering Aβ, however, does not recapitulate the physiological process of gradual Aβ accumulation which occurs in Tg2576 mice (Kawarabayashi et al., 2001), and which biomarker modelling suggests occurs in human Alzheimer's disease (Jack et al., 2013). Consequently, the hedonic deficit presented in this chapter specifically provides evidence that the gradual Aβ accumulation inherent to Alzheimer's disease can produce a symptom of depression. This is not to say that pathological aspects of Alzheimer's disease other than Aβ, such as tau pathology and neurodegeneration, have no relation to depression symptoms. For example, the depressive phenotype seen in 3xTgAD mice could plausibly be attributed to some effect of either Aβ or tau pathology (or both) (Romano et al., 2015). Behavioural tests in transgenic mice exclusively harbouring human tau mutations have revealed depressive phenotypes (Egashira et al., 2005; Koss et al., 2016; Van der Jeugd et al., 2013), suggesting pathological tau species can induce depression symptoms in their own right, though these are based on mutations underlying a non-Alzheimer's disease dementia (Hutton et al., 1998; Sperfeld et al., 1999). A cross-sectional analysis of psychiatric symptoms in cases of probable Alzheimer's disease found that while major depression frequency decreased as disease severity increased, anhedonia frequency increased (Lopez et al., 2003). This suggests that pathological events downstream of Aβ, such as tau species or neurodegeneration, could also underlie certain depression symptoms. Taken together, the results presented herein and published elsewhere suggest that Aβ accumulation alone could be sufficient to account for a depression symptom such as anhedonia, but this is not to imply that other pathological features of Alzheimer's disease have no relevance.

An outstanding point of discussion is how lick cluster analysis compares with other standard behavioural tests of depression. The forced swim test and tail suspension tests, perhaps the most commonly used of such assays, were initially conceived of as tools for screening potential anti-depressant compounds in rodents (Porsolt et al., 1977; Steru, Chermat, Thierry, & Simon, 1985). Time spent immobile in these tests is generally interpreted as evidence of a depressive phenotype, because certain anti-depressants reduced immobility time in both of these paradigms (Porsolt et al., 1977; Steru et al., 1985). It does not follow, however, that validity as a screening tool for a drug necessitates validity as a test for revealing the condition treated by that drug (Stanford, 2017). This

logical point aside, it is also the case that drugs not thought to exert an anti-depressant effect have also reduced immobility time in the forced swim test (Schechter & Chance, 1979), and that anxiolytic and anti-psychotic agents have increased immobility in the tail suspension test (Cryan, Mombereau, & Vassout, 2005). These latter findings suggest that these tests could produce false negative and false positive results, as differences in calmness and docility, for example, could either mask or be mistaken for a depressive phenotype. Refinements to the forced swim test have been proposed, however, to improve its reliability (Castagné, Porsolt, & Moser, 2009; Sunal, Gümüşel, & Kayaalp, 1994). Other interpretations of immobility in these tests include implementing a stress-coping strategy in the forced swim test (Commons, Cholanians, Babb, & Ehlinger, 2017), and motor dysfunction in the tail suspension test (Mori, Ohashi, Nakai, Moriizumi, & Mitsumoto, 2005). In sum, the inference that reduced mobility or struggling represents 'depressive-like behaviour' may not always be appropriate, especially as confounding motor differences are not always examined in such studies. In addition, the feelings of despair, and 'searching' rather than 'waiting', which forced swim and tail suspension tests purportedly measure (Porsolt et al., 1977; Steru et al., 1985), are not core clinical symptoms of depression. In comparison to these two commonly used behavioural tests, lick cluster analysis includes the ability to record and examine potentially confounding variables, such as inter-lick interval and amount consumed per lick (Lydall et al., 2010; McNamara et al., 2016). If these variables fail to account for lick cluster size differences between groups, then such a difference can be interpreted as one of hedonics. One major advantage of using lick cluster analysis, then, is that other variables which could account for a depressive phenotype can be investigated and, when appropriate, discounted. This approach allows for the possibility of false negative and false positive results to be considered. Another considerable advantage of using lick cluster analysis is that it can plausibly claim to directly index hedonic response (Dwyer, 2012), and thus can reveal anhedonia, an important and core clinical symptom of depression.

Behavioural tests based on measuring the consumption of palatable substances, including sucrose preference testing or overall intake testing, are assumed to reveal hedonic or depressive changes (Katz, 1982; Muscat & Willner, 1992; Papp, Willner, & Muscat, 1991; Willner et al., 1987). While this may be a more appropriate approach than forced swim or tail suspension testing, as it aims to measure a core component of depression, consumption can be influenced by factors other than hedonic response. In fact, consumption and hedonics can sometimes dissociate from one another. For example, consumption of a palatable substance can be reduced while hedonic reactions to that substance remain intact (Pelchat et al., 1983). Indeed, a major dissociation between consumption and hedonic response was shown in this chapter, in which Tg2576 mice consume much more sucrose solution than wild-type mice, while displaying a diminished hedonic response. Thus

testing sucrose preference or overall consumption may reveal hedonic changes in certain circumstances, but does not necessarily always do so. In contrast, lick cluster analysis provides a measure of hedonic response which is not derived from consumption, and also allows consumption to be measured alongside lick cluster size. Lick cluster analysis, then, allows for a fuller examination of rodent appetitive behaviour, providing measures of both total intake and hedonic response within that intake. Consumption and preference tests also do not typically investigate confounding variables such as motoric influences on licking; lick cluster analysis therefore both provides a clearer measure of hedonic behaviour, and reveals whether lick cluster size differences are simply artefacts of motor differences. As a consequence of the advantages offered by lick cluster analysis, the hedonic deficit in Tg2576 mice in this chapter provides clearer evidence of a depressive phenotype in an Alzheimer's disease mouse model than both studies using forced swim or tail suspension testing (Abdel-Hafiz et al., 2018; Filali et al., 2009; Iascone et al., 2013; Romano et al., 2015), and studies using consumption or preferences tests (Nobili et al., 2017; Romano et al., 2015).

One observation yet to be discussed is the peculiar occurrence in Tg2576 mice of a lower body weight coupled with greater sucrose consumption, something which may initially appear counter-intuitive. Low body weight and increased food consumption have both been previously documented in transgenic mouse models of Alzheimer's disease (Pugh, Richardson, Bate, Upton, & Sunter, 2007; Vloeberghs et al., 2008), which may be evidence of metabolic alterations present in APP overexpressing mice. Indeed, Tg2576 mice show a range of metabolic disturbances, including increased energy expenditure, increased metabolic rate, reduced plasma leptin, and abnormal hypothalamic responses to both low plasma leptin and fasting (Ishii et al., 2014). Given that leptin is involved in the regulation of food intake, energy expenditure and body weight (Friedman & Halaas, 1998; Schwartz, Woods, Porte, Seeley, & Baskin, 2000), it seems likely that some type of metabolic alteration underlies the low body weight and increased consumption shown by Tg2576 mice in this chapter. That fasting produces different hypothalamic responses in Tg2576 and wild-type mice is interesting, as food deprivation was used in Experiments 1 and 2 of this chapter. However, this effect of fasting on the hypothalamus was observed after 48 hours of food deprivation (Ishii et al., 2014), whereas experiments in this chapter used 6-8 hours of deprivation, and similar consumption and lick cluster size results were seen at 16 months whether food was deprived or present. Therefore it is unlikely that the results of Experiments 1 and 2 are a product of the differential effects of fasting on Tg2576 and wild-type mice. The potential presence of metabolic disturbances in Alzheimer's disease model mice underscores the importance of using lick cluster analysis rather than standard consumption or preference testing. Differences in hunger, satiety signalling or energy requirements could produce meaningful differences in intake, such that sucrose consumption is

being largely driven by non-hedonic factors, as appears to be the case in Experiments 1 and 2. Only when metabolic and other differences can be excluded, can consumption-based tests potentially provide a good index of hedonic behaviour.

In sum, previous studies examining depressive behaviour in Alzheimer's disease model mice have either used methods which, at best, measure a non-core symptom of depression, or have attempted to measure hedonic change with an imprecise technique. In addition, prior studies have failed to explicitly investigate the effect of age, and thus it has been unclear what the original molecular cause of a depressive phenotype could be. The results in this chapter therefore provide clearer evidence of a depressive state in Tg2576 mice, specifically a state consistent with an anhedonic phenotype, which appears age-dependent and attributable to Aβ. The biological changes which may underlie this state will be examined in Chapter 5. The failure of ketamine to induce a change in the hedonic response of Tg2576 mice will also be explored in Chapter 5, as this topic is better addressed when the biochemical effects of ketamine have been investigated.

Chapter 4: Cognitive profile of Tg2576 mice

4.1. Introduction

One component of memory which is degraded in Alzheimer's disease is recognition memory – that is, the remembrance of things previously encountered (Mandler, 1980). Patients with Alzheimer's disease display impaired recognition memory across a range of stimuli, including colours, patterns, faces, pictures and spatially repositioned objects (Abrisqueta-Gomez, Bueno, Oliveira, & Bertolucci, 2002; Eslinger & Damasio, 1986; Moss, Albert, Butters, & Payne, 1986). Changes in recognition memory may be an early event in Alzheimer's disease, as they also appear in mild cognitive impairment (Barbeau et al., 2004; Hudon, Belleville, & Gauthier, 2009), and may have some value in predicting conversion to Alzheimer's disease (Didic et al., 2013). Considered an example of declarative memory (Manns, Hopkins, Reed, Kitchener, & Squire, 2003), recognition memory is a mnemonic ability well-suited for studying in non-human animals, which investigate objects and spaces as part of their behavioural repertoire. The investigation of recognition memory in primates and rodents has yielded much insight into its underlying processes (Dere, Huston, & De Souza Silva, 2007; Warburton & Brown, 2010).

Recognition memory in non-human animals can be fractionated into a number of subtypes, including memory for object novelty (Ennaceur & Delacour, 1988), relative object recency (Mitchell & Laiacona, 1998), object location novelty (Ennaceur et al., 1997), and object-in-place conjunctions (Dix & Aggleton, 1999). While a detailed description of the neural substrates of these different forms of recognition memory will not be presented here, a brief overview is as follows: object information is processed by the perirhinal cortex (Brown & Aggleton, 2001; Wan, Aggleton, & Brown, 1999), while the hippocampus is critical to forms of object recognition memory that involve a spatial or temporal dimension (Barker & Warburton, 2011). In addition, the medial prefrontal cortex is an important site which may integrate object information from the perirhinal cortex with spatial or temporal information from the hippocampus (Barker, Bird, Alexander, & Warburton, 2007; Barker & Warburton, 2015), and is critical to remembrance of object-in-place associations and relative object recency.

The object-in-place task, introduced in Chapter 2, is one of particular relevance to Tg2576 mice. Tg2576 mice display an impairment in successfully integrating object identity with spatial

location, at ages ranging from 10-12 months to 21 months of age (Good & Hale, 2007; Good, Hale, et al., 2007; Hale & Good, 2005). This cognitive deficit does not seem to result from a general inability to process objects or sustain attention, as aged Tg2576 mice can successfully distinguish between novel and familiar objects, and familiar and novel locations (Good & Hale, 2007). Nor does this object-in-place deficit likely result from task complexity per se, as the intact novel object and location preferences previously mentioned were in response to 4 object arrays, the same array size on which Tg2576 mice display an object-in-place impairment (Good & Hale, 2007). Rather, Tg2576 mice appear to have a selective deficit for a task requiring the integration of object identity with specific spatial locations; where wild-type mice preferentially explore objects which exchange positions, Tg2576 mice do not. While this has been investigated in multiple single studies, a longitudinal profile of Tg2576 object-in-place memory has yet to be captured. Indeed a longitudinal examination of object-in-place memory has only been attempted in one Alzheimer's disease mouse model to date (Evans et al., 2019). A longitudinal object-in-place memory profile in Tg2576 mice would allow the notion that Aβ is responsible for this cognitive deficit to be indirectly tested, in that a deficit would be expected to occur as Aβ accumulated in Tg2576 mice with age (Kawarabayashi et al., 2001). An aging study of this nature would also complement Chapter 3, in which a hedonic deficit was revealed as Tg2576 mice aged. If a longitudinal profile of the Tg2576 object-in-place memory deficit could be similarly captured, its emergence and progression relative to the hedonic deficit could prove informative, as regards the vulnerabilities of their respective underlying circuitries to Aβ pathology. To this end, the neural circuitry involved in successful object-in-place memory will now be outlined in more detail.

A number of studies have revealed that object-in-place associative recognition memory is underpinned by at least three structures in primates and rodents, namely the hippocampus, perirhinal cortex and medial prefrontal cortex (Warburton & Brown, 2010). These three structures form an integrated circuit, and manipulations which disrupt the functioning of this circuit lead to deficits in object-in-place memory (Barker & Warburton, 2015). A number of transmitters contribute to successful performance in this task, including glutamatergic (Barker & Warburton, 2015; Evans et al., 2019) and cholinergic (Barker & Warburton, 2009; Sabec, Wonnacott, Warburton, & Bashir, 2018) systems, as well as potentially Rho GTPase regulatory proteins (De Viti, Martino, Musilli, Fiorentini, & Diana, 2010). As described earlier, it seems likely that for successful object-in-place memory, the perirhinal cortex encodes object information, with the hippocampus encoding spatial information about objects, and the medial prefrontal cortex possibly integrating information from the former structures (Barker & Warburton, 2015).

The hypothesis of this chapter is that Tg2576 mice will develop impaired object-in-place memory with age, and that the accumulation of Aβ within the hippocampus may be sufficient to hinder their performance on this task. Experiment 3 will longitudinally examine object-in-place memory in Tg2576 and wild-type mice. As these same cohorts of mice were treated with sub-anaesthetic ketamine and vehicle in Chapter 3, Experiment 4 will examine the effects of ketamine on associative recognition memory in Tg2576 and wild-type mice. While ketamine was selected for its potential anti-depressant effect, given that another NMDA receptor antagonist aids cognition in Alzheimer's disease patients (Danysz & Parsons, 2012), the effect of ketamine on cognition merits investigation. In addition, to the extent that a Tg2576 object-in-place memory deficit is Aβ-dependent, the fact that ketamine may lower brain Aβ levels suggests that it could, in principle, improve cognition in Tg2576 mice (Quiroga et al., 2014). Experiment 5 will examine short-term spatial memory in ketamine- and vehicle-treated Tg2576 and wild-type mice, using a one-trial T-maze task which was introduced due to low object contact times observed in Tg2576 mice.

4.2. Experiment 3

4.2.1 Subjects, apparatus & procedure

Experiment 3 investigates the cognitive ability of the same cohorts of Tg2576 and wild-type mice used in Chapter 3. Mice were tested at 5-6, 8-9, 12-13 and 16-17 months of age, immediately after the individual lick cluster studies that comprised Experiment 1. The combined numbers of each genotype at each time point were as follows: 5-6 months – 42 Tg2576 and 40 wild-type; 8-9 months – 42 Tg2576 and 38 wild-type; 12-13 months – 40 Tg2576 and 38 wild-type; 16-17 months – 34 Tg2576 and 36 wild-type. Housing conditions were as previously described. As the earliest age at which Tg2576 mice had previously displayed a cognitive deficit in the object-in-place test was at 10-12 months (Good, Hale, et al., 2007), it was anticipated that 3 time points would be sufficient to reveal the presence of this deficit. However, an additional testing age was included after the 12-13 month time point. Consequently, the aging study utilised 4 sets of 4 object arrays; the first 3 time points were counterbalanced with respect to object array selection, whereas the final time point used one set of 4 object arrays. Other counterbalancing details, testing apparatus and experimental procedure were as described in Chapter 2. As the squads in which mice were run did not always contain equal numbers of Tg2576 and wild-type mice, on occasion two wild-type mice would be yoked to the same Tg2576 mouse, or there would be a Tg2576 mouse with no yoked wild-type mouse. While the latter could theoretically result in lower overall Tg2576 object sampling, the individual time point analyses would suggest this made no material difference. Nonetheless, to

remove this as a potential influence in Experiment 4, no unyoked Tg2576 mice were used. This involved, on occasion, one wild-type mouse being yoked to two Tg2576 mice and being given the average of their object sampling time.

4.2.2 Data analysis

Data were initially collected as described in Chapter 2. Object-in-place data were cleaned to remove low or unreliable object contact measurements, as described in Section 2.3.2. Data cleaning resulted in excluded data of 4 mice at 8-9 months, 3 mice at 12-13 months, and 3 mice at 16-17 months. Only the subset of mice which were present at every time point until (and including) 16-17 months of age after data cleaning (n = 26 Tg2576, 35 wild-type mice), informed the aging analysis. Failure of recording equipment caused one additional Tg2576 mouse at 8-9 months to be excluded from the habituation data. The analyses of each individual time point were also conducted and are presented after the aging data.

For the aging analysis, habituation and object-in-place test phase data were analysed using mixed ANOVA, with a between-subjects factor of genotype, and within-subjects factors of age and day, and age and object, respectively. Sample phase data were analysed as total contact time by mixed ANOVA, with a between-subjects factor of genotype, and a within-subjects factor of age. Discrimination ratios were analysed using mixed ANOVA, with a between-subjects factor of genotype, and a within-subjects factor of age. At each individual time point, habituation and object-in-place test phase data were analysed using mixed ANOVA, with a between-subjects factor of genotype, and within-subjects factors of day and object, respectively. Sample phase total contact time data of each genotype were compared by unpaired Student's t-tests. Tg2576 and wild-type discrimination ratios were compared using unpaired t-tests, and the discrimination ratio of each genotype was compared against chance performance (0.5) using one-sample t-tests. Pairwise comparisons between object groupings within each genotype were conducted by paired t-tests. Data are presented as follows: all forms of object contact (habituation, sample phase, and test phase (both raw contact times and discrimination ratios)) are presented graphically in the aging analysis; in the individual time point analysis the presentation is the same, except the sample phase data are described in the text.

4.2.3 Results

4.2.3.1 Aging study

Figure 4.1 presents the mean object contact times during habituation of all Tg2576 and wild-type mice in the aging analysis. Inspection of this figure indicates that contact times were high across genotypes at 5-6 months, with a marked reduction at day 2; both overall object contact and the effect of day were generally relatively low thereafter. Notably, Tg2576 mice consistently explored objects less than wild-type mice, across age and day. ANOVA revealed significant effects of age (F(1.566,90.849) = 111.954, p<.001, MSE = 19888.19, η^2_p=0.659), genotype (F(1,58) = 23.09, p<.001, MSE = 6920.3, η^2_p=0.285) and day (F(1,58) = 60.407, p<.001, MSE = 3462.61, η^2_p=0.510). There was also a significant age × day interaction (F(1.724,99.966) = 30.215, p<.001, MSE = 1990.34, η^2_p= 0.343). No other interaction reached statistical significance (highest F for age × genotype (1.566,90.849) = 2.197, p = .128, MSE = 390.23, η^2_p= 0.036).
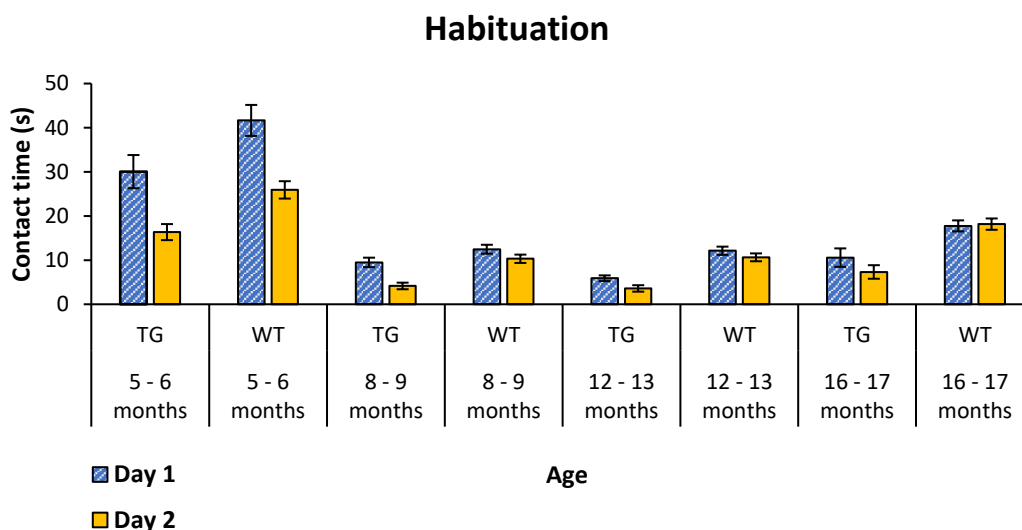
Figure 4.1. Mean contact times (s) of Tg2576 (TG) and wild-type (WT) mice when habituating to objects with age, over two consecutive days and in 10 minute sessions. n= 25 Tg2576 and 35 wild-type mice.

Figure 4.2 presents the mean sample and test phase object contact times, and discrimination ratios, of all Tg2576 and wild-type mice in the aging analysis. Inspection of panel A of Figure 4.2 indicates that, in the sample phase, object contact of all mice markedly decreased after the 5-6 month time point. In addition, Tg2576 mice displayed numerically larger object sampling times than wild-type mice, across age, although this difference appeared negligible after the 5-6 month time point. ANOVA results were consistent with this impression, revealing a significant effect

of age ($F_{(1.975, 116.505)}$ = 42.314, $p < .001$, MSE = 5057.0, η^2_p = 0.418), no significant effect of genotype ($F_{(1,59)}$ = 0.898, p = .347, MSE = 415.1, η^2_p = 0.015), and a non-significant age × genotype interaction ($F_{(1.975, 116.505)}$ = 0.979, p = .378, MSE = 117.1, η^2_p = 0.016). The genotype sampling discrepancy may seem counter-intuitive given that wild-type mice were yoked to match sampling times; this is explained by the fact that cohort attrition and data cleaning impacted Tg2576 mice more than wild-types. For example, by 16-17 months of age several Tg2576 mice had died and could not contribute to the aging analysis, while their yoked wild-type counterparts were included, with the effect of raising the mean Tg2576 contact time. Analyses at individual time points presented in Sections 4.2.4.2-5, however, demonstrate that yoking was effective at matching genotype object contact times in the sample phase.

Inspection of panel B of Figure 4.2 indicates that in the test phases, mice generally explored objects less with age, across genotype and object type. In addition, mice generally spent numerically more time exploring mismatched objects than familiar ones, across genotype and age. As with the habituation data, Tg2576 mice showed lower levels of object exploration than wild-type mice, across age and object type. While the numeric contact time difference between familiar and mismatched objects declined with age for both genotypes, by 16-17 months Tg2576 but not wild-type mice displayed numerically near-identical exploration of both object types. ANOVA revealed significant effects of age ($F_{(2.637, 155.611)}$ = 13.090, $p < .001$, MSE = 796.724, η^2_p = 0.182), object ($F_{(1,59)}$ = 71.202, $p < .001$, MSE = 1344.212, η^2_p = 0.547) and genotype ($F_{(1,59)}$ = 82.89, $p < .001$, MSE = 26448.6, η^2_p = 0.584), but no significant age × genotype interaction ($F_{(2.637, 155.611)}$ = 0.211, p = .866, MSE = 12.836, η^2_p = 0.004). There was a significant object × genotype interaction ($F_{(1,59)}$ = 28.393, $p < .001$, MSE = 536.030, η^2_p = 0.325), but a non-significant age × object × genotype interaction ($F_{(3,177)}$ = 0.375, p = .771, MSE = 4.063, η^2_p = 0.006). No other interaction was statistically significant (highest F for age × object $_{(3,177)}$ = 2.333, p = .076, MSE = 25.303, η^2_p = 0.038).

A   Sample Phase Total Object Contact Time

□ TG ■ WT

B   Test Phase Object Contact Time

▨ Familiar
■ Mismatch

C   Discrimination Ratios

□ TG ■ WT
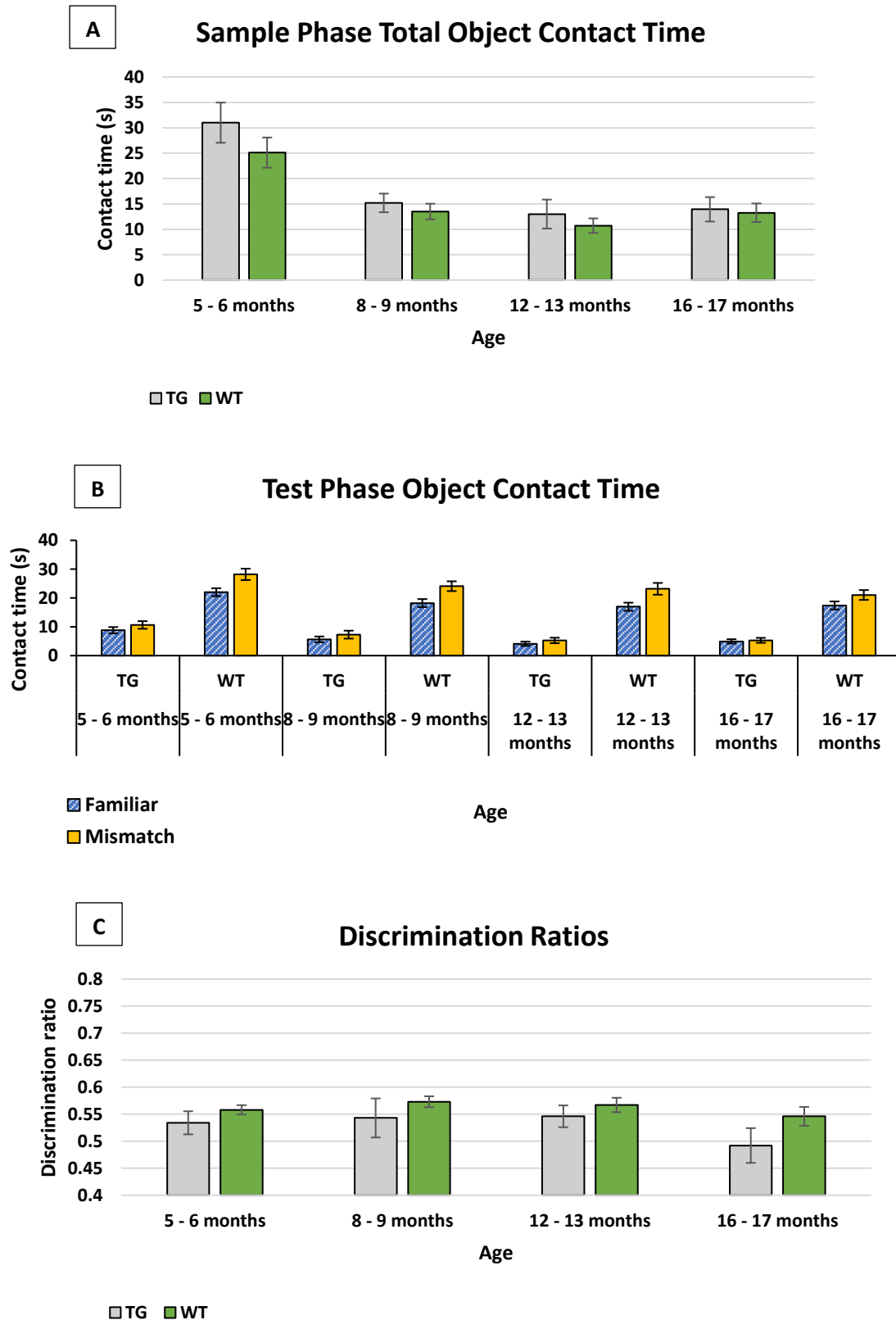
Figure 4.2. Mean object contact times in 10 minute sample (A) and test (B) phases, and discrimination ratios (C), for aging Tg2576 (TG) and wild-type (WT) mice (at 5-6, 8-9, 12-13 and 16-17 months of age). (A) presents total sample phase object contact, while (B) presents separate contact times for familiar and mismatched objects. n = 26 Tg2576 and 35 wild-type mice.

Inspection of panel C of Figure 4.2 indicates that Tg2576 mice display a numerically lower preference ratio for exploring mismatched objects at all ages, though this is numerically most pronounced at 16-17 months of age. In addition, while both wild-type and Tg2576 mice showed an overall numeric discrimination ratio decline with age, this decline appears numerically larger in Tg2576 mice. ANOVA results were partially consistent with this impression, and revealed a significant effect of genotype ($F_{(1,59)} = 4.488$, $p = .038$, MSE = 0.062, $\eta^2_p = 0.071$), but no significant effect of age ($F_{(2.669,157.470)} = 1.680$, $p = .179$, MSE = 0.022, $\eta^2_p = 0.028$), and a non-significant age × genotype interaction ($F_{(2.669,157.470)} = 0.296$, $p = .805$, MSE = 0.004, $\eta^2_p = 0.005$).[1] As occurred with the sample phase data, cohort attrition and data cleaning led to data exclusion of several Tg2576 mice from the aging analysis. This numerically lowered the mean Tg2576 discrimination ratios at the first three time points, and numerically raised it at the fourth. The discrimination ratios presented in the individually analysed time points more accurately represent object discrimination at each age.

While the aging analysis did not provide direct evidence of an age-dependent Tg2576 deficit in associative object-in-place memory, there did appear to be a noticeable numeric decline in both the degree to which mismatched objects were explored more than familiar ones, and in the discrimination ratio, in Tg2576 mice at 16-17 months of age. In Chapter 3, the hedonic responsiveness of Tg2576 and wild-type mice was examined at each individual time point following the aging analysis, in part to determine at which age a hedonic deficit first manifests. To examine whether a cognitive deficit emerges at any individual time point, and to evaluate the time of emergence in relation to the previously established hedonic deficit, cognitive performance at each time point will be analysed next.

4.2.3.2 5-6 month object-in-place test results

Inspection of Figure 4.3 indicates that at 5-6 months of age, both Tg2576 and wild-type mice decreased their object exploration on day 2 of habituation, while Tg2576 mice display less object exploration than wild-type mice across habituation days. ANOVA results were consistent with these

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[1] A second data cleaning strategy was also employed, similar to that described in 4.2.2, but with the additional step that any mouse removed from the test phase data also had its yoked/master counterpart removed. This second strategy produced the same pattern of test results seen in the individual time point analysis, with a Tg2576 deficit absent at the first 3 time points but present at the final time point. However, the significant genotype effect observed in the aging discrimination ratio analysis was not seen (F for main effect of genotype $(1,49) = 2.792$, $p = .101$, MSE = 0.045, $\eta^2_p = 0.054$) (n = 26 Tg2576 and 25 wild-type mice). Therefore, other than this non-significant effect of genotype in the aging discrimination ratio analysis, the precise cleaning strategy made little material difference to the overall interpretation of results. The analysis presented herein for Experiment 3 is that which used the first cleaning strategy.

observations, revealing significant effects of day (F(1,80) = 88.000, p<.001, MSE = 9206.20, η^2_p= 0.524) and genotype (F(1,80) = 22.76, p<.001, MSE = 8157.0, η^2_p= 0.221), with no significant day × genotype interaction (F(1,80) = 0.388, p = .535, MSE = 40.63, η^2_p= 0.005).

Habituation: 5-6 months

Figure 4.3. Mean object contact times (s) from 10 minute habituation sessions over two consecutive days, for 5-6 month old Tg2576 (TG) and wild-type (WT) mice. n = 42 Tg2576 and 40 wild-type mice.

At 5-6 months of age, the yoking procedure produced near-identical object sampling in both genotypes; Tg2576 mice explored objects for 24.91(±2.807) seconds, and wild-type mice for 24.68(±2.632) seconds (t(80) = 0.058, p = .954, d = 0.013). Inspection of panel A of Figure 4.4 indicates that, at 5-6 months of age, both Tg2576 and wild-type mice show greater exploration of mismatched than familiar objects. In addition, Tg2576 mice show numerically lower contact times at both object types compared with wild-type mice, especially on mismatched objects. ANOVA results were consistent with this impression, revealing significant effects of object (F(1,80) = 69.37, p<.001, MSE = 703.72, η^2_p= 0.464) and genotype (F(1,80) = 93.73, p<.001, MSE = 11088.5, η^2_p= 0.540), and a significant object × genotype interaction (F(1,80) = 13.61, p<.001, MSE = 138.03, η^2_p= 0.145). Despite this general reduction in Tg2576 contact time, paired samples t-tests comparing familiar versus mismatched contact time within each genotype revealed a significant difference for both Tg2576 (Student's t(41) = 4.867, p<.001, d = 0.751) and wild-type (Student's t(39) = 6.719, p<.001, d = 1.062) mice. Similarly, inspection of panel B of Figure 4.4 indicates that, when exploration is expressed as a discrimination ratio, Tg2576 mice display a preference for mismatched objects which is numerically greater than wild-type preference, while all mice display a preference above chance performance.

An unpaired t-test revealed no significant difference between Tg2576 and wild-type discrimination ratios (Welch's $t(50.66) = 0.972$, $p = .336$, $d = 0.211$). A Bayesian independent samples t-test provided an indication that the null effect of genotype on discrimination ratio was a genuine absence of effect ($BF_{10} = 0.341$). One sample t-tests comparing discrimination ratios against chance performance (0.5) revealed significant differences for both Tg2576 (Student's $t(41) = 3.464$, $p = .001$, $d = 0.535$) and wild-type (Student's $t(39) = 7.034$, $p<.001$, $d = 1.112$) mice.

Figure 4.4. Mean object contact times (s) for familiar and mismatched objects (A) and discrimination ratios (B) for 5-6 month old Tg2576 (TG) and wild-type (WT) mice, in 10 minute test sessions. n = 42 Tg2576 and 40 wild-type mice.

4.2.3.3 8-9 month object-in-place test results

Inspection of Figure 4.5 indicates that at 8-9 months of age, a similar habituation pattern occurred as at 5-6 months, albeit the reduction in object contact time at habituation day 2 was more pronounced in Tg2576 mice. ANOVA results were consistent with this impression, revealing significant effects of day ($F_{(1,73)}$ = 48.201, p<.001, MSE = 408.806, η^2_p= 0.398) and genotype ($F_{(1,73)}$ = 30.95, p<.001, MSE = 1430.58, η^2_p= 0.298), and a significant day × genotype interaction ($F_{(1,73)}$ = 8.450, p = .005, MSE = 71.668, η^2_p= 0.104).

Habituation: 8-9 months

Figure 4.5. Mean object contact times (s) from 10 minute habituation sessions over two consecutive days, for 8-9 month old Tg2576 (TG) and wild-type (WT) mice. n = 37 Tg2576 and 38 wild-type mice.

At 8-9 months of age, yoking again produced roughly numerically equivalent genotypic object sampling. Tg2576 mice accumulated 12.606(±1.458) seconds and wild-type mice 13.73(±1.439) seconds of object contact time ($t_{(74)}$ = 0.549, p = .585, d = 0.126). Inspection of panel A of Figure 4.6 reveals the same pattern of object contact time results at 8-9 months of age as seen at 5-6 months of age. This impression was confirmed by ANOVA results, which revealed significant effects of object ($F_{(1,74)}$ = 53.46, p<.001, MSE = 481.988, η^2_p= 0.419) and genotype ($F_{(1,74)}$ = 95.66, p<.001, MSE = 9565.8, η^2_p= 0.564), and a significant object × genotype interaction ($F_{(1,74)}$ = 21.14, p<.001, MSE = 190.557, η^2_p= 0.222). As seen at 5-6 months of age, paired samples t-tests comparing familiar versus mismatched contact time within each genotype revealed a significant difference for both Tg2576 (Student's $t_{(37)}$ = 2.169, p = .037, d = 0.352) and wild-type (Student's $t_{(37)}$ = 7.634, p<.001, d = 1.238) mice.

Figure 4.6. Mean object contact times (s) for familiar and mismatched objects (A) and discrimination ratios (B) for 8-9 month old Tg2576 (TG) and wild-type (WT) mice, in 10 minute test sessions. n = 38 Tg2576 and 38 wild-type mice.

Inspection of panel B of Figure 4.6 indicates that Tg2576 mice display a numerically lower discrimination ratio than wild-type mice, while all mice display a preference for mismatched objects which is numerically greater than chance performance. An unpaired t-test revealed no significant difference between Tg2576 and wild-type discrimination ratios (Welch's t(45.33) = 0.938, p = .353, *d* = 0.215). A Bayesian independent samples t-test gave an indication that the null genotype effect on discrimination ratio was a genuine absence of effect (BF$_{10}$ = 0.347). One sample t-tests comparing discrimination ratios against chance performance (0.5) revealed no significant difference for Tg2576

mice (Student's t(37) = 1.662, p = .105, d = 0.270), and a significant difference for wild-type mice (Student's t(37) = 7.856, p<.001, d = 1.274). A Bayesian one sample t-test provided uninformative evidence regarding the Tg2576 discrimination ratio not differing from 0.5 (BF_{10} = 0.612).

4.2.3.4 12-13 month object-in-place test results

Inspection of Figure 4.7 indicates that, at 12 months of age, habituation followed a pattern similar to that reported at previous time points. ANOVA results were consistent with this observation, revealing significant effects of day (F(1,73) = 18.211, p<.001, MSE = 142.549, η^2_p= 0.200) and genotype (F(1,73) = 60.14, p<.001, MSE = 1925.10, η^2_p= 0.452), with no significant day × genotype interaction (F(1,73) = 0.554, p = .459, MSE = 4.338, η^2_p= 0.008).
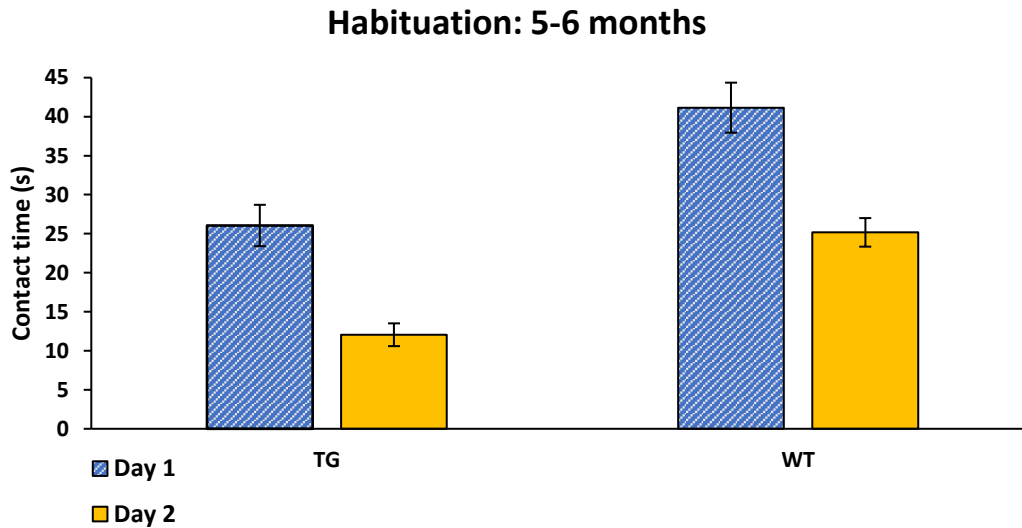
Habituation: 12-13 months
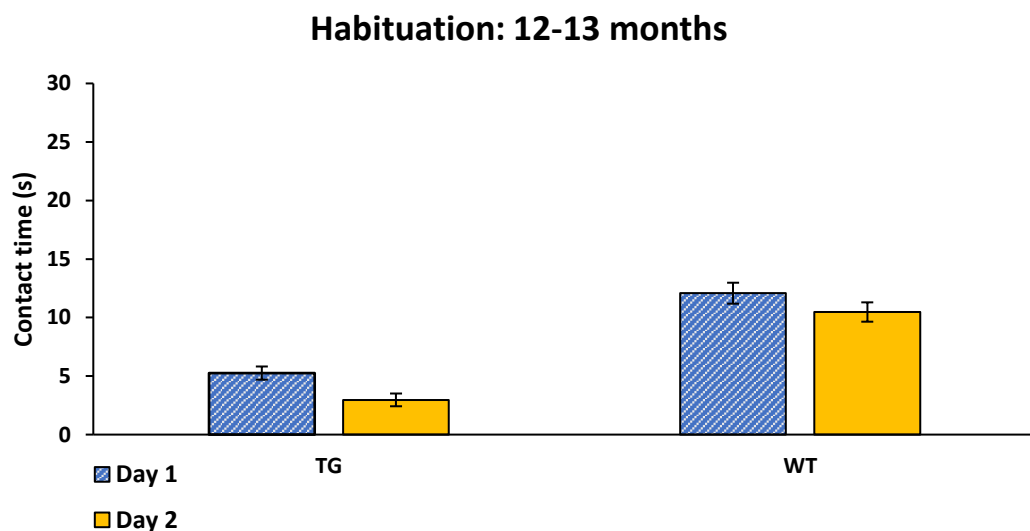
Figure 4.7. Mean object contact times (s) from 10 minute habituation sessions over two consecutive days, for 12-13 month old Tg2576 (TG) and wild-type (WT) mice. n = 37 Tg2576 and 38 wild-type mice.

As at previous ages, yoking at 12-13 months produced numerically near-equivalent total object contact for the two genotypes during the sample phase. Tg2576 mice accrued 10.964(±2.111) seconds, and wild-type mice 11.246(±1.352) seconds, of total object contact (Student's t(73) = 0.113, p = .910, d = 0.026). Inspection of panel A of Figure 4.8 indicates that, at 12-13 months of age, both Tg2576 and wild-type mice display a similar pattern of object contact time results as seen at previous time points. ANOVA results were consistent with this impression, revealing significant effects of object (F(1,73) = 49.76, p<.001, MSE = 645.75, η^2_p= 0.405) and genotype (F(1,73) = 85.78, p<.001, MSE = 9131.0, η^2_p= 0.540), and a significant object × genotype interaction (F(1,73) = 18.94,

p<.001, MSE = 245.78, η^2_p = 0.206). Consistent with prior ages, paired samples t-tests comparing familiar versus mismatched contact time within each genotype revealed significant differences for both Tg2576 (Student's t(36) = 3.958, p<.001, d = 0.651) and wild-type (Student's t(37) = 6.139, p<.001, d = 0.996) mice.
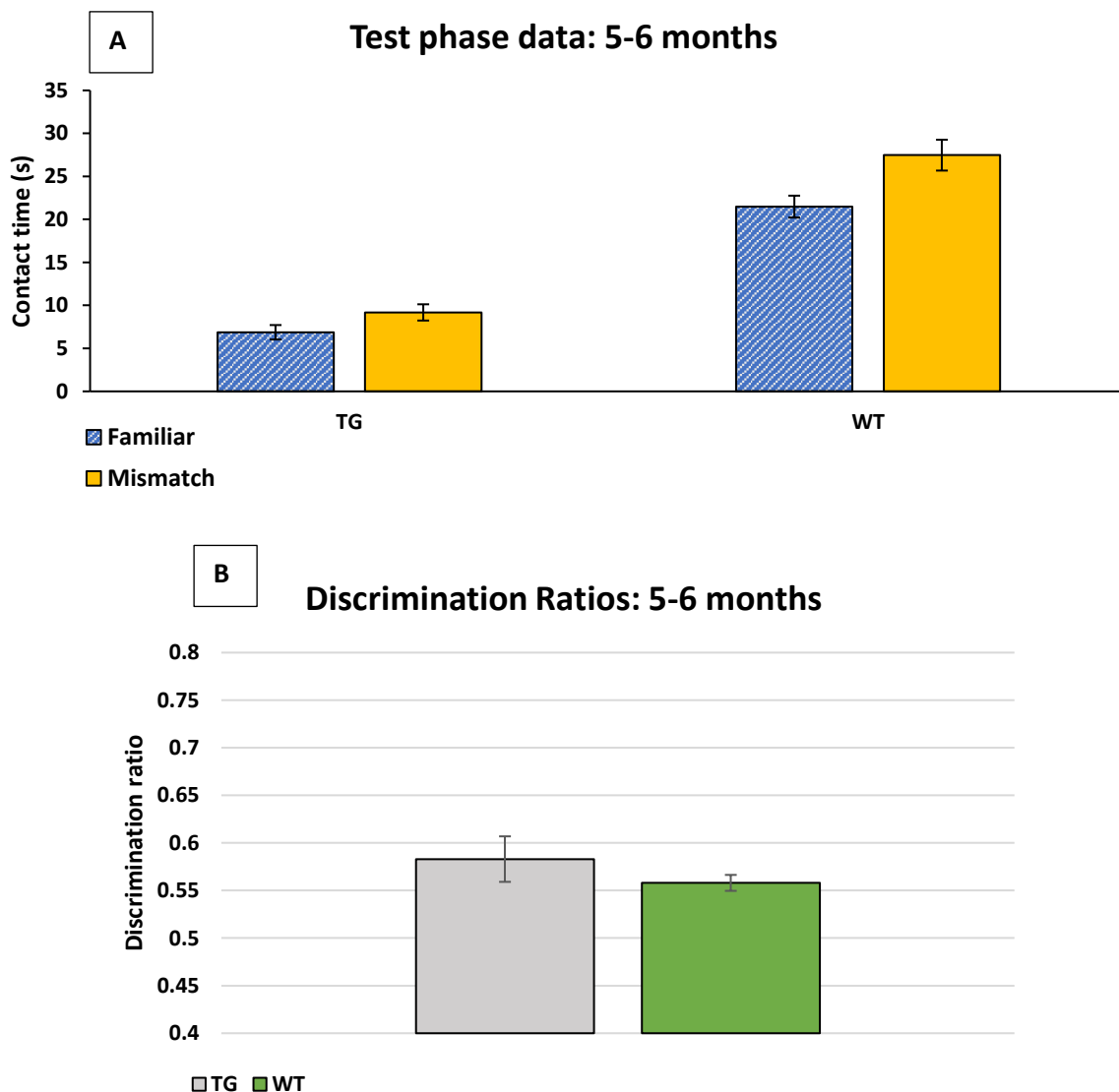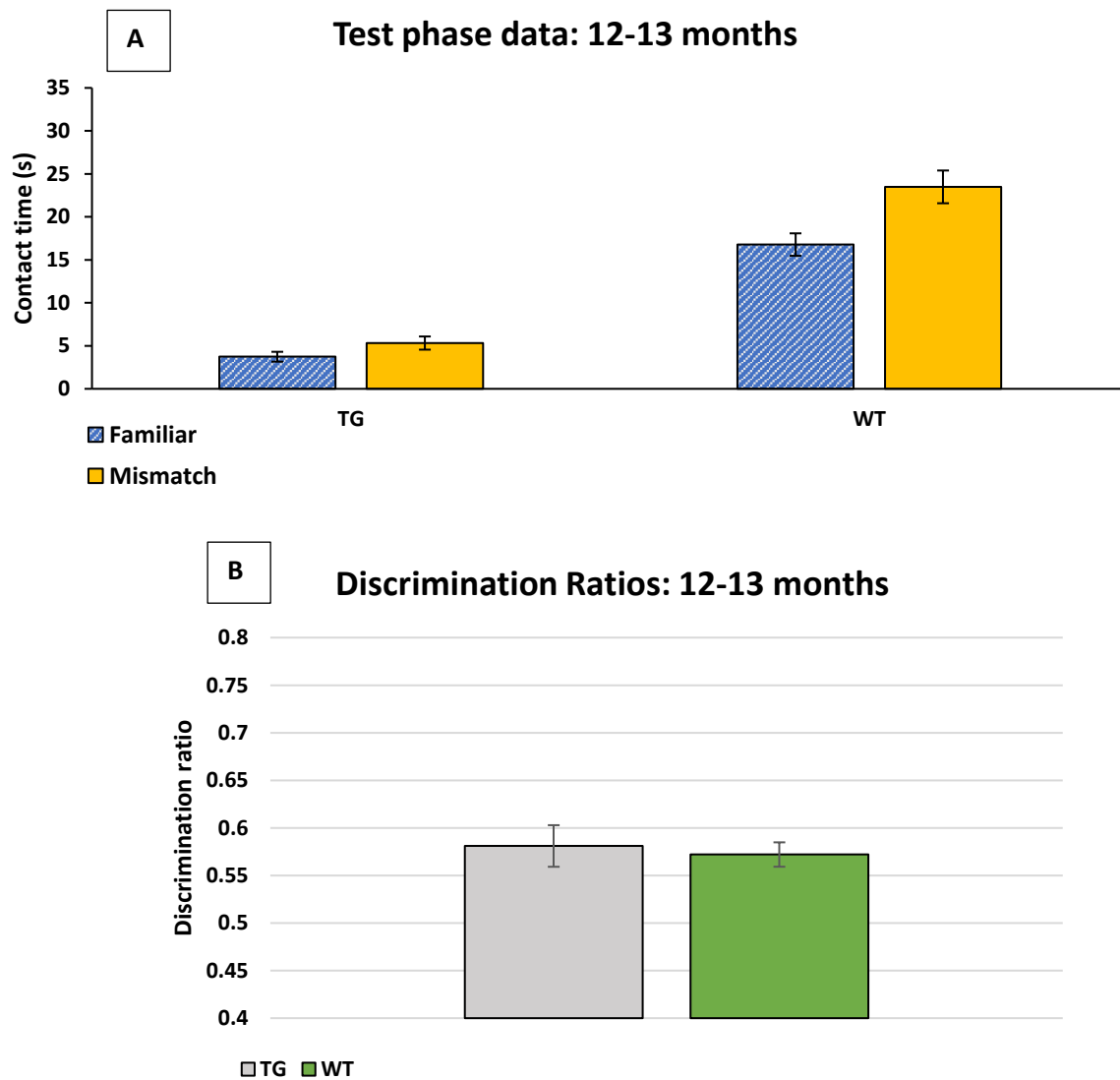

Figure 4.8. Mean object contact times (s) for familiar and mismatched objects (A) and discrimination ratios (B) for 12-13 month old Tg2576 (TG) and wild-type (WT) mice, in 10 minute test sessions. n = 37 Tg2576 and 38 wild-type mice.

Inspection of panel B of Figure 4.8 indicates that Tg2576 mice display a discrimination ratio numerically greater than wild type mice, while all mice continue to display a preference for mismatched objects which is numerically greater than chance performance. An unpaired t-test

revealed no significant difference between Tg2576 and wild-type discrimination ratios (Welch's: t(58.46) = 0.361, p = .720, d = 0.084). A Bayesian independent samples t-test provided evidence which suggested this null genotype effect on discrimination ratio was a genuine null effect (BF_{10} = 0.253). One sample t-tests comparing discrimination ratios against chance performance (0.5) revealed significant differences for both Tg2576 (Student's t(36) = 3.689, p<.001, d = 0.607), and wild-type mice (Student's t(37) = 5.555, p<.001, d = 0.901).

4.2.3.5 16-17 month object-in-place test results

Inspection of Figure 4.9 indicates that, at 16-17 months of age, as seen at prior time points, Tg2576 mice reduced their object exploration on habituation day 2, and showed lower contact times than wild-type mice across habituation days. However, unlike previous time points, 16-17 month old wild-type mice did not reduce their exploration on habituation day 2. In fact, wild-type mice displayed numerically greater contact time on habituation day 2. ANOVA results were largely consistent with this impression, revealing no significant effect of day (F(1,63) = 2.105, p = .152, MSE = 65.24, η^2_p= 0.032), a significant effect of genotype (F(1,63) = 32.88, p<.001, MSE = 3346.6, η^2_p= 0.343), and a non-significant day × genotype interaction (F(1,63) = 3.459, p = .068, MSE = 107.21, η^2_p= 0.052).
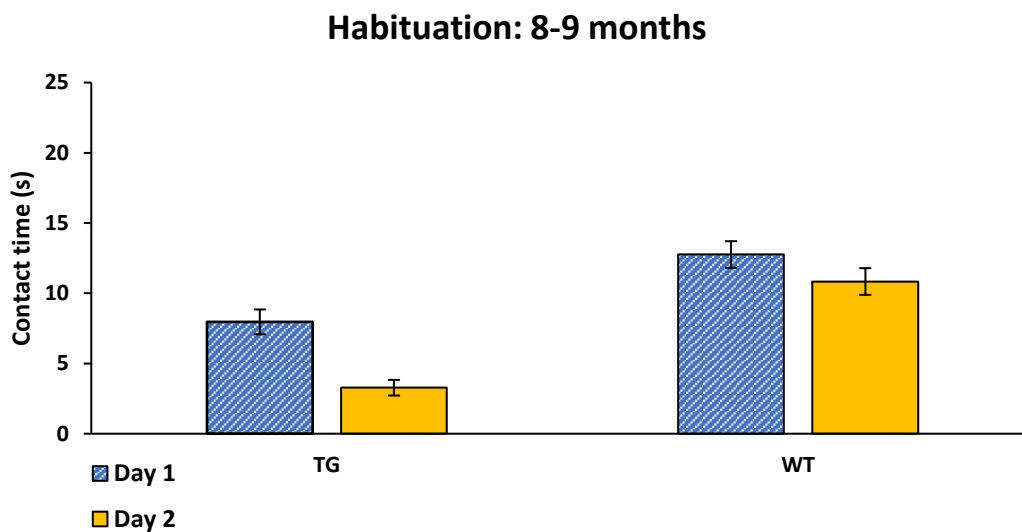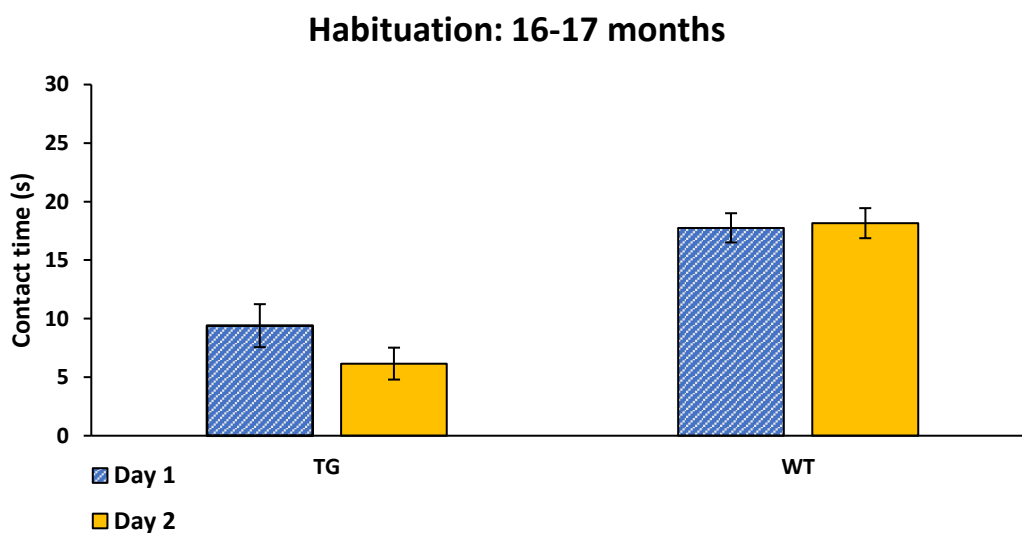

Figure 4.9. Mean object contact times (s) from 10 minute habituation sessions over two consecutive days, for 16-17 month old Tg2576 (TG) and wild-type (WT) mice. n = 30 Tg2576 and 35 wild-type mice.

Once again, yoking at 16-17 months produced numerically near-equivalent total object contact for the two genotypes during the sample phase. Tg2576 mice accumulated 12.822(±2.158) seconds, and wild-type mice 13.256(±1.829) seconds, of total object contact (Student's $t(63) = 0.155$, $p = .878$, $d = 0.038$). Inspection of panel A of Figure 4.10 indicates that, as at previous ages, both Tg2576 and wild-type mice display numerically greater exploration of mismatched objects, with Tg2576 mice displaying lower contact times than wild-type mice across object type. ANOVA results were somewhat consistent with this impression, revealing significant effects of object ($F(1,63) = 8.555$, $p = .005$, MSE = 124.30, $\eta^2_p = 0.120$) and genotype ($F(1,63) = 74.15$, $p<.001$, MSE = 6840.95, $\eta^2_p = 0.541$), and a significant object × genotype interaction ($F(1,63) = 6.214$, $p = .015$, MSE = 90.28, $\eta^2_p = 0.090$). Unlike previous ages, however, paired samples t-tests comparing familiar versus mismatched contact time within each genotype revealed a significant difference only for wild-type mice (Student's $t(34) = 3.123$, $p = .004$, $d = 0.528$), and a non-significant difference for Tg2576 mice (Student's $t(29) = 0.575$, $p = .569$, $d = 0.105$). Inspection of panel B of Figure 4.10 indicates that Tg2576 mice display a discrimination ratio lower than that of wild-type mice, while only wild-type mice continue to display a preference for mismatched objects which is greater than chance. A one-tailed unpaired t-test revealed a significant difference between Tg2576 and wild-type discrimination ratios (Welch's: $t(46.37) = 1.819$, $p = .038$, $d = 0.469$). A one-tailed t-test was used in this analysis because it was anticipated that, based on existing literature, Tg2576 mice would be impaired in object-in-place memory by 16 months of age (Good & Hale, 2007; Hale & Good, 2005). One sample t-tests comparing discrimination ratios against chance performance (0.5) revealed a significant difference for wild-type (Student's $t(34) = 2.651$, $p = .012$, $d = 0.448$), but not Tg2576 (Student's $t(29) = 0.594$, $p = .557$, $d = 0.108$) mice. A Bayesian one sample t-test provided evidence suggesting the Tg2576 discrimination ratio genuinely did not differ from 0.5 ($BF_{10} = 0.229$).

Test phase data: 16-17 months

A

B

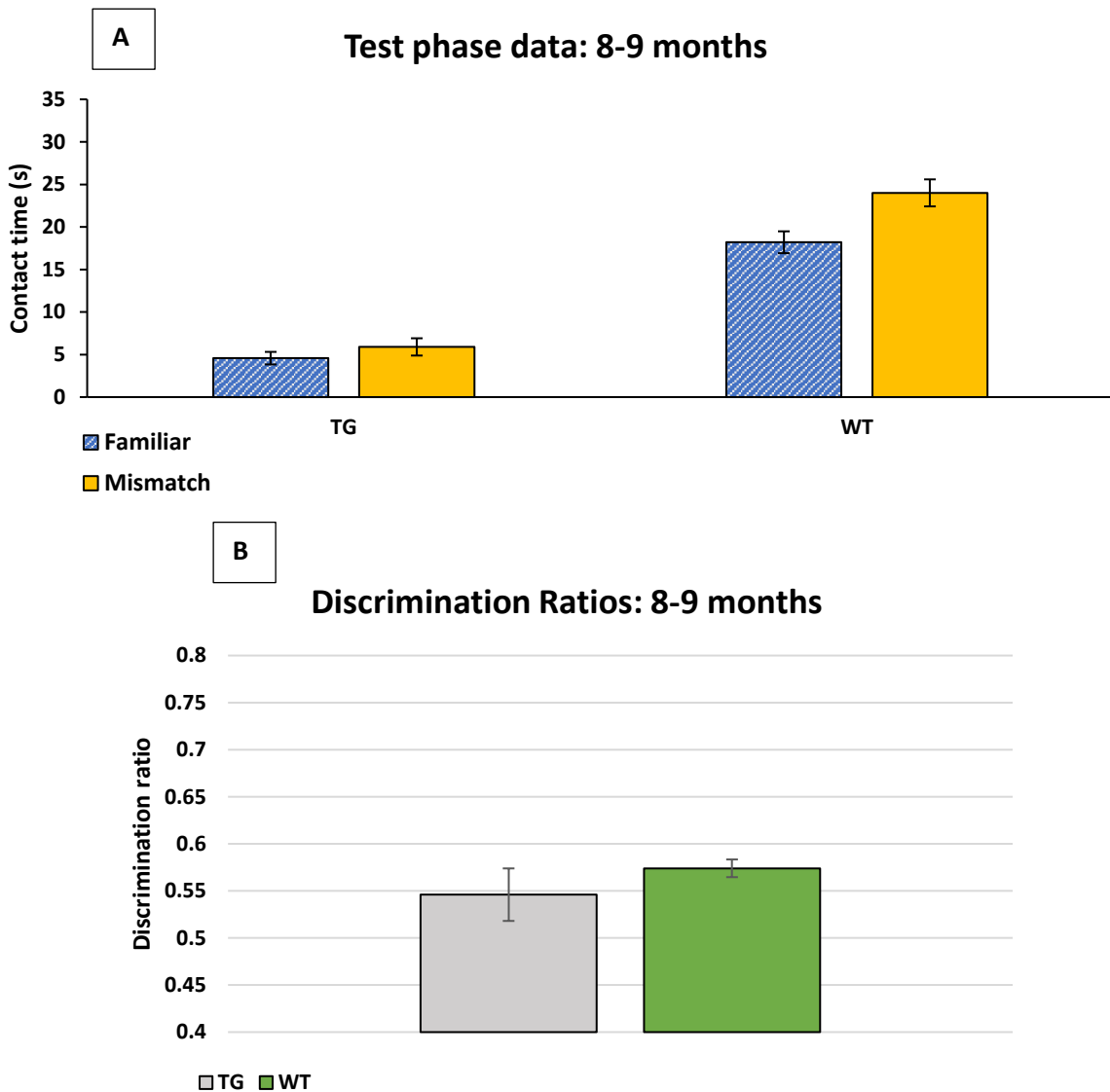## Discrimination Ratios: 16-17 months

Figure 4.10. Mean object contact times (s) for familiar and mismatched objects (A) and discrimination ratios (B) for 16-17 month old Tg2576 (TG) and wild-type (WT) mice, in 10 minute test sessions. n = 30 Tg2576 and 35 wild-type mice.

4.2.4 Summary

Experiment 3 examined the associative object-in-place memory of Tg2576 mice longitudinally, from 5-6 to 16-17 months of age. Both the aging and individual time point analyses presented a largely similar pattern of results. Firstly, the habituation results revealed that object exploration generally decreased with age and on the second habituation day for both genotypes, and that Tg2576 mice consistently explored both object types to a lesser extent than wild-type mice. This general genotype-independent reduction in object exploration over time, and consistently lower object exploration in Tg2576 mice, was also observed in the test phase data. Secondly, and

critically, while Tg2576 and wild-type mice display noticeably numerically greater exploration of mismatched objects up to 12-13 months, this exploratory numeric preference remains clearly noticeable at 16-17 months only in wild-type, but not in Tg2576, mice. However, this genotypic difference coincides with generally low object exploration by Tg2576 mice. When object contact times are converted into discrimination ratios, to account for differences in overall object contact, a similar pattern of results occurs. That is, Tg2576 and wild-type mice display discrimination ratios which are not dissimilar from one another, and numerically above chance performance (0.5), from 5-6 months until (and including) 12-13 months of age. At 16-17 months of age, however, the Tg2576 discrimination ratio is markedly lower than that of wild-type mice, and both numerically below and meaningfully indistinguishable from chance performance (0.5). Importantly, this eventual genotype difference in preference for mismatched objects occurred after Tg2576 and wild-type mice were given approximately equal amounts of object sampling, prior to two objects exchanging locations. The apparent Tg2576 reduction in mismatched object preference, therefore, cannot be attributed to a lower level of object exploration in the sample phase.

4.3 Experiment 4

4.3.1 Subjects, apparatus & procedure

Experiment 4 is a single study of the associative object-in-place memory of 19-20 month old Tg2576 and wild-type mice, featuring a between subjects drug condition. Animals used were the mice which had gone through all time points in Experiment 3, and the experiment occurred directly after the ketamine licking study presented in Experiment 2, Chapter 3 (see Figure 4.11). Mice were kept in the treatment groups assigned in Experiment 3; the numbers of mice entered into the study, within both each genotype and treatment group, were as reported in Experiment 3. Testing apparatus and procedure were as previously described, with the addition of one further dose of ketamine (30 mg/kg) or vehicle being administered 24h prior to each squad of mice beginning the habituation phase. This resulted in all mice having received 3 doses of either ketamine or vehicle when beginning Experiment 4. A new set of 4 object arrays was chosen for Experiment 4; object set and diagonal shift were counterbalanced across genotype, treatment group, and day.
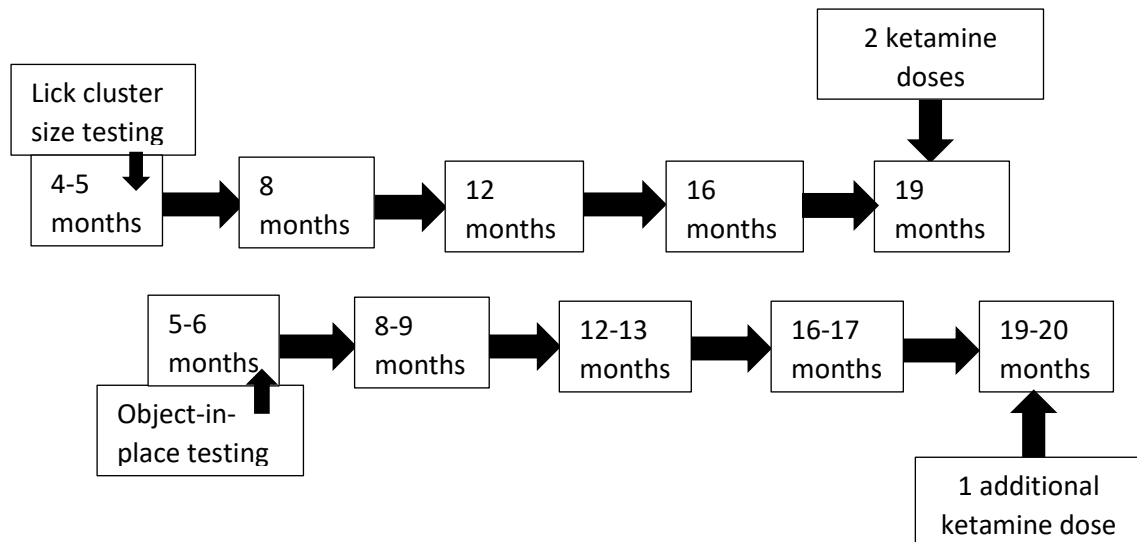
Figure 4.11. Time course of behavioural tests for aging mouse cohorts. Tg2576 and wild-type mice went through both sets of tests, then the T-maze test (Experiment 5, Section 4.4), before being sacrificed for brain biochemistry.

4.3.2 Data analysis

Data were collected and cleaned as for Experiment 3, resulting in 5 vehicle-treated Tg2576 mice having their data removed.[2] Habituation and test data were analysed using mixed ANOVA, with between-subjects factors of genotype and drug, and within-subjects factors of day (for habituation) and object (for test phase). Sample phase data were analysed by ANOVA, with between-subjects factors of genotype and drug. Overall Tg2576 and wild-type discrimination ratios (across treatment group) were compared using unpaired t-tests, and the overall discrimination ratio of each genotype was compared against chance performance (0.5) using one-sample t-tests. Pairwise comparisons between overall object groupings within a genotype were conducted by paired t-tests. Data are presented in the same manner as for Experiment 3. That is, all object contact times or discrimination ratios are presented graphically, except for sample phase contact times which are described in the text.

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[2] The same cleaning strategy used in Experiment 3, described in footnote 1, was also carried out for Experiment 4 test data. As with Experiment 3, this produced the same pattern of results as the cleaning strategy described in the main text. For example, in the discrimination ratio analysis there was a significant effect of genotype ($F(1,51) = 5.789$, $p = .020$, $MSE = 0.104$, $\eta^2_p = 0.102$), a non-significant effect of drug ($F(1,51) = 0.031$, $p = .860$, $MSE = 5.598e^{-4}$, $\eta^2_p = 0.001$), and a non-significant genotype × drug interaction ($F(1,51) = 0.167$, $p = .685$, $MSE = 0.003$, $\eta^2_p = 0.003$) (n = 26 Tg2576 mice (16 ketamine- and 10 vehicle-treated), 29 wild-type mice (16 ketamine- and 13 vehicle-treated)). Therefore the results presented for Experiment 4 are those which resulted from the original cleaning strategy.

4.3.3 Results

Inspection of Figure 4.12 indicates that, in general, ketamine- and vehicle-treated mice displayed the same pattern of habituation results seen at the final time point of Experiment 3. That is, Tg2576 mice exhibited lower object contact times than wild-type mice, regardless of treatment group or habituation day. There was also no obvious effect of ketamine in either genotype. ANOVA results were largely consistent with this observation, revealing no significant effect of day ($F_{(1,53)}$ = 0.014, p = .906, MSE = 0.057, η^2_p<.001), a significant effect of genotype ($F_{(1,53)}$ = 24.414, p<.001, MSE = 402.093, η^2_p= 0.315), and no significant day × genotype interaction ($F_{(1,53)}$ = 0.920, p = .342, MSE = 3.719, η^2_p= 0.017). There was no significant effect of ketamine treatment ($F_{(1,53)}$ = 0.098, p = .756, MSE = 1.610, η^2_p= 0.002), and no other interaction term reached statistical significance (highest F for day × genotype $(1,53)$ = 0.920, p = .342, MSE = 3.719, η^2_p= 0.017).
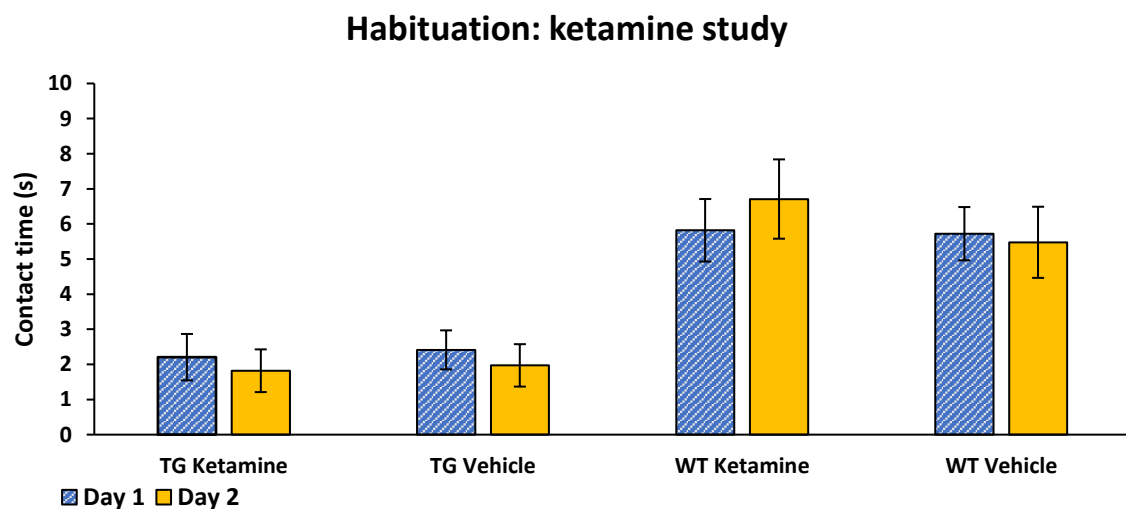
Figure 4.12. Mean object contact times (s) from 10 minute habituation sessions, over two consecutive days, for 19-20 month old, ketamine- and vehicle-treated, Tg2576 (TG) and wild-type (WT) mice. n = 26 Tg2576 (16 ketamine- and 10 vehicle-treated) and 31 wild-type (16 ketamine- and 15 vehicle-treated) mice.

At 19-20 months of age, yoking produced numerically near-equivalent object contact times between genotypes during the sample phase within both ketamine and vehicle treatment groups. In addition, ketamine-treated mice of both genotypes displayed numerically lower sample phase object contact times than their vehicle-treated counterparts. Within ketamine-treated mice, Tg2576 mice accrued 10.53(±2.233) seconds and wild-type mice 10.77(±1.643) seconds of total object contact time in the sample phase, while in vehicle-treated animals Tg2576 mice accumulated 14.31(±2.645) seconds and wild-type mice 15.32(±2.072) seconds of total object contact time. ANOVA results

revealed no significant effect of genotype ($F_{(1,53)} = 0.084$, $p = .773$, MSE = 5.373, $\eta^2_p = 0.002$), a non-significant effect of ketamine ($F_{(1,53)} = 3.734$, $p = .059$, MSE = 237.827, $\eta^2_p = 0.066$), and a non-significant genotype × drug interaction ($F_{(1,53)} = 0.032$, $p = .858$, MSE = 2.053, $\eta^2_p = 0.001$).
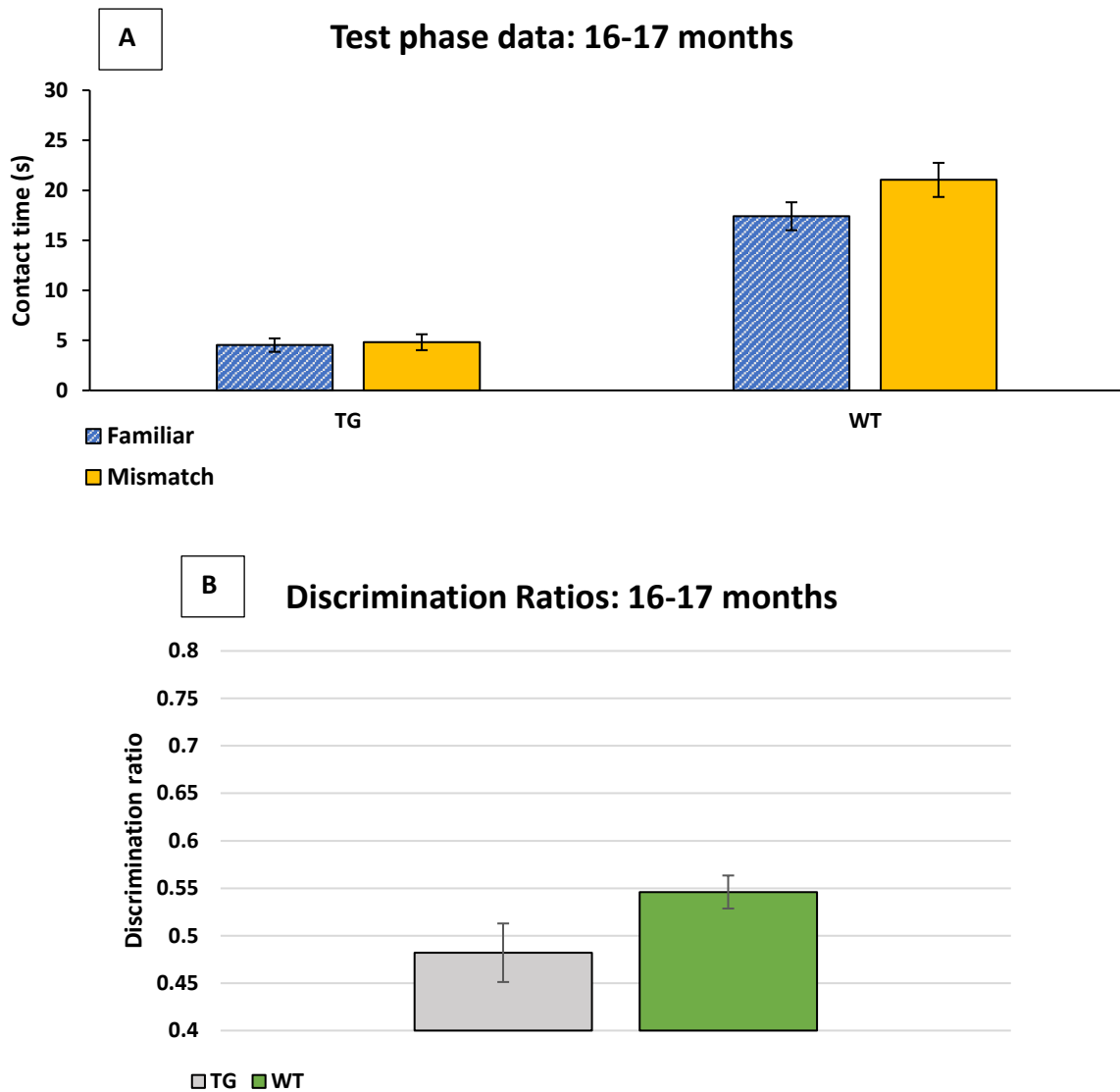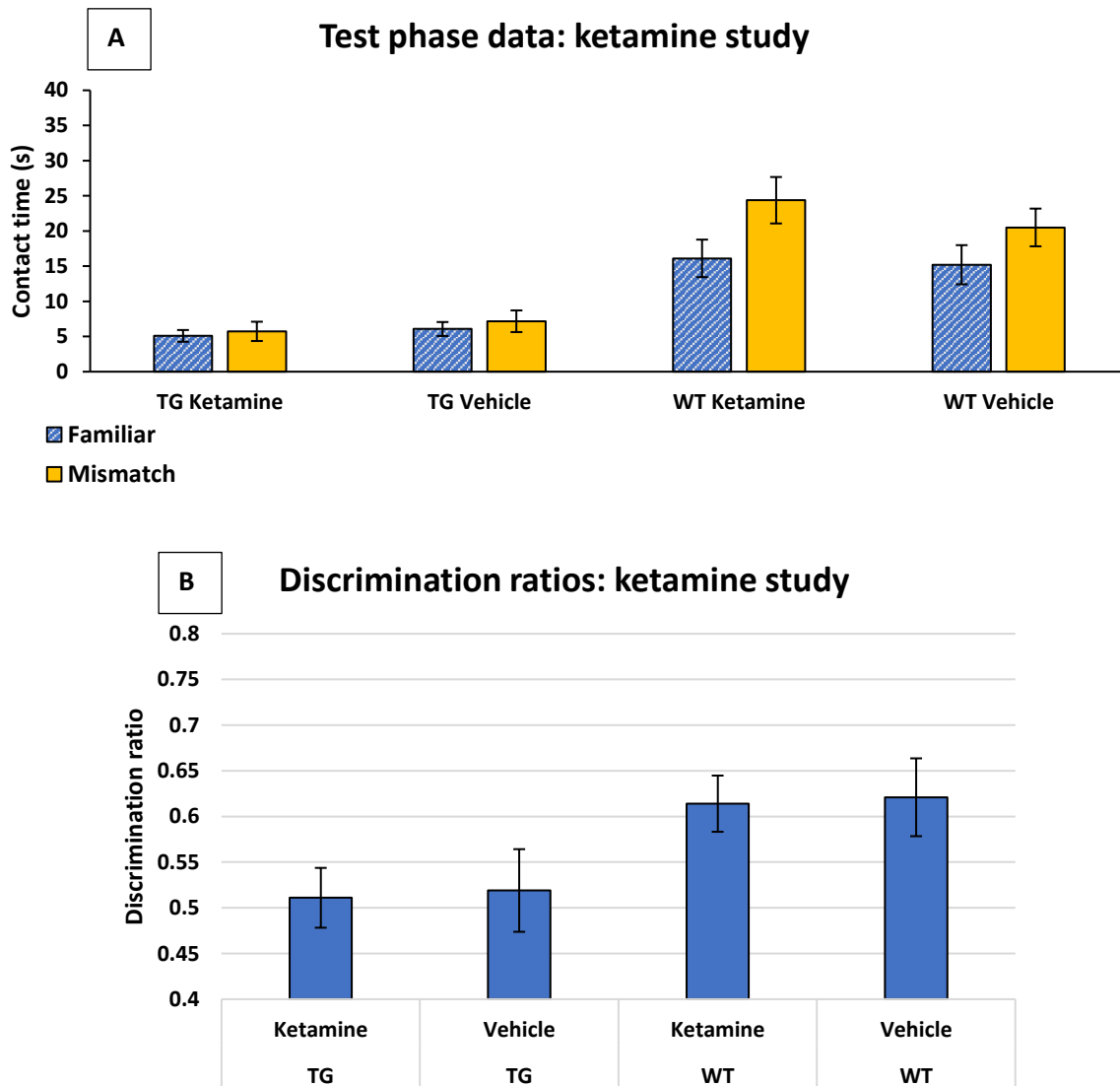

Figure 4.13. Mean object contact times for familiar and mismatched objects (A) and discrimination ratios (B) for 19-20 month old, ketamine- and vehicle-treated, Tg2576 (TG) and wild-type (WT) mice, in 10 minute test sessions. n = 26 Tg2576 (16 ketamine- and 10 vehicle-treated) and 31 wild-type (16 ketamine- and 15 vehicle-treated) mice.

Inspection of panel A of Figure 4.13 indicates that, as in Experiment 3, in the test phase all groups of mice display numerically greater contact times with mismatched than familiar objects, especially so in wild-type mice, while Tg2576 display much lower contact times than wild-type mice, regardless of object type. In addition, ketamine-treated mice exhibited small differences in contact

times compared with vehicle-treated mice; a numeric decrease in Tg2576 and a numeric increase in wild-type mice. ANOVA results were broadly consistent with this impression, revealing significant effects of object ($F(1,53) = 9.075$, $p = .004$, MSE = 402.03, $\eta^2_p = 0.146$) and genotype ($F(1,53) = 40.934$, $p<.001$, MSE = 4657.901, $\eta^2_p = 0.436$), and a significant object × genotype interaction ($F(1,53) = 5.403$, $p = .024$, MSE = 239.35, $\eta^2_p = 0.093$). There was no significant effect of ketamine treatment ($F(1,53) = 0.084$, $p = .773$, MSE = 9.529, $\eta^2_p = 0.002$), and no other interaction reached statistical significance (highest F for genotype × drug $(1,53) = 0.779$, $p = .381$, MSE = 88.677, $\eta^2_p = 0.014$). A Bayesian mixed ANOVA here provided uninformative evidence regarding the null effect of ketamine on object contact times ($BF_{10} = 0.735$), and the null genotype × drug interaction ($BF_{10} = 1.776$). Paired samples t-tests comparing familiar versus mismatched contact time within each genotype (across treatment groups) revealed a significant difference only for wild-type mice (Student's $t(30) = 3.122$, $p = .004$, $d = 0.561$), and a non-significant difference for Tg2576 mice (Student's $t(25) = 1.182$, $p = .248$, $d = 0.232$).

Inspection of panel B on Figure 4.13 indicates that mice of all genotypes and treatment groups displayed discrimination ratios numerically greater than chance performance, though only narrowly in the case of Tg2576 mice. In addition, Tg2576 mice displayed notably smaller discrimination ratios than wild-type mice, regardless of treatment group. ANOVA results were consistent with this impression, revealing a significant effect of genotype $(1,53) = 7.294$, $p = .009$, MSE = 0.144, $\eta^2_p = 0.121$), no significant effect of ketamine treatment $(1,53) = 0.041$, $p = .841$, MSE = $8.056e^{-4}$, $\eta^2_p = 0.001$), and no significant genotype × drug interaction $(1,53) = 4.849e^{-4}$, $p = .983$, MSE = $9.593e^{-6}$, $\eta^2_p<.001$). A Bayesian ANOVA provided evidence suggesting that the null effect of ketamine on discrimination ratios represented a true null drug effect ($BF_{10} = 0.294$), and supporting the absence of a genotype × drug interaction ($BF_{10} = 0.095$). An unpaired t-test comparing discrimination ratios of Tg2576 and wild-type mice, across treatment groups, revealed a significant genotype difference (Student's $t(55) = 2.816$, $p = .007$, $d = 0.749$). One sample t-tests comparing these same overall genotype discrimination ratios against chance performance (0.5) revealed a significant difference for wild-type (Student's $t(30) = 4.588$, $p<.001$, $d = 0.824$), but not Tg2576 (Student's $t(25) = 0.533$, $p = .599$, $d = 0.105$), mice. A Bayesian one sample t-test provided evidence suggesting that the overall Tg2576 discrimination ratio genuinely did not differ from chance performance ($BF_{10} = 0.236$).

4.3.4 Summary

Experiment 4 examined the associative object-in-place memory of ketamine- and vehicle-treated Tg2576 and wild-type mice at 19-20 months of age. Habituation results revealed that the general trend observed in Experiment 3, of object exploration decreasing after the first time point, had continued, with overall habituation-phase contact times in Experiment 4 at or below that seen at the end of Experiment 3. The pattern of test phase results was largely similar to that seen at the final time point in Experiment 3, in that wild-type mice displayed a greater exploration of mismatched objects while Tg2576 mice did not. When object contact times were expressed as discrimination ratios, wild-type mice clearly performed above chance (0.5), while Tg2576 mice did not, and were in fact not distinguishable from chance in their performance. In addition, overall Tg2576 mice displayed a significantly lower discrimination ratio than that of wild-type mice. There was no significant effect of ketamine on test phase data, whether expressed as object contact times or discrimination ratios.

4.4 Experiment 5

4.4.1 Subjects, apparatus & procedure

Experiment 5 is a single study of the short-term spatial memory of 19-20 month old Tg2576 and wild-type mice, featuring a between-subjects drug condition. Animals used were the same mice from Experiment 4, with each squad of mice entering Experiment 5 24h after the final object-in-place test day for that squad. Mice were kept in the treatment groups assigned in Experiments 2 and 4; 31 Tg2576 and 31 wild-type mice (16 ketamine- and 15 vehicle-treated in each genotype) were entered into the experiment. The apparatus was a T-maze with clear Perspex walls and a wooden base, with a start arm 52cm long, left and right goal arms 26cm long, and an opaque Perspex guillotine door for closing off one arm. Test sessions were recorded via a small camera connected to a monitor and DVD recorder mounted on the ceiling directly above the T-maze. The testing procedure was a modified version of a Y-maze task (Sanderson et al., 2007), and occurred in a new, quiet, room with extra-maze cues distinct from those used in the object-in-place tests. The T-maze was elevated 40cm from the ground, and the testing procedure was as follows: all mice received a 5 minute exposure phase, during which access to one of the left and right arms (the novel arm) was blocked by the Perspex guillotine, while the remaining two arms could be freely explored. Mice were returned to their home cage for 1 minute, during which the T-maze was cleaned with 70% isopropyl alcohol, then received a 2-minute test phase during which the two familiar arms and the novel arm could be freely explored. Mice were placed at the base of the start arm prior to exposure and test

phases, which began once mice had left the start arm. The amount of time that mice spent in each of the three arms was manually recorded; a mouse was considered to have entered and exited an arm once all four of its paws had crossed the threshold of that arm. To prevent the longer start arm distorting the results (as a mouse would likely take more time to reach the end of the start arm compared with the other arms), only time spent in the length of start arm equal to that of the other arms was recorded. Selection of novel arm (left or right) was counterbalanced across genotype and treatment group.

4.4.2 Data analysis

Time spent in arms was analysed using mixed ANOVA, with between-subjects factors of genotype and drug, and a within-subjects factor of arm. Time taken to leave the start arm, and discrimination ratios derived from time spent in arms were analysed by ANOVA, with between-subjects factors of genotype and drug. Overall Tg2576 and wild-type discrimination ratios (across treatment group) were compared using unpaired Student's t-tests, and the overall discrimination ratio of each genotype was compared against chance performance (0.33) using one-sample t-tests. Discrimination ratios were calculated by the following formula: (time spent in novel T-maze arm)/(time spent in all three T-maze arms). Two mice (one ketamine-treated Tg2576 mouse and one vehicle-treated wild-type mouse) had their data lost due to recording error; the final number of mice analysed was 30 Tg2576 (15 ketamine- and 15 vehicle-treated) and 30 wild-type (16 ketamine- and 14 vehicle-treated) mice.

4.4.3 Results

Tg2576 mice, across treatment groups, took longer to leave the start arm during the test phase than wild-type mice. Within Tg2576 mice, ketamine-treated mice took 54.26(±22.536) seconds and vehicle-treated mice took 82.42(±36.375) seconds to leave the start arm. For wild-type mice, ketamine-treated mice took 10.37(±4.215) seconds and vehicle-treated mice took 14.30(±11.449) seconds to leave the start arm. ANOVA revealed a significant effect of genotype $(F(1,56) = 6.371, p = .014, MSE = 46948, \eta^2_p = 0.102)$ but no significant effect of drug $(F(1,56) = 0.523, p = .473, MSE = 3854, \eta^2_p = 0.009)$ and no significant genotype × drug interaction $(F(1,56) = 0.298, p = .587, MSE = 2197, \eta^2_p = 0.005)$. Inspection of panel A of Figure 4.14 indicates that mice of all genotypes and treatment groups spent the greatest amount of time in the novel T-maze arm, less time in the other (familiar) T-arm, and less time still in the start arm, with no obvious effect of drug treatment. Other than the difference between time spent in the start arm and the other two arms

being more marked in Tg2576 than wild-type mice, there was no obvious genotype difference. ANOVA results were broadly consistent with this impression, revealing a significant effect of arm ($F_{(1.556, 87.140)} = 22.458$, $p<.001$, MSE = 11473.98, $\eta^2_p = 0.286$), and no significant effects of either drug ($F_{(1,56)} = 1.646$, $p = .205$, MSE = 205.53, $\eta^2_p = 0.029$) or genotype ($F_{(1,56)} = 0.543$, $p = .464$, MSE = 67.85, $\eta^2_p = 0.010$). The arm × genotype interaction was non-significant ($F_{(1.556, 87.140)} = 2.777$, $p = .081$, MSE = 1418.99, $\eta^2_p = 0.047$), and no other interaction reached statistical significance (highest F for arm × drug $_{(1.556, 87.140)} = 0.359$, $p = .646$, MSE = 183.50, $\eta^2_p = 0.006$).
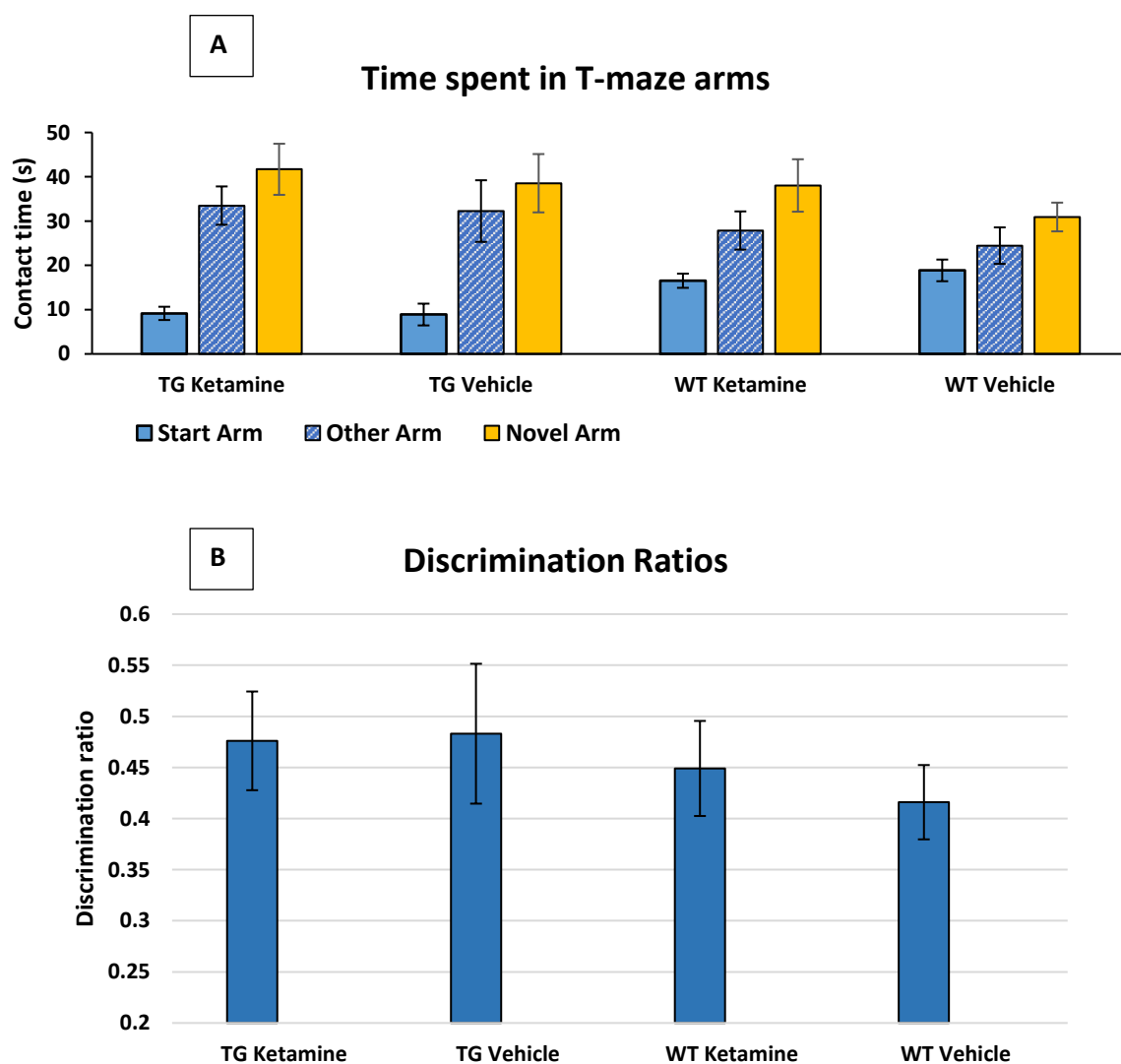
Figure 4.14. Mean time spent in T-maze arms (A) and derived discrimination ratios showing preference for the novel arm (B) for 19-20 month old, ketamine- and vehicle-treated, Tg2576 (TG) and wild-type (WT) mice. n = 30 Tg2576 (15 ketamine- and 15 vehicle-treated) and 30 wild-type (16 ketamine- and 14 vehicle-treated) mice.

Inspection of panel B of Figure 4.14 indicates that mice of all genotypes and treatment groups display discrimination ratios above chance performance (0.33), with no obvious differences between genotypes or treatment groups. ANOVA results were consistent with this impression, revealing no significant effects of genotype ($F_{(1,56)} = 0.833$, $p = .365$, MSE = 0.033, $\eta^2_p = 0.015$) or drug ($F_{(1,56)} = 0.064$, $p = .802$, MSE = 0.003, $\eta^2_p = 0.001$), and no significant genotype × drug interaction ($F_{(1,56)} = 0.153$, $p = .697$, MSE = 0.006, $\eta^2_p = 0.003$). An unpaired t-test comparing discrimination ratios of Tg2576 and wild-type mice, across treatment groups, revealed no significant genotype difference ($t(58) = 0.906$, $p = .369$, $d = 0.234$). One sample t-tests comparing these same overall genotype discrimination ratios against chance performance (0.33) revealed significant differences for both Tg2576 ($t(29) = 3.554$, $p = .001$, $d = 0.649$) and wild-type ($t(29) = 3.371$, $p = .002$, $d = 0.615$) mice. A Bayesian independent samples t-test examining the genotype difference in discrimination ratios lent support to the notion that there was no overall difference between genotypes ($BF_{10} = 0.370$).

However, as this analysis included the non-counterbalanced start arm, a further analysis was conducted which was restricted to the two counterbalanced choice arms, presented in Figure 4.15. Inspection of Figure 4.15 indicates that, as in Figure 4.14, all genotype treatment groups display discrimination ratios numerically above chance performance (0.5), with no obvious differences between genotypes or treatment groups. ANOVA results were consistent with this impression, revealing no significant effects of genotype ($F_{(1,56)} = 0.001$, $p = .971$, MSE = $6.274e^{-5}$, $\eta^2_p < 0.001$) or drug ($F_{(1,56)} = 0.196$, $p = .660$, MSE = 0.009, $\eta^2_p = 0.003$), and no significant genotype × drug interaction ($F_{(1,56)} = 0.157$, $p = .694$, MSE = 0.007, $\eta^2_p = 0.003$). An unpaired t-test comparing discrimination ratios of Tg2576 and wild-type mice, across treatment groups, revealed no significant genotype difference ($t(58) = 0.036$, $p = .972$, $d = 0.009$). One sample t-tests comparing these same overall genotype discrimination ratios against chance performance (0.5) revealed a non-significant difference for Tg2576 mice ($t(29) = 1.466$, $p = .153$, $d = 0.268$) and a significant difference for wild-type mice ($t(29) = 2.084$, $p = .046$, $d = 0.381$). While the Tg2576 mouse discrimination ratio was not significantly different from 0.5, a Bayesian independent samples t-test examining the genotype difference in discrimination ratios suggested there was no meaningful difference between the performance of the two genotypes ($BF_{10} = 0.263$).

T-maze Discrimination Ratios (2 way)
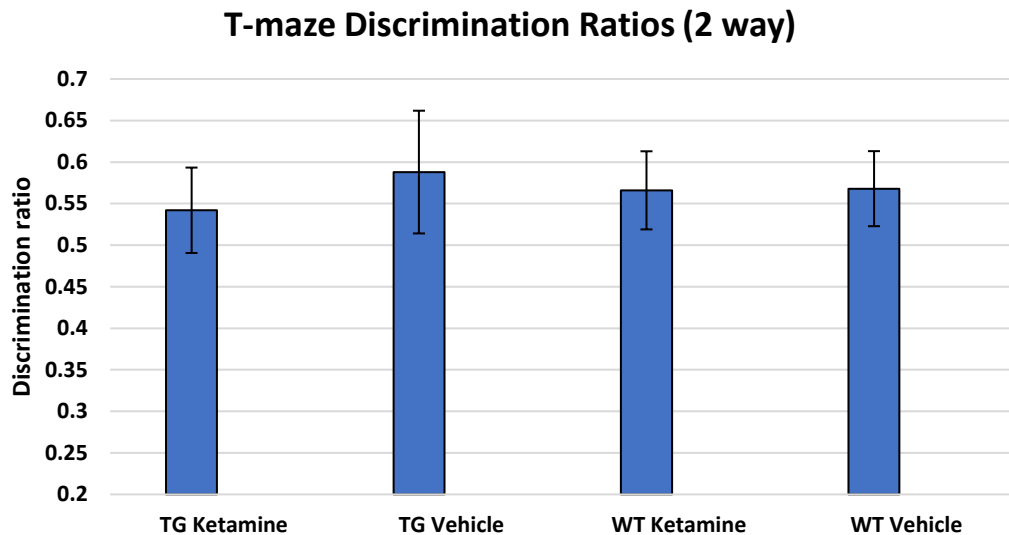
Figure 4.15. Mean discrimination ratios showing preference for the novel arm for 19-20 month old, ketamine- and vehicle-treated, Tg2576 (TG) and wild-type (WT) mice. n = 30 Tg2576 (15 ketamine- and 15 vehicle-treated) and 30 wild-type (16 ketamine- and 14 vehicle-treated) mice.

4.4.4 Summary

Experiment 5 examined the short-term spatial memory of ketamine- and vehicle-treated Tg2576 and wild-type mice at 19-20 months of age, immediately subsequent to Experiment 4. Results revealed that both Tg2576 and wild-type mice spent the greatest amount of time in the novel T-maze arm, and lesser amounts of time in the start and familiar T-arm, with no apparent genotype or drug effects. Although there was a suggestion from the overall ANOVA of a difference in how the two genotypes explored the T-maze arms, this looks to have been driven by differences in exploration of the two previously explored arms, rather than a difference in the tendency to spend more time in the novel arm. When time spent in the novel arm was expressed as a ratio of time spent in all arms, overall Tg2576 and wild-type mice ratios did not significantly differ from one another, and were greater than chance (0.33) performance.

4.5. Discussion

Experiment 3 demonstrated that Tg2576 mice exhibit impaired object-in-place associative memory function at 16-17 months of age. While the age-dependency of this memory deficit was not statistically evident, the cognitive ability of Tg2576 mice was nonetheless comparable to that of wild-type mice in the object-in-place task at 5-6, 8-9 and 12-13 months of age, prior to its decline. Despite the aging analysis not explicitly demonstrating an age-related cognitive deficit in Tg2576

mice, the eventual appearance of a deficit at 16-17 months of age is consistent with a memory impairment which requires a period of aging to manifest. Critically, this Tg2576 memory deficit occurred after both genotypes had received approximately equal amounts of object sampling prior to objects exchanging locations, meaning that the impaired Tg2576 performance could not be attributed to lesser object contact time in the sample phase. Experiment 4 revealed that this deficit in object-in-place associative memory was still present in Tg2576 mice at 19-20 months of age, and was not alleviated by sub-anaesthetic ketamine treatment. Ketamine treatment also failed to alter the performance of wild-type mice in the object-in-place task, whose discrimination ratios remained greater than both those of Tg2576 mice and chance performance, regardless of treatment group. Experiment 5 revealed that, despite an eventually impaired ability to form specific object-location associations, at 19-20 months aged Tg2576 mice remain sensitive to spatial novelty per se.

This study marks the first time performance in the object-in-place task has been longitudinally investigated in Tg2576 mice. Previous studies at single time points have revealed Tg2576 mice to be deficient in this task at 14 months of age (Hale & Good, 2005), 10-12 months of age (Good, Hale, et al., 2007), and at 16 and 21 months of age (Good & Hale, 2007). While the longitudinal Tg2576 cognitive tests shown in Experiment 3 differ somewhat from these individual experiments, in that a deficit was not apparent at 12-13 months of age, they still revealed a deficit at 16-17 months of age. In addition, Experiment 4 confirmed this cognitive deficit was still present at 19-20 months of age. Therefore Experiments 3 and 4 taken together are broadly in keeping with previous reports of a failure of object-in-place associative memory in aged Tg2576 mice. Critically, this memory deficit is unlikely to simply reflect a generalised impairment in object processing or recognition memory more broadly, as aged Tg2576 mice still showed intact object novelty detection (Good & Hale, 2007). While an age-related cognitive impairment in Tg2576 mice was not statistically proven, taken together Experiments 3 and 4 are nonetheless suggestive of an age-related cognitive decline in Tg2576 mice. This cognitive deficit would not appear to be the result of a floor effect due to a low level of object contact; Tg2576 mice displayed similar levels of object contact at both 12-13 and 16-17 months, at which time points a deficit was apparent only at the latter age. In addition, Tg2576 object contact was numerically greater at 19-20 months of age than at 12-13 months of age, with a deficit present at 19-20 months of age, while at 12-13 months of age Tg2576 mice were able to discriminate between familiar and mismatched objects.

It was anticipated that Experiment 3 would reveal the age of emergence of the Tg2576 object-in-place memory deficit. Taking the results of that experiment at face value, it appears that this deficit emerges later than other studies have suggested. The earliest a deficit has been previously detected is 10-12 months of age (Good, Hale, et al., 2007), whereas in the present

experiment memory was not impaired until 16-17 months of age. As the number of mice at each individual time point in Experiment 3 was greater than these previous studies, which typically used 10-11 mice of each genotype, it could be argued that Experiment 3 provides a better estimate of the time point at which a deficit first manifests. However, the repeated testing used in Experiment 3 may have influenced the general exploratory behaviour of the mice, in particular in Tg2576 mice. For example, Figure 4.1 and panels A and B of Figure 4.2 demonstrate that, after the first time point, habituation of both genotypes to objects was markedly reduced, and Tg2576 mice explored objects less in both the sample and test phases, respectively. Both the decreased object exploration of Tg2576 mice after 5-6 months, and the yoking process itself, may have increased 'noise' in the results. Therefore, interpretation of the results of Experiment 3 as regards the precise time of a deficit first appearing should be undertaken with caution. It seems sensible to interpret the results of Experiment 3 as evidence of a cognitive deficit that is absent at 5-6 months of age, and which emerges by 16-17 months of age at the latest.

One implication of Experiment 3 could be that the aging process alters the general or object-related exploratory behaviour of Tg2576 mice, causing them to generally explore objects less with age. This account seems implausible, however, as in one previous report 21 month-old Tg2576 mice showed greater amounts of test phase object contact than a similar cohort at 16 months of age (Good & Hale, 2007). It seems more likely that, when given multiple exposures to objects over a series of object-related tests, mice (in particular Tg2576 mice) will generally explore objects to a lesser extent after the first test; this appears to be borne out in panel B of Figure 4.2, and the associated main effect of age. Given that repeated testing itself may cause low object contact times in Tg2576 mice after the first testing, longitudinally assessing cognition in Tg2576 mice with a test which relies on object exploration should perhaps be approached cautiously in future studies. However, it is worth noting that in Experiment 3, despite there being generally and similarly low Tg2576 object contact at both 12-13 and 16-17 months of age, at the former age Tg2576 mice could distinguish familiar from mismatched objects, while at the latter age they could not. This suggests that, while object contact was reduced in Tg2576 mice, it was still at or above the minimum level sufficient to reveal a deficit.

One final, and interesting, point about the nature of repeated object-in-place testing is that it may in itself influence when a deficit is detected. It has been reported that giving Tg2576 mice 6 days of training in the Morris water maze is sufficient to improve their associative memory as measured by contextual fear conditioning, both 24h and 28 days after initial fear conditioning training, alongside reducing Aβ levels, enhancing hippocampal LTP, causing dendritic remodelling and other changes (Jiang et al., 2015). While the object-in-place test may not be training per se, it is

possible that the added stimulation and test process itself could induce similar types of changes in Tg2576 mice. Such changes could potentially explain why the Tg2576 cognitive deficit in Experiment 3 manifested later than may have been anticipated based on previous reports. An additional and complementary possibility is that the food deprivation regime used in Chapter 3, Experiments 1 and 2, may have interacted with Tg2576 cognitive ability. For example, when an APP knock-in mouse model of Alzheimer's disease was subjected to an intermittent fasting regime, it improved spatial working memory in the Y-maze, spatial learning and memory in a water maze task, and enhanced hippocampal LTP (Liu et al., 2019). There was also a suggestion that this intervention may reduce Aβ accumulation in the hippocampus. While this intermittent fasting involved longer periods of food deprivation than were used in Experiments 1 and 2, nonetheless food deprivation may have also had a beneficial effect on Tg2576 cognition.

In addition to broadly confirming earlier reports of an associative recognition memory deficit in Tg2576 mice, the present results are in keeping with reports from other Alzheimer's disease mouse models. For example, PDAPP mice show an age-dependent deficit in the object-in-place task at 14-16 months of age, at both short and long delays, while object novelty detection remains intact (Evans et al., 2019). Similarly, APPswe/PS1dE9 mice are impaired in the object-in-place task at 5 months of age, while object novelty and relative recency detection are unimpaired (Bonardi, Pardon, & Armstrong, 2016). Taken together, these studies and the results from Tg2576 mice demonstrate that a number of APP overexpressing mouse models exhibit a selective deficit in associative recognition memory.

This selective deficit may be the result of Aβ disrupting the neural circuitry underlying the object-in-place task, for example by causing synaptic loss and impairment in the hippocampus (Li et al., 2009, 2011; Mucke & Selkoe, 2012). APPswe/PS1dE9 mice display deposition of Aβ in the hippocampus from an early age (Garcia-Alloza et al., 2006; Jankowsky et al., 2004), and hippocampal soluble Aβ levels in these mice correlate with spatial and associative memory deficits (Zhang et al., 2011). When APP processing was altered in PDAPP mice by the monoclonal antibody 2B3, soluble Aβ levels were reduced in the hippocampus (along with other biochemical changes), and the PDAPP object-in-place memory deficit was both reversed by acute treatment and prevented by chronic treatment (Evans et al., 2019). These findings in APPswe/PS1dE9 and PDAPP mice are consistent with Aβ ultimately underlying their object-in-place memory deficit. The possibility of this being the case in Tg2576 mice will be examined next.

Given that the hippocampus is a critical structure for object-in-place memory (Barker & Warburton, 2011, 2015; Good, Barnes, Staal, McGregor, & Honey, 2007), the presence of Aβ in the

hippocampus of Tg2576 mice may well account for their deficit in associative recognition memory (Hsiao et al., 1996). Synapse density is decreased in Tg2576 dentate gyrus, a subfield of the hippocampus, in the presence and vicinity of Aβ plaques, suggesting synapse loss may be caused by Aβ (Dong et al., 2007). Dendritic spine density loss in Tg2576 dentate gyrus coincides with LTP impairment at 4 months of age, and is closely followed by a deficit in an associative memory task (Jacobsen et al., 2006). Synaptic loss and disruption in the hippocampus due to Aβ (Li et al., 2009, 2011; Mucke & Selkoe, 2012), over time, could account for the object-in-place memory deficit in Tg2576 mice, by weakening the hippocampal component of the underlying and interdependent circuitry. Further, a specific Aβ assembly has been identified in Tg2576 mice which could be particularly important in impairing cognition (Lesné et al., 2006). This dodecameric Aβ assembly, termed Aβ*56, is present in Tg2576 mouse brain, its level correlates with a spatial memory impairment and, when isolated and administered to rats, it impairs performance on a spatial memory task (Lesné et al., 2006). This Aβ assembly and others may be particularly impactful in impairing cognition in Tg2576 mice, and could underlie their object-in-place memory deficit. This prospect, and other Aβ-related biochemical changes which could underlie this deficit, will be explored in more depth in Chapter 5. Similarly, the lack of an effect of ketamine on both cognitive tests will be discussed in Chapter 5, once the biochemical effects (or lack of effects) of ketamine in the brain have been presented.

Aged Tg2576 mice displaying an intact preference for spatial novelty in the T-maze task is in keeping with object recognition studies in which object-in-place memory is impaired but preference for objects in novel locations is intact (Good & Hale, 2007). However, such a result seems to be contrary to other studies of spatial working memory, using T-maze forced choice alternation or Y-maze continuous alternation, in which Tg2576 mice appear impaired (Cacucci, Yi, Wills, Chapman, & O'Keefe, 2008; Chapman et al., 1999; Deacon et al., 2008; Hale & Good, 2005; Hsiao et al., 1996; Wilcock et al., 2004), though these deficits have not been consistently replicated (Stewart, Cacucci, & Lever, 2011). However, this apparent discrepancy may be due to the relative complexities of the different tasks. The one-trial spatial novelty task used in Experiment 5 was a simple and absolute novel versus familiar task, whereas spontaneous alternation and forced choice alternation both rely upon relative novelty judgements, and forced choice alternation also requires rule-learning. The demands of these latter tasks may require a more sophisticated recollective ability which the hippocampus is unable to support in Tg2576 mice, while cruder environmental representations which allow absolute novel versus familiar judgements can still be created.

In sum, the results of this chapter are consistent with Aβ accumulation disrupting the ability of Tg2576 mice to form or recall stable and specific object-location associations, something which

will be explored further when some related receptors are examined in Chapter 5. It does not appear, however, that the build-up of Aβ over time results in a global impairment in Tg2576 mouse memory, suggesting that some degree of hippocampal functionality, or ability to distinguish between absolute spatial novelty and familiarity, remains intact.

Chapter 5: Biochemical changes in Tg2576 mice

5.1. Introduction

Chapter 1 briefly outlined some of the broad neurotransmitter systems of interest to this thesis, namely the glutamatergic, serotonergic and opioidergic systems; the former primarily in relation to cognition, and the latter two in relation to depression. These systems were chosen because they may underlie or contribute to the hedonic and cognitive deficits displayed in Chapters 3 and 4, as well as potentially reveal information about the usefulness of ketamine in alleviating these deficits.

Glutamate, an excitatory amino acid, is the major excitatory neurotransmitter in the mammalian brain (Niciu, Kelmendi, & Sanacora, 2012), and is known to play a critical role in learning and memory (McEntee & Crook, 1993). In particular, ionotropic glutamate receptors, notably NMDA and AMPA receptors, facilitate memory encoding and retrieval (Bast, da Silva, & Morris, 2005; Lopez, Gamache, Schneider, & Nader, 2015), and underlie long term potentiation (LTP), a cellular model for learning and memory (Bliss, Collingridge, Morris, & Reymann, 2018; Nicoll, 2017). The NMDA receptor (NMDAR) in particular has long been recognised as integral to learning and memory (Leuner, Falduto, & Shors, 2003; Morris, 1989; Morris, Anderson, Lynch, & Baudry, 1986; Steele & Morris, 1999), and this is thought to be due to certain properties which characterise the receptor, described below. In addition to their importance to mnemonic processes, NMDARs also mediate excitotoxicity when overstimulated by glutamate (Hardingham & Bading, 2003).

NMDARs are expressed widely throughout the mammalian brain (Moriyoshi et al., 1991; Young & Fagg, 1990), and are populous in structures essential for learning and memory such as the hippocampus (Greenamyre, Olson, Penney, & Young, 1985; Monaghan, Holets, Toy, & Cotman, 1983). While postsynaptic NMDARs are inactive under baseline conditions due to magnesium (Mg^{2+}) blockade, postsynaptic membrane depolarisation alleviates this blockade, allowing presynaptically released glutamate to bind to the NMDAR (Traynelis et al., 2010). Glutamate binding opens the ion channel, allowing cations (notably calcium) to enter the postsynaptic bouton (Lee et al., 2014). Calcium influx then leads to a series of cellular events, including activation of calcium/calmodulin-dependent protein kinase II (CaMKII) and a resultant increase in AMPARs at the synapse (Herring & Nicoll, 2016). This NMDAR-dependent process allows for a form of synaptic plasticity, which may

enable learning and memory, to occur. The voltage-dependent nature of the Mg^{2+} blockade allows the NMDAR to act as a coincidence detector, only allowing Ca^{2+} entry when postsynaptic depolarisation coincides with presynaptic glutamate release and NMDAR binding (Traynelis et al., 2010). This quality of the NMDAR marks it as a good candidate for the mechanistic explanation of LTP (Bliss & Collingridge, 1993).

NMDARs are structurally heterogeneous; their composition can entail a number of subunits, of which seven have been identified, categorised into three subfamilies (NR1, NR2A-D, and NR3A/B) (Paoletti, Bellone, & Zhou, 2013). This subunit variety allows for distinct heterotetrameric combinations, in which NR1 subunits largely associate with NR2 subunits, with different NMDAR subtypes allowing the fulfilment of different functions (Paoletti et al., 2013). For example, in the induction and expression of LTP, di-heteromeric NR1/NR2A receptors may have a higher channel open probability for allowing Ca^{2+} influx, while the presence of the NR2B subunit on tri-heteromeric NR1/NR2A/NR2B receptors may be responsible for CaMKII recruitment and LTP expression (Paoletti et al., 2013). These subunits undergo post-translational modifications, such as phosphorylation by various kinases, which alter NMDAR trafficking and distribution (Wang et al., 2014).

Given that NMDARs play a critical role in cognitive processes, the interaction of Aβ with the glutamate system broadly, and NMDARs specifically, is highly consequential. Aβ appears to increase glutamate availability, both by enhancing its release from astrocytes (Talantova et al., 2013), and by impairing neuronal glutamate uptake (Li et al., 2009), though decreases in glutamate have also been reported (Danysz & Parsons, 2012). In addition to altering glutamate availability, Aβ oligomers induce toxic effects in cell culture and entorhinal-hippocampal organotypic slice experiments, via NMDAR activation (Alberdi et al., 2010; Texidó, Martín-Satué, Alberdi, Solsona, & Matute, 2011). Here the NMDAR subunit specificity is important, as the NR2B subunit appears to mediate excitotoxicity (Liu et al., 2007), and longer hippocampal slice exposure to Aβ oligomers results in a reduction of NR2B presence at the synapse (Li et al., 2011). These findings are in keeping with the notion that the chronic presence and accumulation of Aβ, as occurs in Alzheimer's disease, could result in an NMDAR-induced slow excitotoxicity (Danysz & Parsons, 2012; Ong, Tanaka, Dawe, Ittner, & Farooqui, 2013). Such a process could reasonably be expected to result in compensatory NMDAR changes. This tonic, rather than phasic, NMDAR over-activation due to Aβ could underlie early memory impairment, and explains why the NMDAR antagonist memantine is a useful therapeutic agent, as it may block tonic Aβ-induced NMDAR activation while still allowing non-pathological synaptic activation to occur (Danysz & Parsons, 2012). Given that NR1 subunit endocytosis has been observed in cultured Tg2576 cortical neurons, and that Aβ exposure can provoke NR2B endocytosis and dephosphorylation (Snyder et al., 2005), chronic Aβ accumulation in Tg2576 mice may be

expected to result in changes to the NR1 and NR2B subunits. These NMDAR subunits therefore merit further investigation in this thesis. Further, given that the NR1 and NR2B subunits are implicated in rodent short-term memory tasks with a spatial component (Evans et al., 2019; Niewoehner et al., 2007), NR2B specifically in the object-in-place task (Evans et al., 2019), these subunits may plausibly underlie the cognitive deficit documented in Chapter 4.

AMPA receptors (AMPARs) are a critical component of NMDAR-dependent synaptic plasticity (Huganir & Nicoll, 2013; Nicoll, 2017), and as such are highly salient in processes facilitating learning and memory. As previously described, the trafficking and synaptic insertion of AMPARs is one of the features of LTP (Shepherd & Huganir, 2007). As with NMDARs, the subunit composition of AMPARs varies, with physiological consequences. AMPARs comprise four homologous subunits (GluR1-4, or GluRA-D), which form tetrameric complexes, of which the major forms in the hippocampus include GluR1/2 and GluR2/3 heteromers and GluR1 homomers (Huganir & Nicoll, 2013; Malinow & Malenka, 2002). AMPAR function and synaptic plasticity are regulated by subunit phosphorylation, with serine, threonine and tyrosine residues being phosphorylated by a range of protein kinases, including CaMKII, protein kinase A (PKA) and protein kinase C (PKC) (Lu & Roche, 2012; Shepherd & Huganir, 2007). The GluR1 subunit appears to be particularly important in synaptic plasticity; GluR1 presence appears to be necessary for activity-dependent delivery of AMPARs to synapses (Hayashi et al., 2000; Shi, Hayashi, Esteban, & Malinow, 2001), with phosphorylation at serine831 and serine845 by CaMKII and PKA, respectively, being important events (Esteban et al., 2003). Consistent with these findings, a number of studies have revealed that GluR1 deletion in mice results in impaired spatial working memory (Reisel et al., 2002; Schmitt, Deacon, Seeburg, Rawlins, & Bannerman, 2003; Schmitt et al., 2005). In addition to interacting with NMDARs, Aβ also influences AMPAR trafficking and functionality. For example, Aβ depresses synapses via removing AMPARs from the synapse by endocytosis (Hsieh et al., 2006). In addition, hippocampal synaptic AMPAR content is decreased with age in a double knock-in Alzheimer's disease mouse model (Chang et al., 2006), consistent with Aβ having a role in disrupting AMPAR function. A decrease in synaptically available GluR1 is seen in Tg2576 primary neurons and mouse hippocampus (Almeida et al., 2005; Cavallucci et al., 2013; D'Amelio et al., 2011), and dephosphorylation of GluR1 at the serine845 residue is also seen in Tg2576 mouse hippocampus (Cavallucci et al., 2013; D'Amelio et al., 2011). Moreover, there is evidence that NMDAR function may also be compromised in Tg2576 mice (Snyder et al., 2005), and that both NMDAR and AMPAR transmission are needed for object-in-place task performance (Barker & Warburton, 2015). Thus there is a basis for investigating both NMDAR and AMPAR expression in Tg2576 and wild-type mice

in relation to the cognitive deficit described in Chapter 4. In particular, the NR1, NR2B and GluR1 subunits merit examination, especially in the hippocampus.

In addition to NMDARs and AMPARs themselves, scaffolding proteins such as postsynaptic density protein-95 (PSD-95) may play a role in any NMDAR and AMPAR changes in Tg2576 mice. PSD-95 belongs to a group of proteins known as membrane-associated guanylate kinases (MAGUKS), and stabilises NMDAR and AMPAR presence at the synaptic membrane surface (MacGillavry, Song, Raghavachari, & Blanpied, 2013; Nair et al., 2013; Roche et al., 2001). Soluble Aβ can reduce PSD-95 levels (Liu et al., 2010), and PSD-95 reductions are seen in Tg2576 cultured neurons and cortical tissue (Almeida et al., 2005; Oulès et al., 2012). Thus cognitive deficits in Tg2576 mice could be caused by synaptic NMDAR and/or AMPAR unavailability owing to a reduced anchoring or stabilisation by PSD-95, and this scaffolding protein should also be investigated.

Neurotransmitter systems that may mediate the depressive phenotype characterised in Chapter 3 also require investigating. To this end, Chapter 5 will also examine the serotonergic and opioidergic systems. Chapter 1 observed that Alzheimer's disease includes a degradation of the serotonergic system (Garcia-Alloza et al., 2005; Halliday et al., 1992; Palmer et al., 1987; Yamamoto & Hirano, 1985). However, these results were discovered in post-mortem cases, meaning that they were revealed following years of pathology which included neurodegeneration as well as effects of Aβ and tau. Preclinical studies suggest that there may be a relationship between serotonin and Aβ, and as a result serotonergic changes could potentially occur in early Alzheimer's disease and relate to depression. For example, acute application of both SSRIs and serotonin to the hippocampus of APP/PS1 mice reduced the Aβ content in the interstitial fluid, and chronic SSRI administration reduced cortical and hippocampal plaque load and CSF Aβ40 and Aβ42 (Cirrito et al., 2011). This demonstrates a relationship in one direction between serotonin and Aβ. Evidence that Aβ itself may disturb various aspects of serotonin signalling is seen in studies of transgenic Alzheimer's disease model mice; a reduced number of serotonin transporter (5HTT or SERT) binding sites and SERT mRNA-positive cells are seen in the dorsal raphe of APP/PS1 mice (Metaxas et al., 2018). A follow-up study revealed a reduced SERT density in frontal and parietal cortex of APP/PS1 mice, diminished SERT activity in APP/PS1 mouse neocortex, and that synthetic soluble Aβ40 can inhibit SERT activity (Metaxas et al., 2019). In addition, a reduction in both SERT and the 5HT$_{1B}$ receptor has been observed in Tg2576 mouse hippocampus (Tajeddinn et al., 2015). This latter finding is especially intriguing as the 5HT$_{1B}$ receptor may be an important component of ketamine's mechanism of action as an antidepressant (Yamanaka et al., 2014).

While depression is almost certainly not reducible to disturbed serotonergic signalling alone, alterations in certain serotonin receptors and SERT do occur in depression (Gryglewski, Lanzenberger, Kranz, & Cumming, 2014; Stockmeier, 2003), and reducing serotonin availability in recovered depressed patients provokes a return of depressive symptoms (Cowen, 2008). Whether serotonergic signalling relates specifically to anhedonia in depression is unclear, as patients taking antidepressants, including SSRIs, have described feeling a lack of positive emotions and reduced enjoyment of hobbies and interests which is consistent with anhedonia (Goodwin et al., 2017; Price et al., 2009). This could represent either an unfortunate side effect or consequence of SSRIs, or a sign that SSRIs have little or limited impact on anhedonia in depression. Some limited evidence from chronically stressed mice has shown that hippocampal SERT expression is lower in anhedonic mice than in non-anhedonic and control mice, and that fluoxetine treatment restores both hedonic behaviour and SERT expression in treatment-responsive anhedonic mice (Tang, Lei, Sun, Liu, & Zhao, 2013). This does not, however, directly prove a connection between SERT and anhedonia. Given that SERT could possibly relate to anhedonia and appears reduced in Tg2576 mice in a prior study (Tajeddinn et al., 2015), it would seem prudent to investigate its abundance in Tg2576 mice which have consistently displayed a hedonic deficit. In addition, as ketamine did not improve hedonic responsiveness in these same Tg2576 mice (Chapter 3, Experiment 2), and part of ketamine's mechanism of action may involve the $5HT_{1B}$ receptor (which may be reduced in Tg2576 mice), the $5HT_{1B}$ receptor should be investigated as a potential explanation of this null result.

A further serotonin receptor which may relate Aβ to both depression and cognition is the $5HT_4$ receptor. Individuals with a family history of major depressive disorder display lower striatal $5HT_4$ receptor binding, and lower $5HT_4$ binding was associated with greater number of depressed relatives in both the striatum and limbic region in these individuals (Madsen et al., 2014). In addition, the Flinders Sensitive Line rat model of depression shows reduced $5HT_4$ receptor binding in the hippocampus and lateral globus pallidus (Licht et al., 2009). Taken together, these studies suggest that the $5HT_4$ receptor may be involved in the risk of developing depression, as well as potentially being a component of the condition itself. The fact that $5HT_4$ receptor knockout mice show a reduced sucrose intake (Amigó et al., 2016), and that a $5HT_4$ receptor agonist improves sucrose intake in treatment-responsive chronically stressed rats (Lucas et al., 2007), provides an additional suggestion that this receptor may contribute to anhedonia specifically. It is notable in this context that $5HT_4$ receptors are highly expressed in both human and rodent brain in reward-related sites such as the nucleus accumbens, as well as in the hippocampus (Varnäs, Halldin, Pike, & Hall, 2003; Waeber, Sebben, Nieoullon, Bockaert, & Dumuis, 1994). In addition, stimulating $5HT_4$ receptors improves performance in hippocampal-dependent learning and memory tasks in rodents

(Hagena & Manahan-Vaughan, 2017), and post-mortem investigation reveals that Alzheimer's disease brains display a loss of $5HT_4$ receptors in regions including the hippocampus (Reynolds et al., 1995). Interestingly, among newly-diagnosed living Alzheimer's disease patients, those with a detectable insoluble Aβ burden show an increase in brain $5HT_4$ receptor levels (Madsen et al., 2011). Also, chronic activation of $5HT_4$ receptors in young APP/PS1 mice lowers soluble and insoluble hippocampal Aβ40 and Aβ42, as well as hippocampal plaque count (Tesseur et al., 2013). Thus the $5HT_4$ receptor elevation seen in the aforementioned Alzheimer's disease patients could reflect a compensatory response to both the presence of Aβ, and also symptoms such as memory loss and potentially depression. Due to its links to the Aβ peptide, Alzheimer's disease, cognition and potentially anhedonia, the $5HT_4$ receptor may represent a point of convergence for the major interests of this thesis, and as such strongly warrants investigation in Tg2576 mice.

The final neurotransmitter system that this chapter aims to explore in Tg2576 mice is the opioidergic system. Opioid receptors and their endogenous ligands are expressed widely throughout the mammalian brain; there are three opioid receptors, known as mu, kappa and delta, and a number of endogenous peptides that bind to these receptors, namely β-endorphin, dynorphins and enkephalins, respectively (Le Merrer, Becker, Befort, & Kieffer, 2009; Pasternak & Pan, 2013). Important sites of opioid receptor expression include components of the reward network such as the nucleus accumbens, ventral pallidum, orbitofrontal cortex and parabrachial nucleus, as well as other structures such as the hippocampus (Castro & Berridge, 2014, 2017; Le Merrer et al., 2009). Opioidergic signalling is involved in various physiological events, including nociception (pain), substance addiction, and, importantly, sensations of pleasure and reward (Berridge & Kringelbach, 2015; Le Merrer et al., 2009). In the main, mu opioid receptor agonism is thought to induce or enhance a positive affective state (Kelley et al., 2002; Peciña & Smith, 2010), while kappa opioid receptor agonism mediates dysphoric, depressive and potentially anhedonic states (Lalanne, Ayranci, Kieffer, & Lutz, 2014; Taylor & Manzella, 2016). In addition, delta opioid receptor agonism is also thought to have an antidepressant effect in rodents (Pradhan, Befort, Nozaki, Gavériaux-Ruff, & Kieffer, 2011). Broadly speaking then, mu and delta receptor signalling can be thought to work in opposition to kappa receptor signalling. One caveat to this broad view of the opioid system is that in minute subregions within many of the aforementioned reward sites, all three opioid receptors can amplify or suppress hedonic reactions in rodents when activated (Berridge & Kringelbach, 2015; Castro & Berridge, 2014, 2017; Peciña et al., 2006). Perturbed opioidergic signalling could thus in principle underlie a hedonic deficit such as that present in Tg2576 mice in Chapter 3. Here it is noteworthy that reward sites such as the nucleus accumbens, orbitofrontal cortex and parabrachial nucleus display Aβ plaques and NFTs in post mortem studies of Alzheimer's disease brain tissue

(German et al., 1987; Parvizi et al., 1998; Suenaga et al., 1990; Van Hoesen et al., 2000). In addition, post mortem studies also reveal changes in opioid receptor availability in Alzheimer's disease brain. While a non-specific ligand revealed a decrease in the overall availability of opioid receptors in certain parts of the hippocampal formation (Jansen, Faull, Dragunow, & Synek, 1990), in general there is an increase in kappa opioid receptor binding in several brain regions, while mu and delta availability is generally reduced or unchanged (Barg et al., 1993; Hiller, Itzhak, & Simon, 1987; Mathieu-Kia, Fan, Kreek, Simon, & Hiller, 2001) (though see Ikeda, Mackay, Dewar, & McCulloch, 1993). The fact that Alzheimer's disease pathology accumulates in reward regions, and that even advanced pathology seems to generally include an increase in kappa receptor availability and possibly changes in mu and delta receptor expression, raises the possibility that opioid receptors in reward sites may be altered in Alzheimer's disease. If such changes occurred early in Alzheimer's disease, i.e. initially as a function of Aβ accumulation, then they may be seen in Tg2576 mice, and could underlie or contribute to the hedonic deficit observed in Chapter 3. Chapter 5 will therefore examine all three opioid receptors in Tg2576 and wild-type mice, in order to obtain a general overview of how the opioidergic system may be functioning in mice with typical and diminished hedonic responsiveness.

5.2. Experiment 6

5.2.1 Subjects, apparatus, procedure & data analysis

Experiment 6 investigates the effect of sub-anaesthetic ketamine treatment on soluble and insoluble Aβ levels in the hippocampus and cortex of the Tg2576 mice used in Experiments 2, 4 and 5. As the principal Aβ species which accumulates in Tg2576 mice, and which are absent in wild-type mice, are human Aβ, and the ELISAs used are specific to human Aβ, only Tg2576 mouse brains were examined. Apparatus, procedure and data collection are as described in Sections 2.4 and 2.5. Due to low sample volume following the extraction procedure, only 11 ketamine- and 11 vehicle-treated Tg2576 hippocampal samples underwent ELISA quantification. All 16 ketamine- and 15 vehicle-treated Tg2576 cortex samples were analysed by ELISA. Soluble and insoluble Aβ40 and Aβ42 levels were statistically analysed using Student's t-tests, with drug treatment as the dependent variable.

5.2.2 Results

5.2.2.1 Hippocampus Aβ levels

Inspection of Figure 5.1 indicates that for both soluble (panel A) and insoluble (panel B) hippocampal Aβ40, there was no appreciable effect of ketamine treatment in Tg2576 mice. Student's t-tests revealed no significant differences in soluble and insoluble Aβ40 between ketamine- and vehicle-treated Tg2576 mice ($t(20) = 0.018$, $p = .986$, $d = 0.008$) and ($t(20) = 0.368$, $p = .717$, $d = 0.157$), respectively. Bayesian independent samples t-tests were suggestive of a genuine absence of effect of ketamine on either soluble or insoluble Aβ40 ($BF_{10} = 0.385$ and 0.404, respectively).

Figure 5.1. Mean hippocampal soluble (A) and insoluble (B) human Aβ40 concentrations (pg/mg) for ketamine- (Tg Ket) and vehicle-treated (Tg Veh) 19-20 month old Tg2576 mice. n = 11 ketamine- and 11 vehicle-treated Tg2576 mice.

Inspection of panel A of Figure 5.2 indicates that ketamine-treated Tg2576 mice display a markedly lower hippocampal soluble Aβ42 concentration than vehicle-treated Tg2576 mice, which a Student's t-test revealed to be statistically significant (t(20) = 2.190, p = .041, *d* = 0.934). In contrast, inspection of panel B of Figure 5.2 indicates that ketamine treatment had no meaningful effect on hippocampal insoluble Aβ42 in Tg2576 mice. A Student's t-test revealed the difference in insoluble Aβ42 between treatment groups to be non-significant (t(20) = 0.465, p = .647, *d* = 0.198). A Bayesian independent samples t-test was consistent with, though not conclusive evidence of, a genuinely null ketamine effect on insoluble Aβ42 (BF_{10} = 0.416).
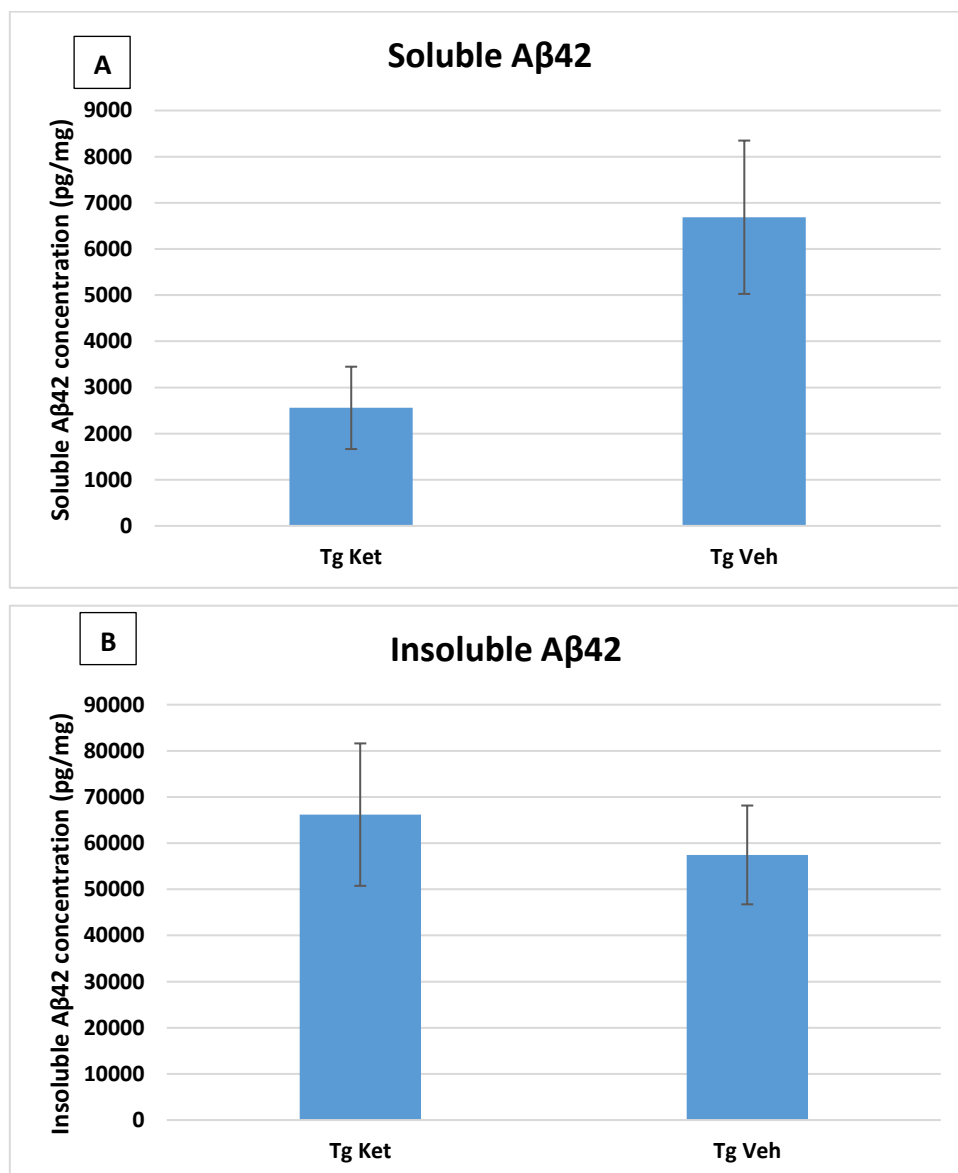
Figure 5.2. Mean hippocampal soluble (A) and insoluble (B) human Aβ42 concentrations (pg/mg) for ketamine- (Tg Ket) and vehicle-treated (Tg Veh) 19-20 month old Tg2576 mice. n = 11 ketamine- and 11 vehicle-treated Tg2576 mice.

5.2.2.2 Cortex Aβ levels

Inspection of Figure 5.3 indicates that ketamine treatment had no notable effect on soluble (panel A) and insoluble (panel B) cortical Aβ40 concentrations in Tg2576 mice. Student's t-tests revealed that for neither Aβ aggregation state was there a significant difference between ketamine- and vehicle-treated Tg2576 mice; for soluble Aβ40 ($t(29) = 0.267$, $p = .791$, $d = 0.096$) and for insoluble Aβ40 ($t(29) = 0.934$, $p = .358$, $d = 0.336$). Bayesian independent samples t-tests were suggestive of no effect of ketamine on soluble Aβ40 ($BF_{10} = 0.350$), though less conclusive regarding the effect of ketamine on insoluble Aβ40 ($BF_{10} = 0.474$), in Tg2576 cortex.
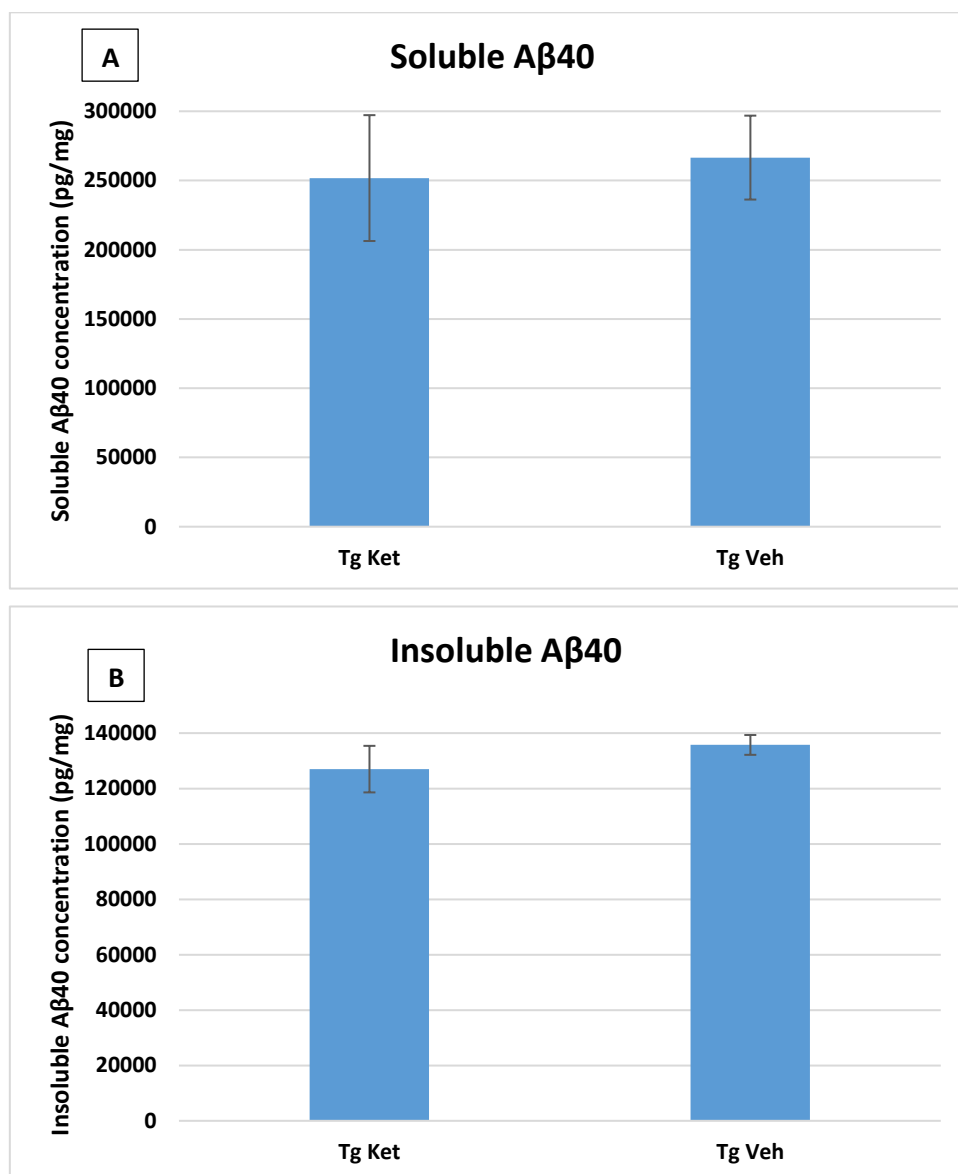
Figure 5.3. Mean cortical soluble (A) and insoluble (B) human Aβ40 concentrations (pg/mg) for ketamine- (Tg Ket) and vehicle-treated (Tg Veh) 19-20 month old Tg2576 mice. n = 16 ketamine- and 15 vehicle-treated Tg2576 mice.

Inspection of panel A of Figure 5.4 indicates that ketamine-treated Tg2576 mice display markedly lower soluble cortical Aβ42 concentrations than vehicle-treated Tg2576 mice. Panel B of Figure 5.3 indicated that cortical insoluble Aβ42, however, was not meaningfully altered by ketamine treatment in Tg2576 mice. A Student's t-test revealed the soluble Aβ42 difference between treatment groups to be significant ($t(29) = 2.597$, $p = .015$, $d = 0.933$), while there was no significant treatment group difference for insoluble Aβ42 concentrations ($t(29) = 0.373$, $p = .712$, $d = 0.134$). A Bayesian independent samples t-tests was generally consistent with the absence of a ketamine effect on insoluble Aβ42 ($BF_{10} = 0.359$).
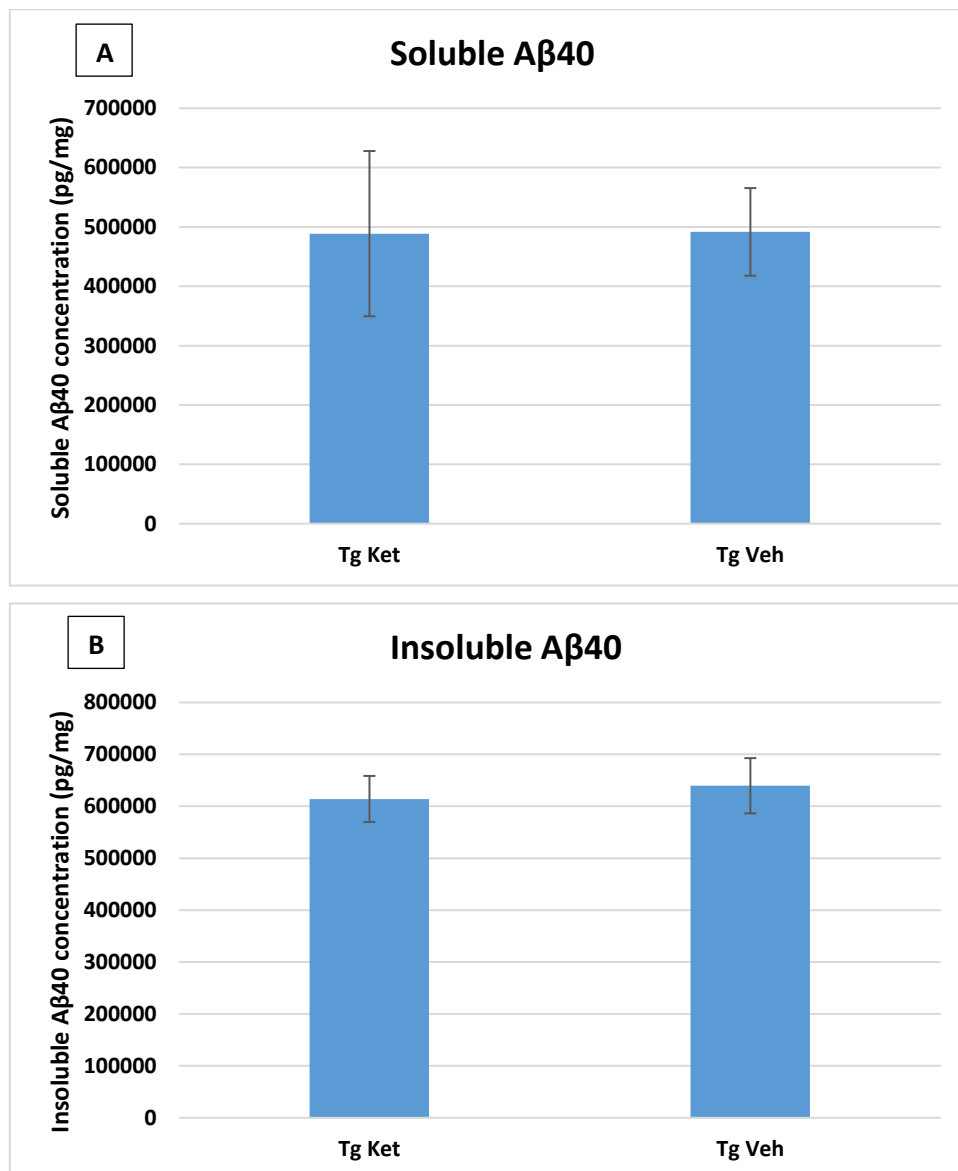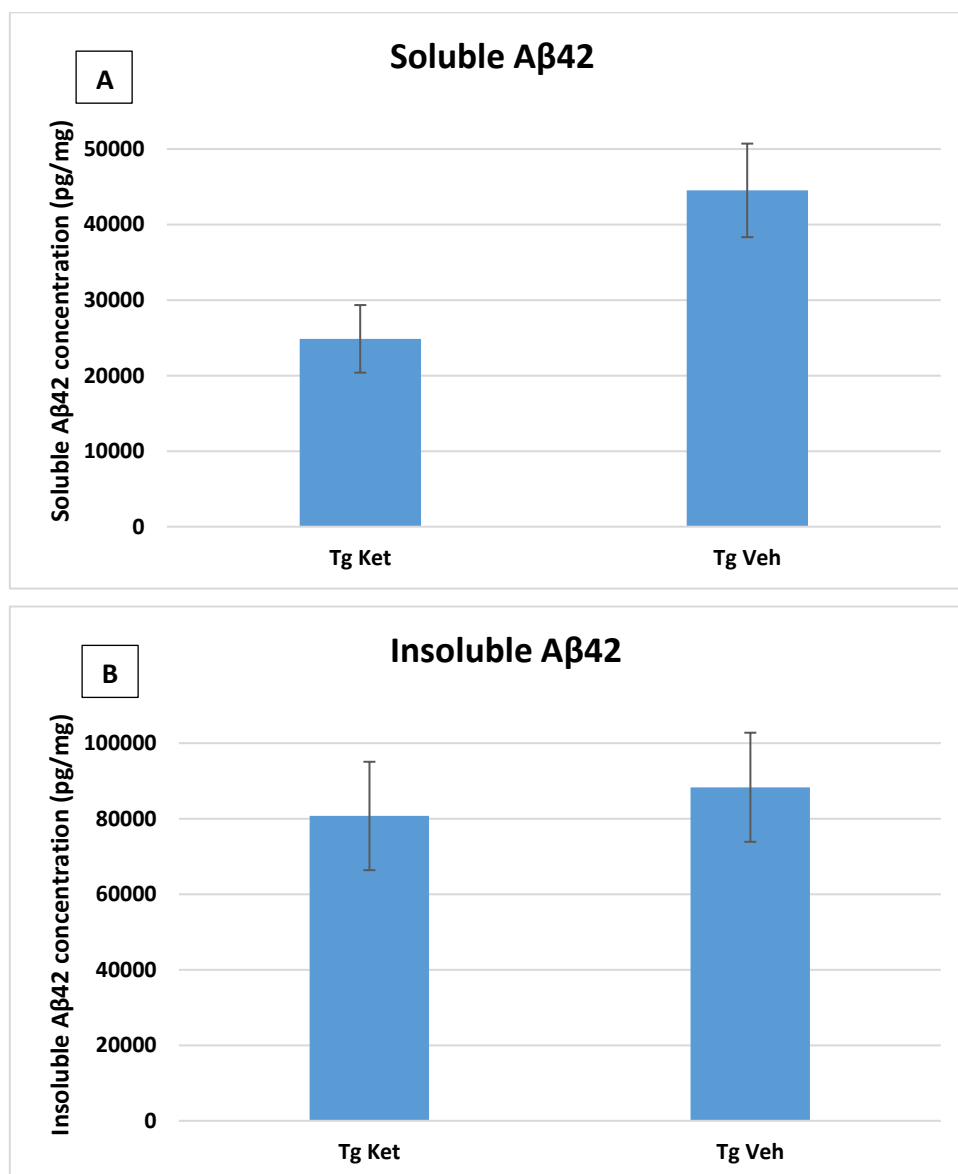

Figure 5.4. Mean cortical soluble (A) and insoluble (B) human Aβ42 concentrations (pg/mg) for ketamine- (Tg Ket) and vehicle-treated (Tg Veh) 19-20 month old Tg2576 mice. n = 16 ketamine- and 15 vehicle-treated Tg2576 mice.

5.2.3 Summary

Experiment 6 revealed that sub-anaesthetic ketamine treatment significantly reduced soluble, but not insoluble, Aβ42 levels in both the hippocampus and cortex of Tg2576 mice. However, ketamine treatment had no effect on either soluble or insoluble Aβ40 concentrations in Tg2576 mice, in both the hippocampus and cortex. Bayesian analyses generally supported the view that the non-reductions of Aβ in ketamine-treated Tg2576 mice were genuine null effects of ketamine.

5.3 Experiment 7

5.3.1 Subjects, apparatus, procedure & data analysis

Experiment 7 investigates the effects of both genotype and ketamine treatment on a number of glutamate receptor subunits in synaptically enriched hippocampal and cortical extracts, as well as a post-synaptic scaffolding protein involved in glutamatergic neurotransmission and synaptic plasticity. The precise numbers of mice from each genotype and treatment group analysed by Western blot were dependent upon sample volume and prior usage at the time of probing for a particular protein; these numbers are reported with each figure. Apparatus, procedure and data collection are as described in Sections 2.4 and 2.6.

5.3.2 Results

5.3.2.1 Hippocampal glutamatergic signalling proteins

Inspection of panel A of Figure 5.5 indicates that hippocampal expression of the NMDA receptor NR1 subunit was not meaningfully altered by either genotype or ketamine treatment. ANOVA results were consistent with this impression, revealing non-significant effects of genotype ($F(1,45) = 0.107$, $p = .745$, MSE = 0.101, $\eta^2_p = 0.002$) and drug ($F(1,45) = 0.924$, $p = .342$, MSE = 0.872, $\eta^2_p = 0.020$), and a non-significant genotype × drug interaction ($F(1,45) = 0.802$, $p = .375$, MSE = 0.757, $\eta^2_p = 0.018$). Bayesian ANOVA results suggested the absence of a genotype effect ($BF_{10} = 0.292$), but were less conclusive regarding a null effect of ketamine ($BF_{10} = 0.404$), and absence of a genotype × drug interaction ($BF_{10} = 0.492$). Panel B of Figure 5.5 displays the same pattern of no apparent genotype- or ketamine-induced alterations in NMDA receptor NR2B subunit expression. ANOVA results were again consistent with this impression, revealing non-significant effects of genotype ($F(1,45) = 1.012$, $p = .320$, MSE = 0.580, $\eta^2_p = 0.022$) and drug ($F(1,45) = 1.759$, $p = .191$, MSE = 1.007, $\eta^2_p = 0.038$), and a non-significant genotype × drug interaction ($F(1,45) = 1.515$, $p =$

.225, MSE = 0.868, η^2_p= 0.033). Bayesian ANOVA results were again suggestive of an absent genotype effect (BF_{10} = 0.394), while being less clear regarding a null ketamine effect (BF_{10} = 0.499), and absence of a genotype × drug interaction (BF_{10} = 0.629). Similarly, Panel C of Figure 5.5 is indicative of no notable genotype or treatment group differences in expression of the NR2B subunit phosphorylated at tyrosine 1472. ANOVA results were again consistent with this impression, revealing non-significant effects of genotype ($F(1,45)$ = 0.083, p = .775, MSE = 0.017, η^2_p= 0.002) and drug ($F(1,45)$ = 1.452, p = .235, MSE = 0.290, η^2_p= 0.031), and a non-significant genotype × drug interaction ($F(1,45)$ = 1.399, p = .243, MSE = 0.279, η^2_p= 0.030). Bayesian ANOVA results were suggestive of the absence of a genotype effect (BF_{10} = 0.289), but were less informative regarding a null effect of drug (BF_{10} = 0.497), or the absence of a genotype × drug interaction (BF_{10} = 0.637). However, panel D of Figure 5.5 indicates that, across treatment groups, Tg2576 mice display a lower ratio of p-Y1472:total NR2B levels than their wild-type counterparts, with no appreciable impact of ketamine treatment. ANOVA results were consistent with this impression, revealing a significant effect of genotype ($F(1,45)$ = 5.563, p = .023, MSE = 0.514, η^2_p= 0.110), a non-significant effect of drug ($F(1,45)$ = 1.055, p = .310, MSE = 0.097, η^2_p= 0.023), and a non-significant genotype × drug interaction ($F(1,45)$ = 0.253, p = .618, MSE = 0.023, η^2_p= 0.006). Bayesian ANOVA results were consistent with the absence of a drug effect (BF_{10} = 0.342), though less informative regarding the absence of a genotype × drug interaction (BF_{10} = 0.405).
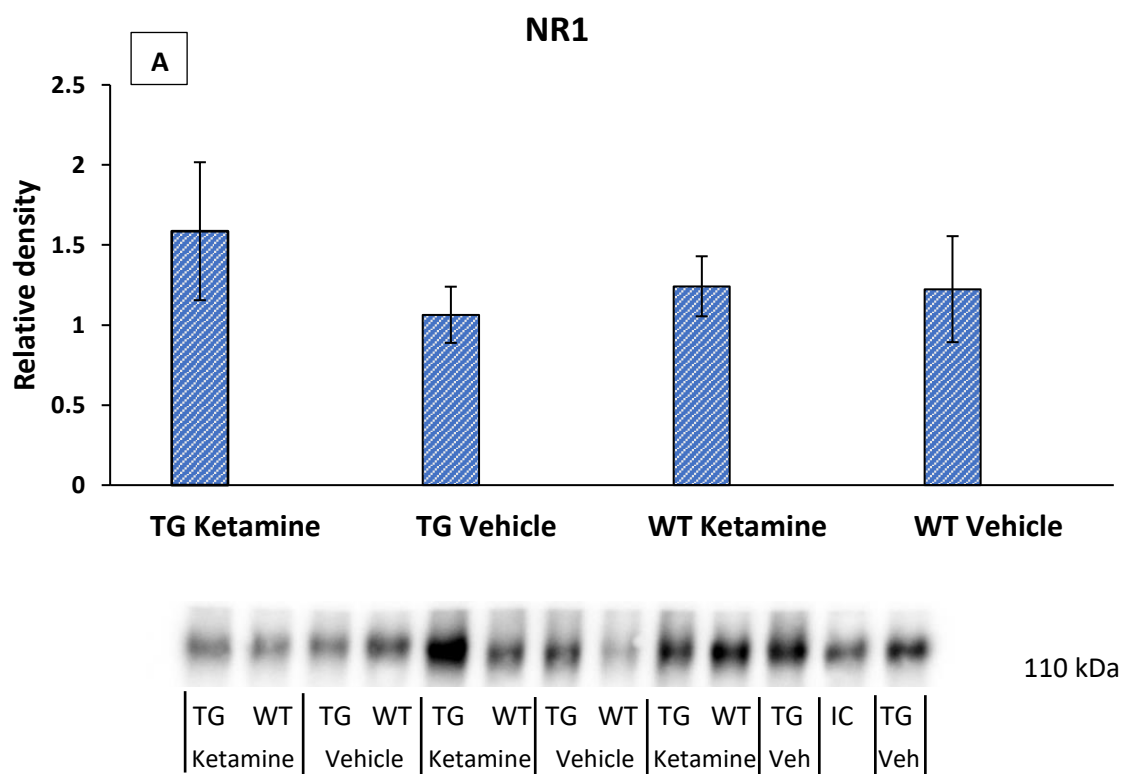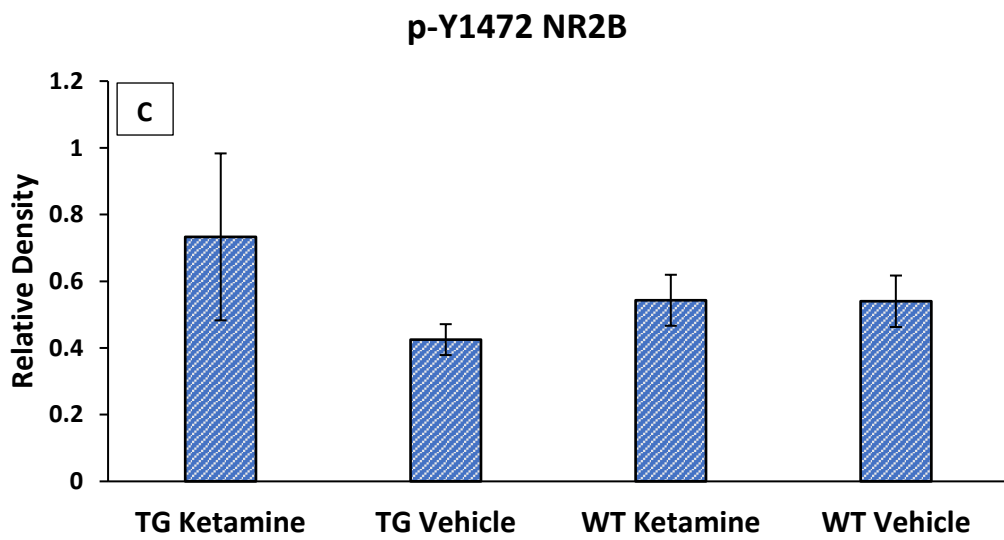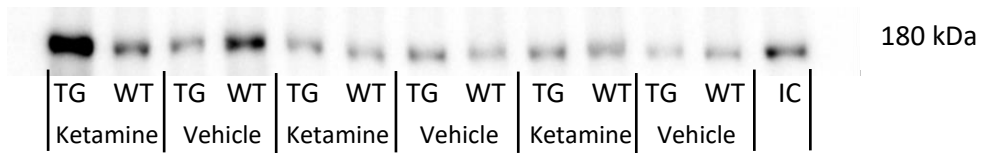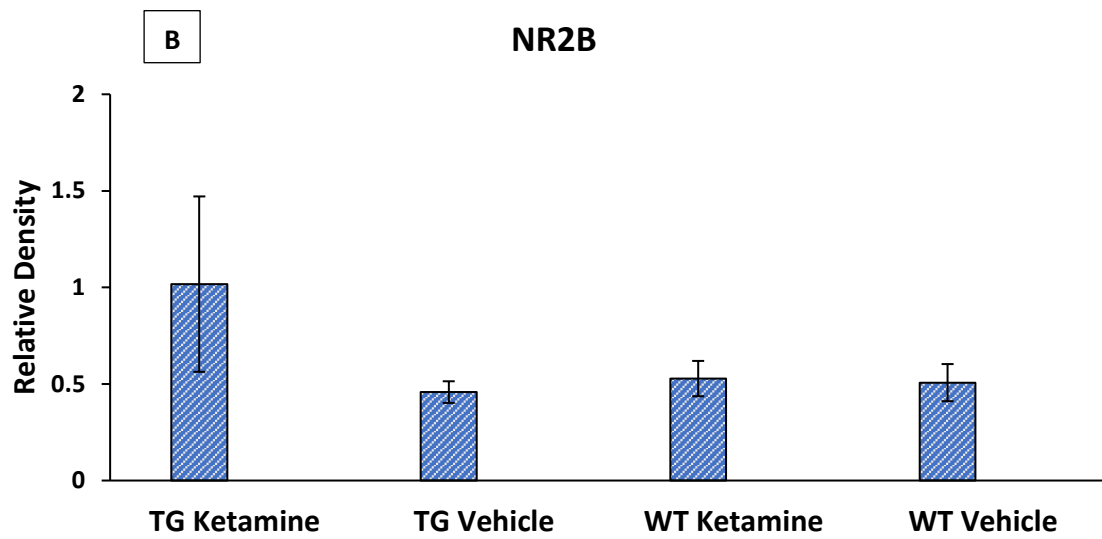
NR2B

p-Y1472 NR2B

Figure 5.5. Mean relative densities and representative Western blot images of NMDA receptor subunits NR1 (A), NR2B (B), pY1472 NR2B (C), and the ratio of pY1472:total NR2B (D) in hippocampal synaptosomal extracts from 19-20 month old Tg2576 and wild-type mice. n = 11 ketamine- and 13 vehicle-treated Tg2576 mice and 15 ketamine- and 10 vehicle-treated wild-type mice. IC = internal control.

Inspection of Figure 5.6 indicates that hippocampal PSD-95 expression was largely unaltered by either genotype or ketamine treatment. ANOVA results were consistent with this impression, revealing non-significant effects of genotype ($F(1,45) = 0.328$, $p = .570$, MSE = 0.093, $\eta^2_p = 0.007$) and drug ($F(1,45) = 0.800$, $p = .376$, MSE = 0.228, $\eta^2_p = 0.017$), and a non-significant genotype × drug interaction ($F(1,45) = 1.064$, $p = .308$, MSE = 0.303, $\eta^2_p = 0.023$). Consistent with this interpretation, Bayesian ANOVA results were suggestive of the absence of effects of genotype ($BF_{10} = 0.318$) and drug ($BF_{10} = 0.374$), though less clear regarding an absent genotype × drug interaction ($BF_{10} = 0.539$).

PSD-95

Figure 5.6. Mean relative densities and representative Western blot image of post-synaptic density 95 (PSD-95) in hippocampal synaptosomal extracts from 19-20 month old Tg2576 and wild-type mice. n = 11 ketamine- and 13 vehicle-treated Tg2576 and 15 ketamine- and 10 vehicle-treated wild-type mice. IC = internal control.

Inspection of panel A of Figure 5.7 indicates that hippocampal expression of the AMPA receptor GluR1 subunit was increased by ketamine treatment in both Tg2576 and wild-type mice. GluR1 expression was not, however, meaningfully different between Tg2576 and wild-type mice, regardless of treatment group. ANOVA results were consistent with this impression, revealing a non-significant effect of genotype ($F(1,32) = 1.267$, $p = .269$, MSE = 0.285, $\eta^2_p = 0.038$), a significant effect of drug ($F(1,32) = 5.590$, $p = .024$, MSE = 1.258, $\eta^2_p = 0.149$), and a non-significant genotype × drug interaction ($F(1,32) = 0.129$, $p = .722$, MSE = 0.029, $\eta^2_p = 0.004$). Bayesian ANOVA results were generally consistent with this interpretation, though did not conclusively reveal a null genotype effect ($BF_{10} = 0.463$), or the absence of a genotype × drug interaction ($BF_{10} = 0.451$).

GluR1

A

Relative density

- 1.8
- 1.6
- 1.4
- 1.2
- 1
- 0.8
- 0.6
- 0.4
- 0.2
- 0

TG Ketamine | TG Vehicle | WT Ketamine | WT Vehicle

100 kDa

TG Ket | TG Vehicle | WT Ketamine | TG Vehicle | WT Ketamine | WT Veh | IC

p-Ser845 GluR1

B

Relative Density

- 5
- 4.5
- 4
- 3.5
- 3
- 2.5
- 2
- 1.5
- 1
- 0.5
- 0

TG Ketamine | TG Vehicle | WT Ketamine | WT Vehicle

100 kDa

TG Ketamine | TG Vehicle | WT | WT Ketamine | WT | TG Veh | IC
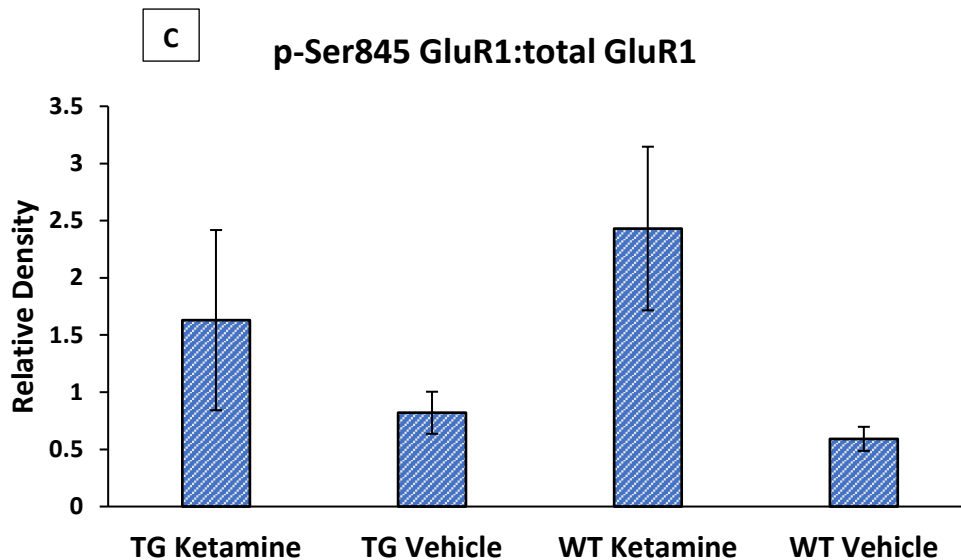
Figure 5.7. Mean relative densities and representative Western blot images of AMPA receptor subunit GluR1 (A), p-GluR1 (B), and the p-GluR1:total GluR1 ratio (C) in hippocampal synaptosomal extracts from 19-20 month old Tg2576 and wild-type mice. n = 9 ketamine- and 9 vehicle-treated Tg2576 mice and 10 ketamine- and 8 vehicle-treated wild-type mice. IC = internal control.

Panels B and C of Figure 5.7 indicate that ketamine also increased p-GluR1 expression, and the p-GluR1:total GluR1 ratio, in both Tg2576 and wild-type mice, while no notable genotype difference was apparent. ANOVA results were consistent with this impression, revealing for p-GluR1 a non-significant effect of genotype ($F_{(1,32)}$ = 0.133, p = .718, MSE = 1.069, η^2_p= 0.004), a significant effect of drug ($F_{(1,32)}$ = 4.424, p = .043, MSE = 35.688, η^2_p= 0.121), and a non-significant genotype × drug interaction ($F_{(1,32)}$ = 0.011, p = .919, MSE = 0.086, η^2_p=<.001). Bayesian ANOVA results for p-GluR1 were also consistent with this interpretation, suggesting the absence of a genotype effect (BF_{10} = 0.329), though less clearly suggesting the absence of a genotype × drug interaction (BF_{10} = 0.406). For the p-GluR1:total GluR1 ratio, ANOVA revealed a non-significant effect of genotype ($F_{(1,32)}$ = 0.251, p = .620, MSE = 0.735, η^2_p= 0.008), a significant effect of drug ($F_{(1,32)}$ = 5.349, p = .027, MSE = 15.684, η^2_p= 0.143), and a non-significant genotype × drug interaction ($F_{(1,32)}$ = 0.809, p = .375, MSE = 2.372, η^2_p= 0.025). Bayesian ANOVA results for p-GluR1:total GluR1 were consistent with this interpretation, suggesting the absence of a genotype effect (BF_{10} = 0.378), though less clearly suggesting the absence of a genotype × drug interaction (BF_{10} = 0.536).

5.3.2.2 Cortical glutamatergic signalling proteins

Inspection of panel A of Figure 5.8 indicates that, despite numerical differences, there were no clear genotype- or ketamine-associated differences in cortical GluR1 expression. ANOVA results

were consistent with this impression, revealing non-significant effects of genotype ($F(1,57) = 2.039$, $p = .159$, MSE = 1.554, $\eta^2_p = 0.035$) and drug ($F(1,57) = 2.359$, $p = .130$, MSE = 1.798, $\eta^2_p = 0.040$), and a non-significant genotype × drug interaction ($F(1,57) = 0.018$, $p = .892$, MSE = 0.014, $\eta^2_p < .001$). Bayesian ANOVA results were partially consistent with this interpretation; though not conclusively revealing null effects of genotype ($BF_{10} = 0.624$) and drug ($BF_{10} = 0.723$), there was a clearer absence of a genotype × drug interaction ($BF_{10} = 0.337$). Panels B and C of Figure 5.8 display a similar, albeit less clear, pattern of results for p-GluR1 and the p-GluR1:total GluR1 ratio in the cortex as seen in the hippocampus. That is, p-GluR1 and p-GluR1:total GluR1 ratio show no notable differences between genotypes, with a possible effect of ketamine increasing these measures, especially in the case of p-GluR1:total GluR1. ANOVA results were somewhat consistent with this impression, revealing for p-GluR1 non-significant effects of genotype ($F(1,57) = 0.040$, $p = .841$, MSE = 0.009, $\eta^2_p = 0.001$) and drug ($F(1,57) = 2.486$, $p = .120$, MSE = 0.532, $\eta^2_p = 0.042$), and a non-significant genotype × drug interaction ($F(1,57) = 0.082$, $p = .776$, MSE = 0.018, $\eta^2_p = 0.001$). Bayesian ANOVA results were suggestive of the absence of a genotype effect ($BF_{10} = 0.264$), less informative regarding an absence of drug effect ($BF_{10} = 0.763$), and suggested an absent genotype × drug interaction ($BF_{10} = 0.359$). For the p-GluR1:total GluR1 ratio, ANOVA revealed non-significant effects of genotype ($F(1,57) = 0.065$, $p = .800$, MSE = 0.011, $\eta^2_p = 0.001$) and drug ($F(1,57) = 3.773$, $p = .057$, MSE = 0.619, $\eta^2_p = 0.062$), and a non-significant genotype × drug interaction ($F(1,57) = 0.433$, $p = .513$, MSE = 0.071, $\eta^2_p = 0.008$). Bayesian ANOVA results were suggestive of the absence of a genotype effect ($BF_{10} = 0.268$), but were less informative regarding the presence of a drug effect ($BF_{10} = 1.334$), and the absence of a genotype × drug interaction ($BF_{10} = 0.430$).

GluR1

A

B
p-Ser845 GluR1
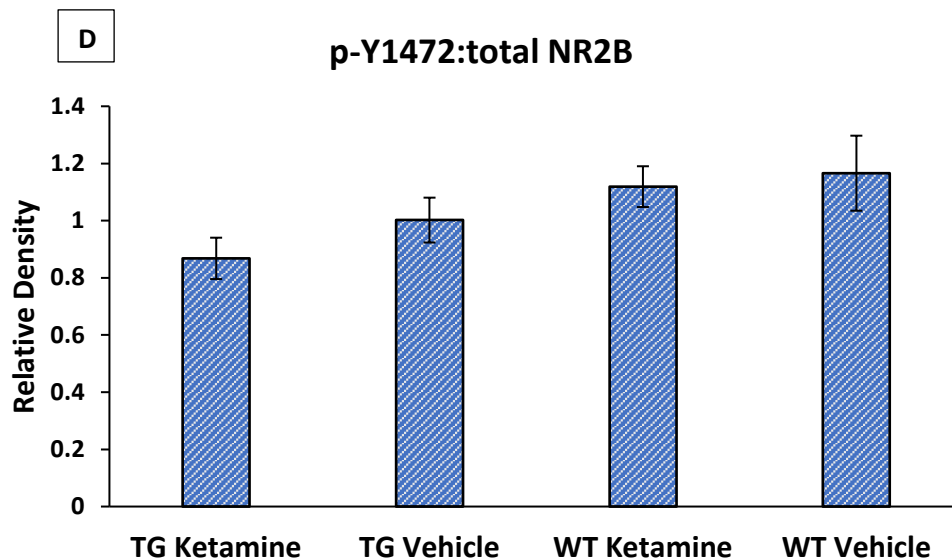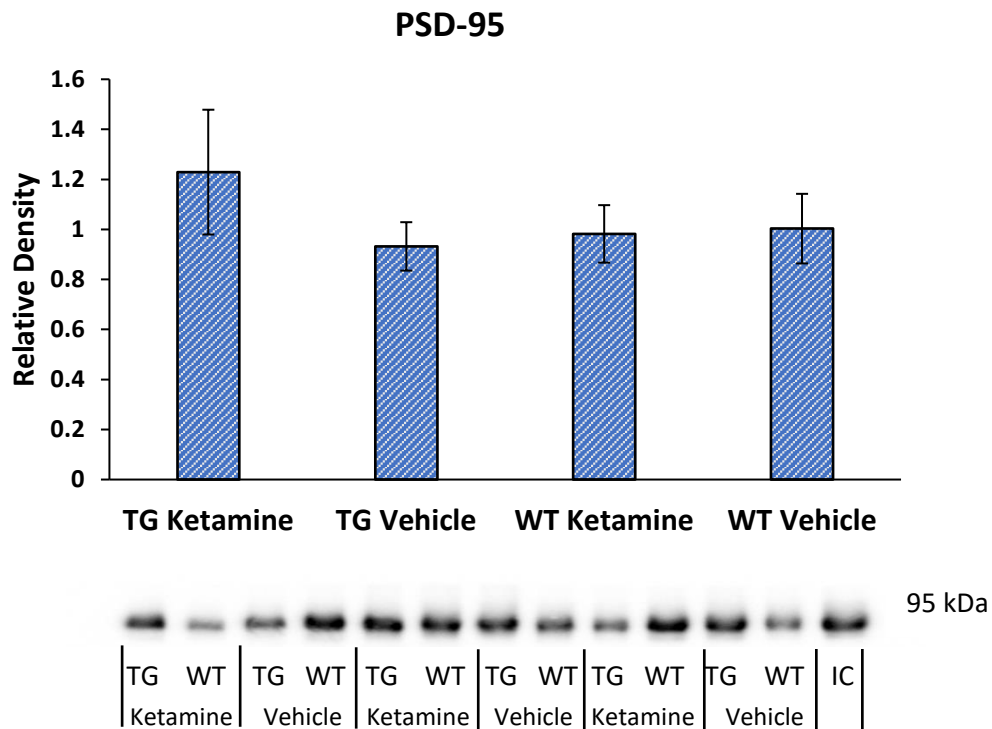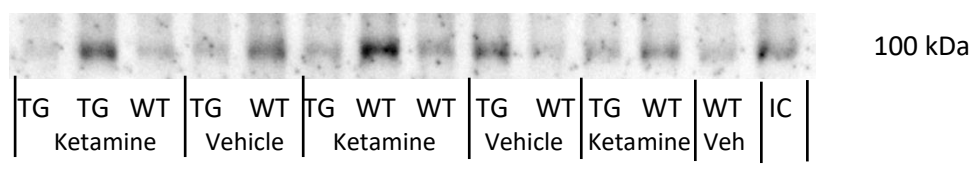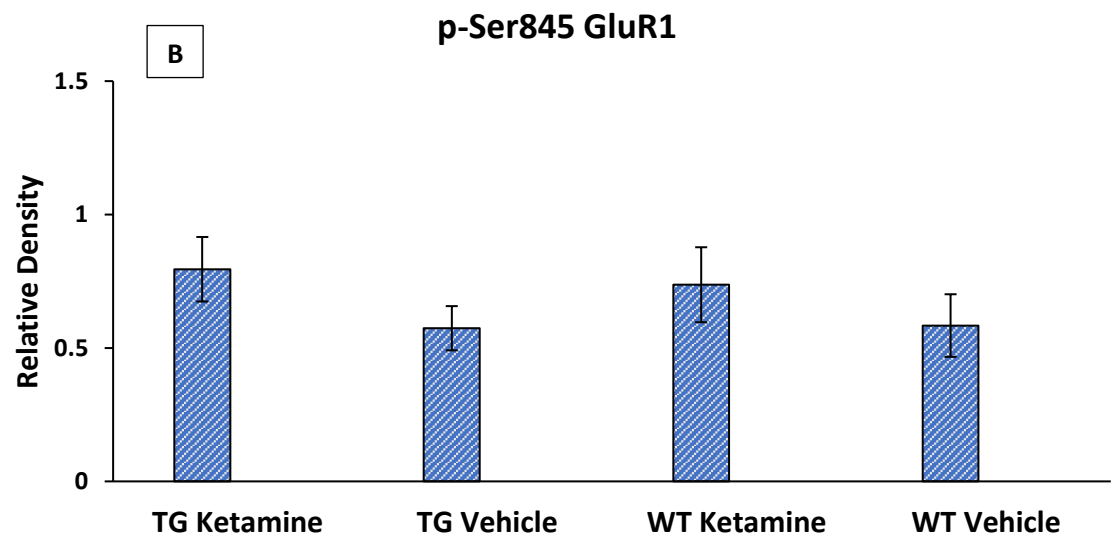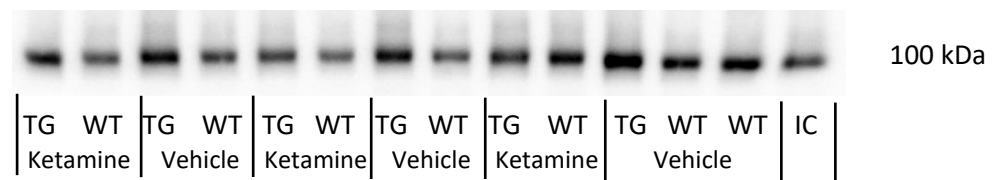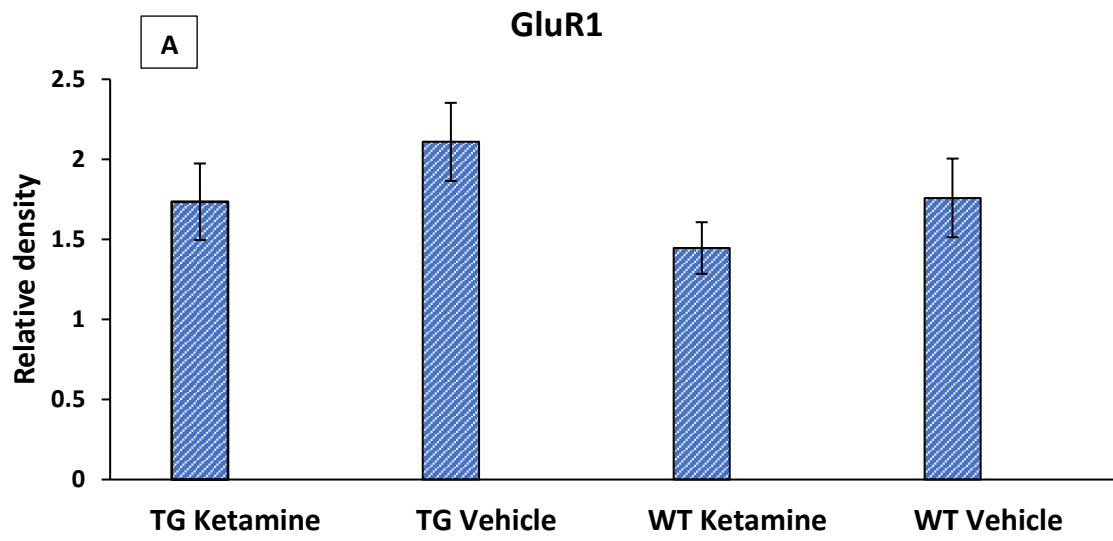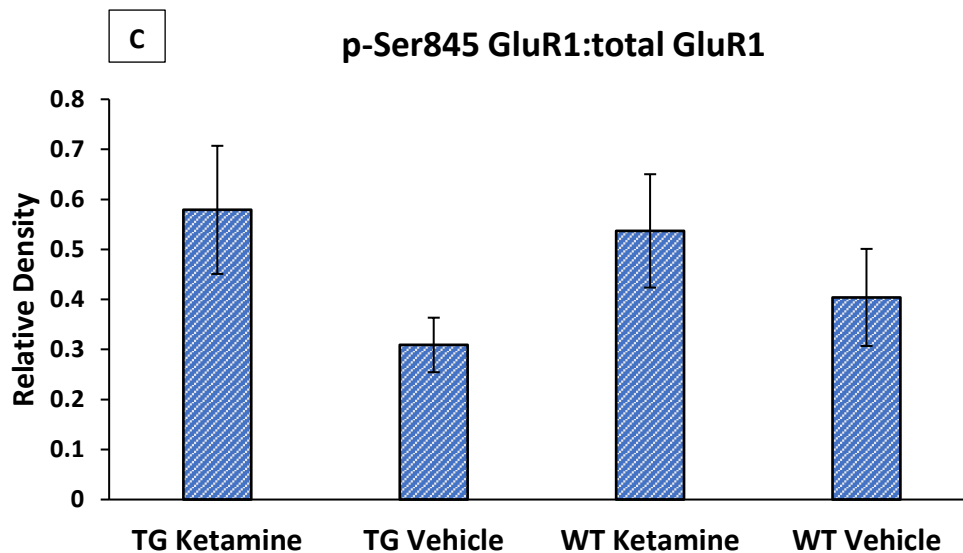
Figure 5.8. Mean relative densities and representative Western blot images of AMPA receptor subunit GluR1 (A), p-GluR1 (B), and the pGluR1:total GluR1 ratio (C) in cortical synaptosomal extracts from 19-20 month old Tg2576 and wild-type mice. n = 16 ketamine- and 15 vehicle-treated Tg2576 mice and 16 ketamine- and 14 vehicle-treated wild-type mice. IC = internal control.

Inspection of Figure 5.9 indicates that cortical PSD-95 expression was not meaningfully different between both genotypes and treatment groups. ANOVA results were consistent with this impression, revealing non-significant effects of genotype ($F(1,57) = 2.117$, $p = .151$, MSE = 0.052, $\eta^2_p = 0.036$) and drug ($F(1,57) = 1.874$, $p = .176$, MSE = 0.046, $\eta^2_p = 0.032$), and a non-significant genotype × drug interaction ($F(1,57) = 0.473$, $p = .494$, MSE = 0.012, $\eta^2_p = 0.008$). Bayesian ANOVA results were consistent with this interpretation though not conclusively so, not providing clear evidence regarding null effects of genotype ($BF_{10} = 0.676$) and drug ($BF_{10} = 0.575$), and the absence of a genotype × drug interaction ($BF_{10} = 0.417$).
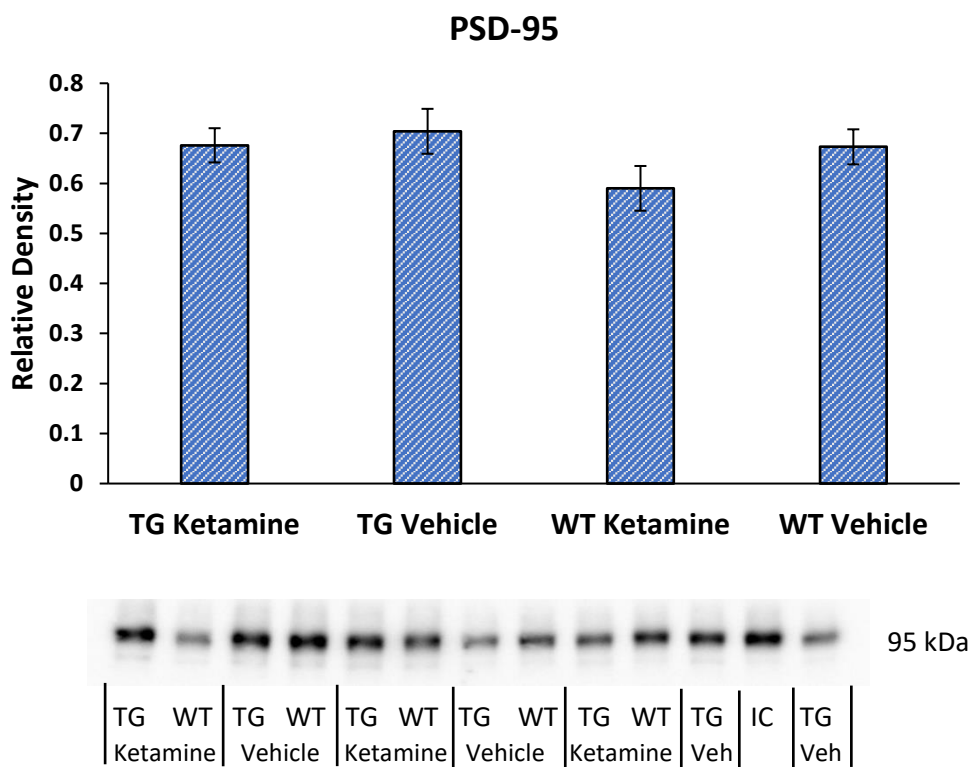
PSD-95

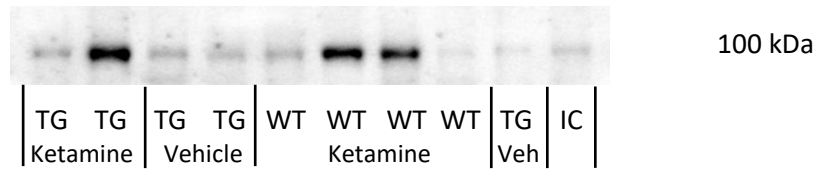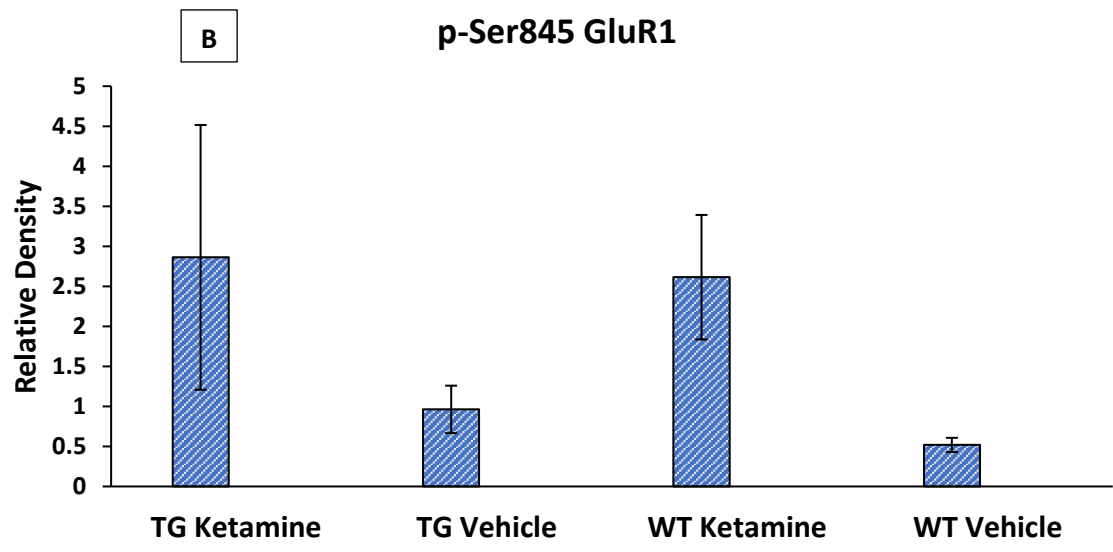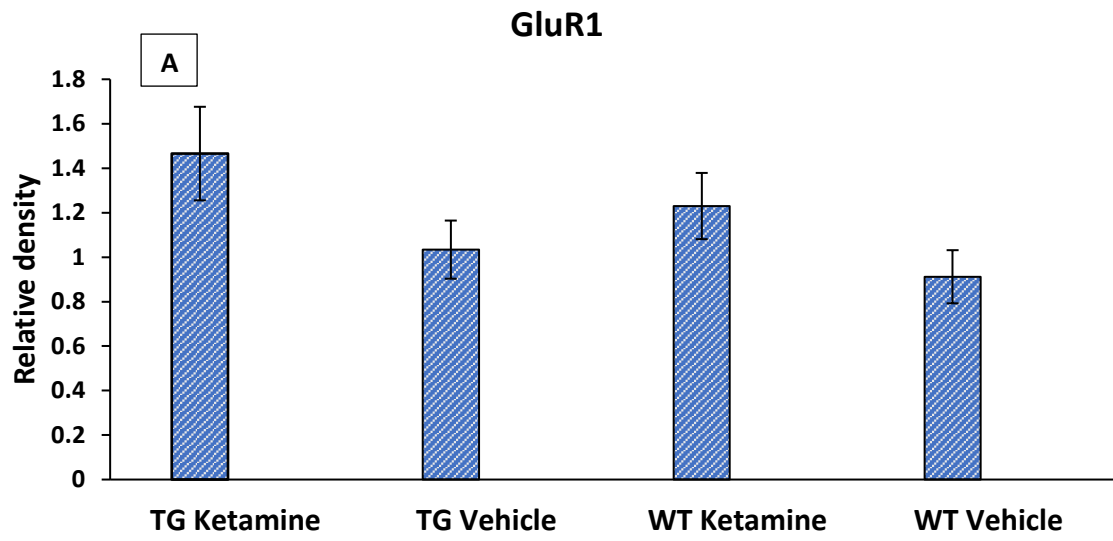
Figure 5.9. Mean relative densities and representative Western blot image of PSD-95 in cortical synaptosomal extracts from 19-20 month old Tg2576 and wild-type mice. n = 16 ketamine- and 15 vehicle-treated Tg2576 mice and 16 ketamine- and 14 vehicle-treated wild-type mice. IC = internal control.

5.3.3 Summary

Experiment 7 revealed that 19-20 month-old Tg2576 mice, across treatment groups, showed a reduction in the hippocampal p-Y1472 NR2B:total NR2B ratio compared with wild-type mice. However, in both the hippocampus and the cortex, the other glutamatergic receptor subunits examined did not differ between genotypes. For hippocampal NMDA receptor subunits NR1, NR2B and p-Y1472 NR2B, the Bayesian analysis generally supported the idea that these subunits were genuinely not different between Tg2576 and wild-type mice. In addition, ketamine treatment had no meaningful impact on any of the NMDAR measures, with the strongest evidence for a true null effect of ketamine found for the p-Y1472 NR2B:total NR2B ratio. Investigation of the AMPA receptor subunit GluR1 revealed no observable differences between Tg2576 and wild-type mice when GluR1, p-Ser845 GluR1 and the p-Ser845 GluR1:total GluR1 ratio were examined, both in the hippocampus and the cortex. Of these GluR1 measures, the strongest evidence for a true null effect of genotype was for p-Ser845 GluR1 and p-Ser845 GluR1:total GluR1 ratio, in both the hippocampus and cortex. Unlike the NMDAR subunits, ketamine treatment did have an effect on GluR1 subunit expression

and phosphorylation across both genotypes, more so in the hippocampus than cortex. Ketamine treatment increased the expression of GluR1, p-Ser845 GluR1, and the p-Ser845 GluR1:total GluR1 ratio, in the hippocampus, while there was also a suggestion of an increase in the p-Ser845 GluR1:total GluR1 ratio in the cortex. PSD-95 expression appeared to neither differ between genotypes, nor be altered by ketamine treatment, in both the hippocampus and the cortex, though the strongest evidence for null effects of genotype and ketamine treatment was in the hippocampal results.

5.4 Experiment 8

5.4.1 Subjects, apparatus, procedure & data analysis

Experiment 8 investigates the effects of both genotype and ketamine treatment on a number of serotonergic and opioidergic receptors in synaptically enriched hippocampal and cortical extracts. As with Experiment 7, the numbers of each genotype and treatment groups are reported with each figure, and apparatus, procedure and data collection are as previously described.

5.4.2 Results

5.4.2.1 Hippocampal serotonergic proteins

Inspection of panel A of Figure 5.10 indicates that $5HT_{1B}$ receptor expression was largely similar between both genotypes and treatment groups. ANOVA results were consistent with this impression, revealing non-significant effects of genotype ($F(1,22) = 0.476$, $p = .497$, MSE = 0.064, $\eta^2_p = 0.021$) and drug ($F(1,22) = 0.025$, $p = .875$, MSE = 0.003, $\eta^2_p = 0.001$), and a non-significant genotype × drug interaction ($F(1,22) = 0.713$, $p = .408$, MSE = 0.096, $\eta^2_p = 0.031$). Bayesian ANOVA results were somewhat supportive of this interpretation, providing unclear evidence regarding a null genotype effect ($BF_{10} = 0.451$), slightly clearer evidence of a null effect of ketamine ($BF_{10} = 0.364$) and some evidence indicating a null genotype × drug interaction ($BF_{10} = 0.223$). Panel B of Figure 5.10 indicates that Tg2576 and wild-type mice show very similar levels of $5HT_4$ receptor expression, with no obvious effect of ketamine treatment. ANOVA results were consistent with this impression, revealing non-significant effects of genotype ($F(1,20) = 0.012$, $p = .915$, MSE = 0.001, $\eta^2_p = 0.001$) and drug ($F(1,20) = 0.023$, $p = .882$, MSE = 0.002, $\eta^2_p = 0.001$), and a non-significant genotype × drug interaction ($F(1,20) = 0.048$, $p = .829$, MSE = 0.005, $\eta^2_p = 0.002$). Bayesian ANOVA results were consistent with this, suggesting some evidence in favour of null effects of genotype ($BF_{10} = 0.378$) and ketamine treatment ($BF_{10} = 0.388$), and less clear evidence of a null genotype × drug interaction

($BF_{10} = 0.530$). However, for the hippocampal $5HT_4$ receptor results, the wild-type vehicle group only consisted of two mice, therefore these particular results should be treated with caution. Panel C of Figure 5.10 indicates that hippocampal SERT expression was largely similar between Tg2576 and wild-type mice, and between ketamine- and vehicle-treated mice. ANOVA results were consistent with this impression, revealing non-significant effects of genotype ($F(1,34) = 0.034$, $p = .855$, MSE = 0.037, $\eta^2_p = 0.001$) and drug ($F(1,34) = 0.369$, $p = .548$, MSE = 0.406, $\eta^2_p = 0.011$), and a non-significant genotype × drug interaction ($F(1,34) = 0.550$, $p = .464$, MSE = 0.606, $\eta^2_p = 0.016$). Bayesian ANOVA results generally supported this interpretation, providing some evidence of an absence of genotype effect ($BF_{10} = 0.320$), some evidence of an absence of ketamine effect ($BF_{10} = 0.383$), with less clear evidence of an absent genotype × drug interaction ($BF_{10} = 0.508$).
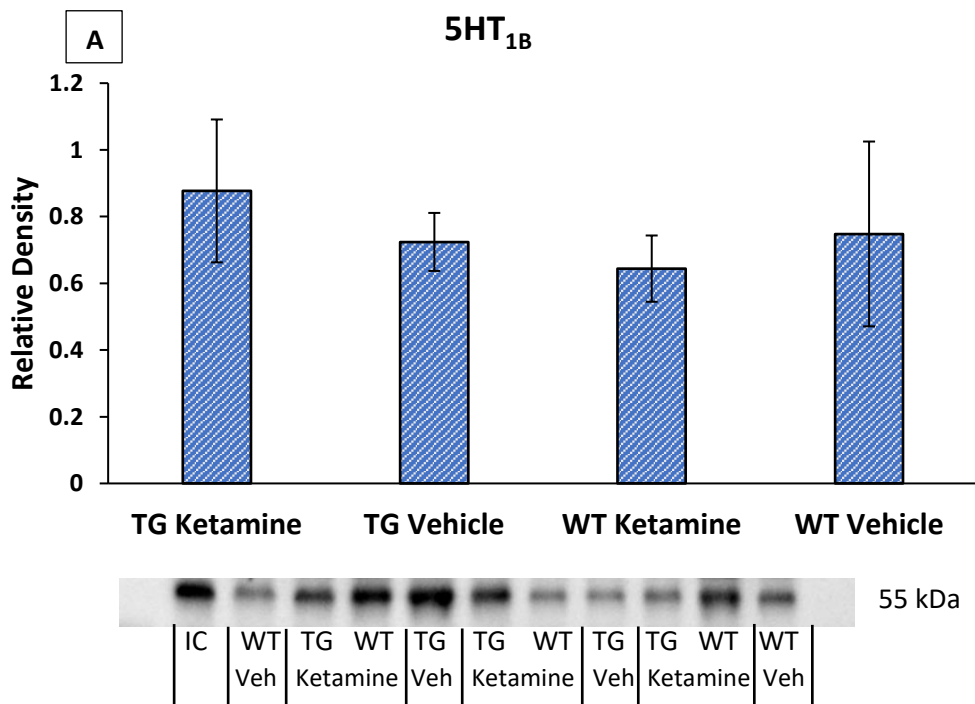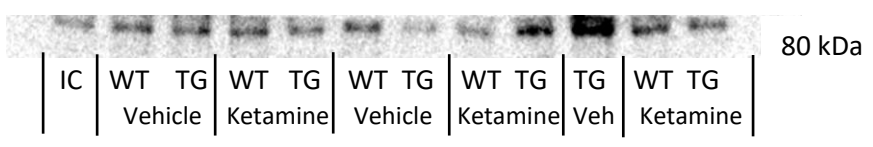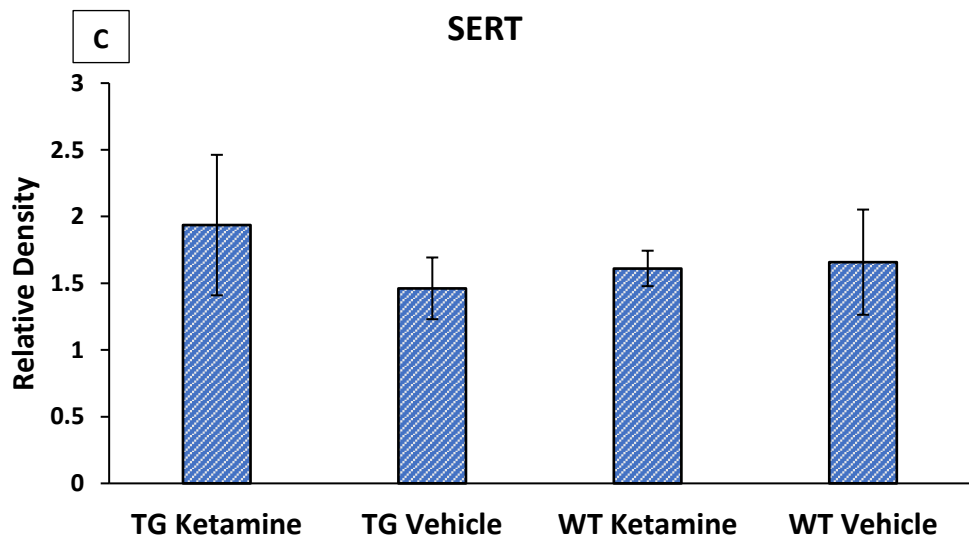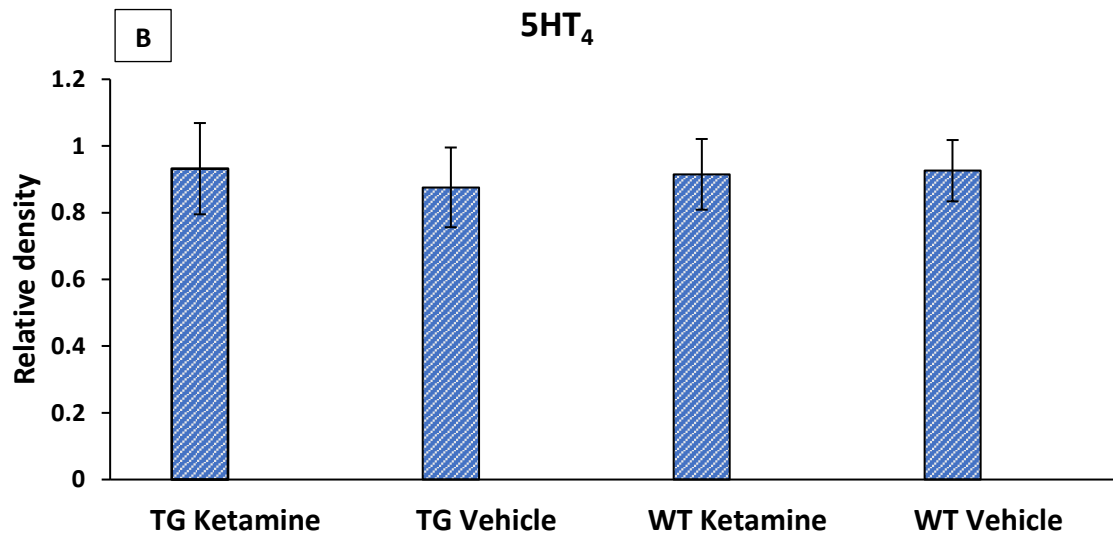
Figure 5.10. Mean relative densities and representative Western blot images of 5HT receptors $5HT_{1B}$ (A), $5HT_4$ (B), and SERT (C) in hippocampal synaptosomal extracts from 19-20 month old Tg2576 and wild-type mice. Numbers of mice in each genotype and treatment group are as follows: $5HT_{1B}$ – 5 ketamine- and 8 vehicle-treated Tg2576 and 9 ketamine- and 4 vehicle-treated wild-type mice; $5HT_4$ – 5 ketamine- and 8 vehicle-treated Tg2576 and 9 ketamine- and 2 vehicle-treated wild-type mice; SERT – 10 ketamine- and 11 vehicle-treated Tg2576 and 11 ketamine- and 6 vehicle-treated wild-type mice. IC = internal control.

5.4.2.2 Hippocampal mu opioid receptors

Inspection of panels A and B of Figure 5.11 indicated that while there was no clear effect of ketamine on mu opioid receptor expression in Tg2576 and wild-type mice, within vehicle-treated mice there was a suggestion of Tg2576 mice displaying greater mu receptor expression. This increased mu opioid receptor expression in vehicle-treated Tg2576 mice was most notable for the 47kDa isoform (panel B). ANOVA results were consistent with this impression, revealing for the 49kDa isoform (panel A) non-significant effects of genotype ($F(1,29) = 1.678$, $p = .205$, $MSE = 2.212$, $\eta^2_p = 0.055$) and drug ($F(1,29) = 3.506e^{-4}$, $p = .985$, $MSE = 4.620e^{-4}$, $\eta^2_p < .001$), and a non-significant genotype × drug interaction ($F(1,29) = 1.498$, $p = .231$, $MSE = 1.974$, $\eta^2_p = 0.049$). Bayesian ANOVA results were unclear regarding a null genotype effect ($BF_{10} = 0.580$), suggested the absence of a drug effect ($BF_{10} = 0.341$), and were less clear regarding a genotype × drug interaction ($BF_{10} = 0.653$). ANOVA results for the 47kDa isoform (panel B) revealed non-significant effects of genotype ($F(1,29) = 2.856$, $p = .102$, $MSE = 3.385$, $\eta^2_p = 0.090$) and drug ($F(1,29) = 0.020$, $p = .889$, $MSE = 0.024$, $\eta^2_p = 0.001$), and a non-significant genotype × drug interaction ($F(1,29) = 3.203$, $p = .084$, $MSE = 3.796$, $\eta^2_p = 0.099$). Bayesian ANOVA results revealed uninformative evidence of a genotype effect ($BF_{10} = 0.814$), were suggestive of a null effect of drug ($BF_{10} = 0.362$), and were not clearly informative regarding a genotype × drug interaction (0.433). As the conventional ANOVA suggested the possibility of a genotype effect which differed in each treatment group, this was investigated with follow-up t-tests. Student's t-tests comparing expression of the 47kDa isoform between genotypes within each treatment group, revealed a non-significant difference between ketamine-treated Tg2576 and wild-type mice ($t(16) = 0.073$, $p = .942$, $d = 0.035$), and a significant difference between vehicle-treated Tg2576 and wild-type mice ($t(13) = 2.388$, $p = .033$, $d = 1.259$). A Bayesian independent samples t-test revealed unclear evidence regarding a null effect of genotype within ketamine-treated mice ($BF_{10} = 0.414$).
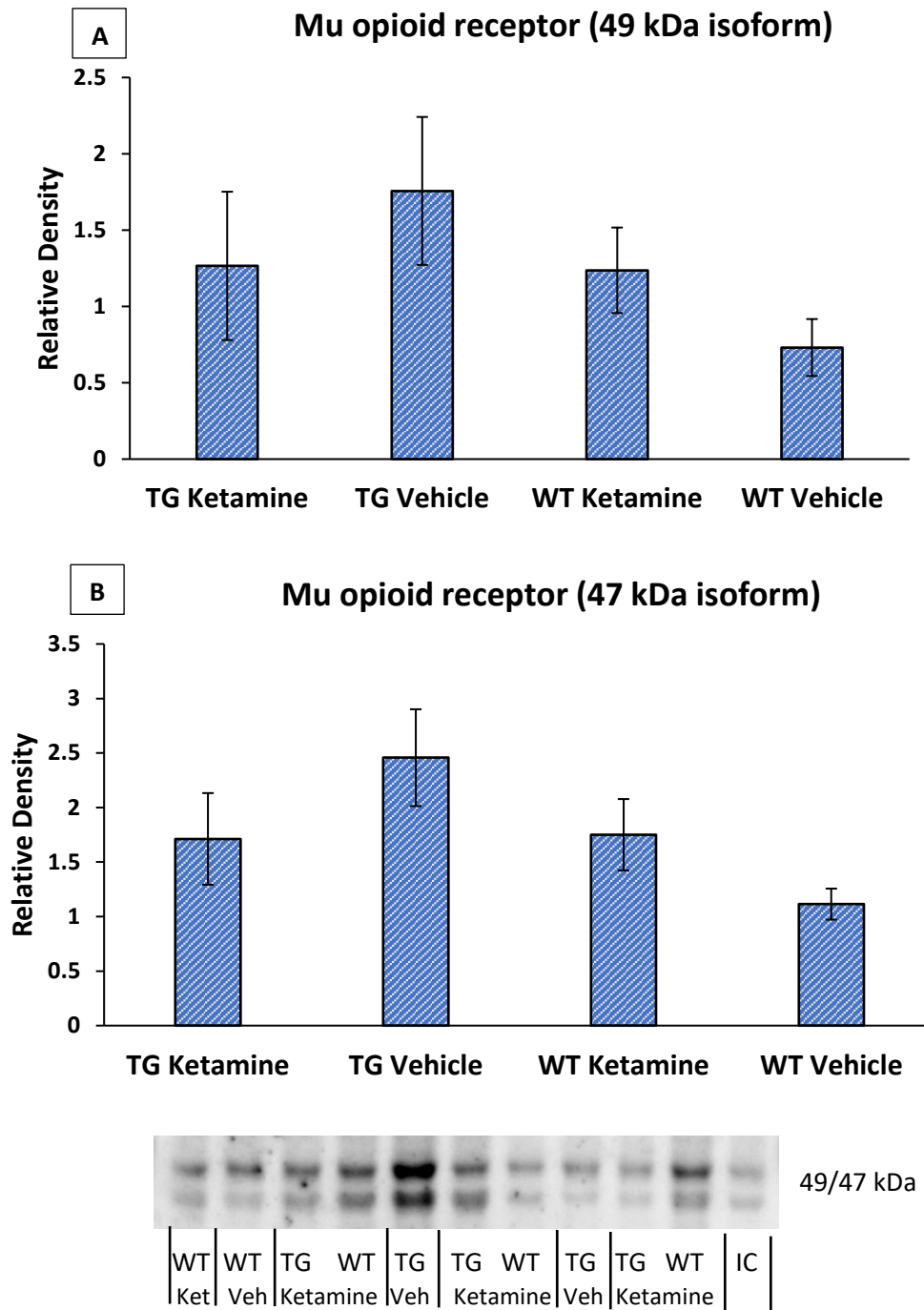
Figure 5.11. Mean relative densities and representative Western blot image of the 49kDa (A) and 47kDa (B) bands for mu opioid receptors in hippocampal synaptosomal extracts from 19-20 month old Tg2576 and wild-type mice. n = 8 ketamine- and 9 vehicle-treated Tg2576 mice and 10 ketamine- and 6 vehicle-treated wild-type mice. IC = internal control.

5.4.2.3 Cortical serotonergic signalling proteins

Inspection of panel A of Figure 5.12 indicates that cortical 5HT$_{1B}$ receptor expression did not meaningfully differ either between genotypes or between treatment groups. ANOVA results were consistent with this impression, revealing non-significant effects of genotype (F(1,57) = 0.082, p =

.775, MSE = 0.021, η^2_p = 0.001) and drug (F(1,57) = 0.405, p = .527, MSE = 0.106, η^2_p = 0.007), and a non-significant genotype × drug interaction (F(1,57) = 0.007, p = .933, MSE = 0.002, η^2_p <.001). Bayesian ANOVA results were consistent with this interpretation, providing evidence in favour of null effects of genotype (BF$_{10}$ = 0.271) and drug (BF$_{10}$ = 0.312), and the absence of a genotype × drug interaction (BF$_{10}$ = 0.353). Inspection of panel B of Figure 5.12 indicates that cortical 5HT$_4$ receptor expression also did not differ between Tg2576 and wild-type mice, and did not appear affected by ketamine treatment. ANOVA results were consistent with this impression, revealing non-significant effects of genotype (F(1,57) = 0.821, p = .369, MSE = 0.045, η^2_p = 0.014) and drug (F(1,57) = 0.731, p = .396, MSE = 0.040, η^2_p = 0.013), and a non-significant genotype × drug interaction (F(1,57) = 0.207, p = .650, MSE = 0.011, η^2_p = 0.004). Bayesian ANOVA results were again consistent with this interpretation, suggestive of null effects of genotype (BF$_{10}$ = 0.373) and drug (BF$_{10}$ = 0.355), and an absent genotype × drug interaction (BF$_{10}$ = 0.396). Inspection of panel C of Figure 5.12 indicates that, while there was no obvious effect of ketamine treatment on SERT expression for either genotype, overall Tg2576 mice displayed a greater cortical expression of SERT than wild-type mice. ANOVA results were consistent with this impression, revealing a significant effect of genotype (F(1,57) = 8.201, p = .006, MSE = 0.751, η^2_p = 0.126), a non-significant effect of drug (F(1,57) = 0.097, p = .756, MSE = 0.009, η^2_p = 0.002), and a non-significant genotype × drug interaction (F(1,57) = 1.292, p = .261, MSE = 0.118, η^2_p = 0.022). Bayesian ANOVA results were consistent with this interpretation, providing evidence supportive of a null drug effect (BF$_{10}$ = 0.269), though less clear regarding a null genotype × drug interaction (BF$_{10}$ = 0.563).

A

5HT$_{1B}$

B
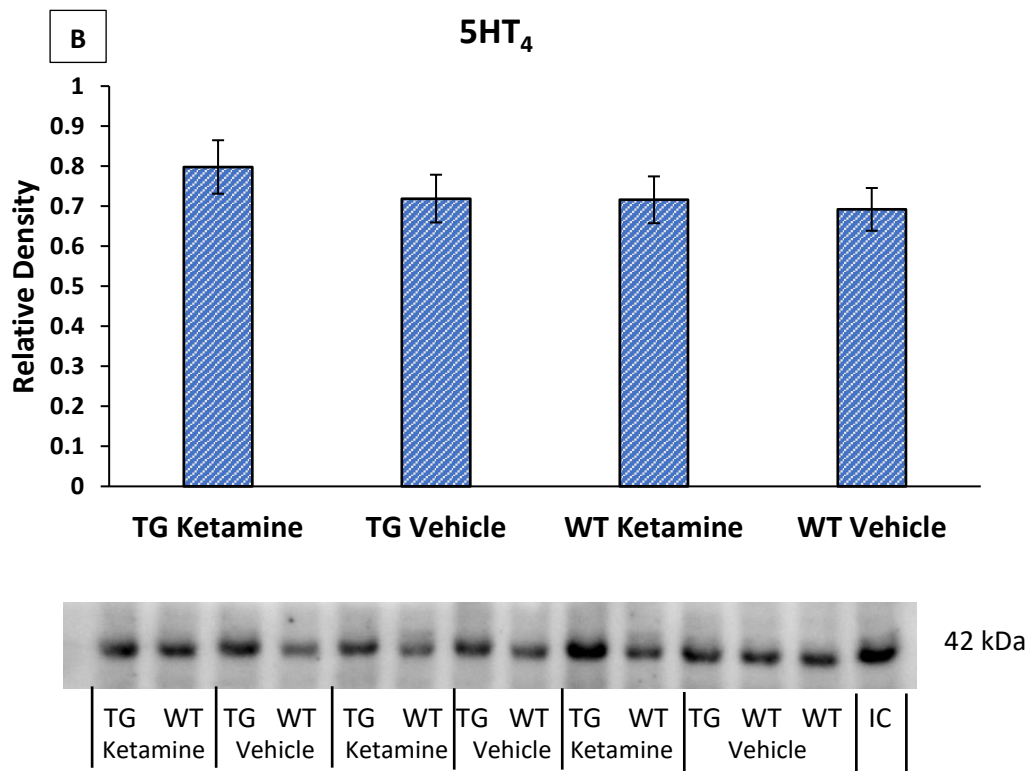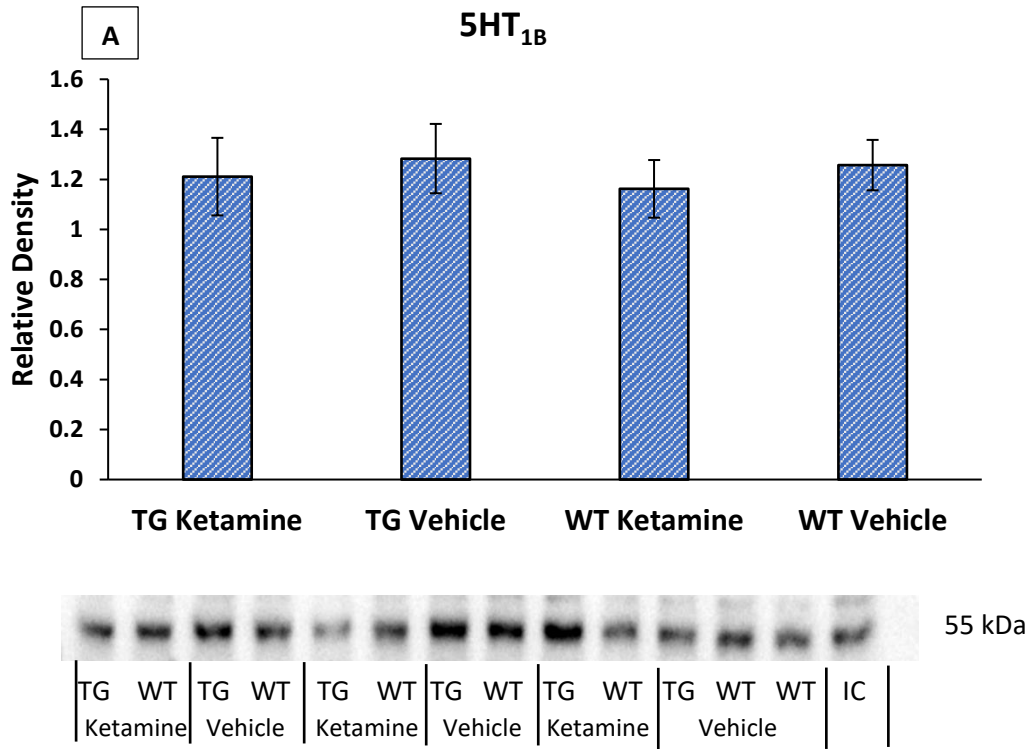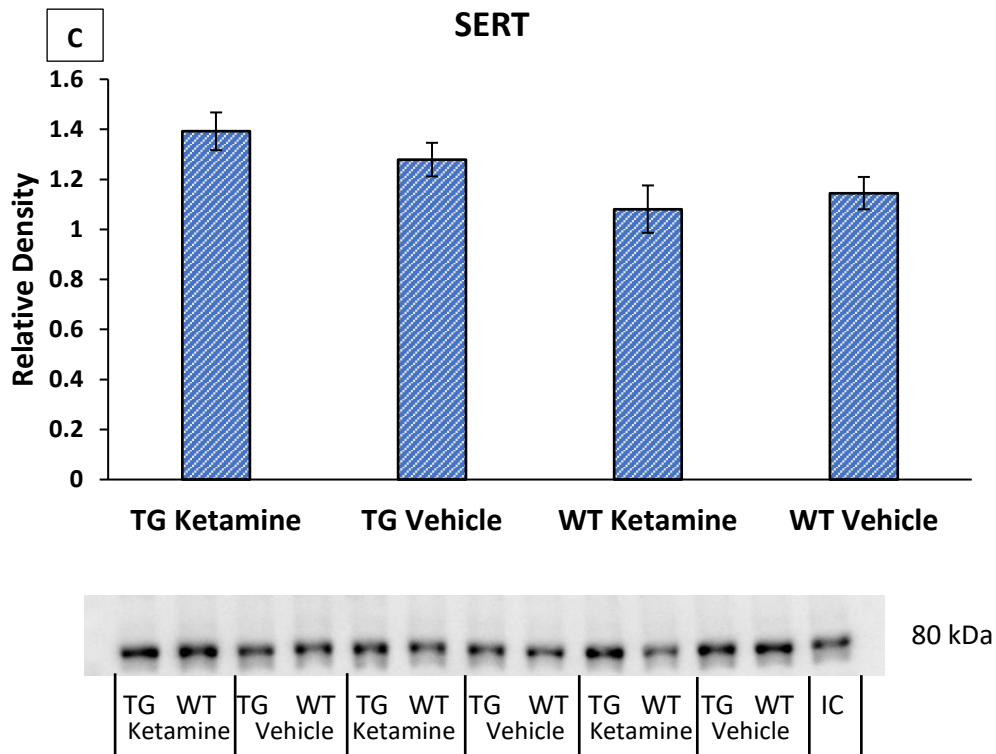
5HT$_4$

Figure 5.12. Mean relative densities and representative Western blot images of 5HT$_{1B}$ receptors (A), 5HT$_4$ receptors (B), and SERT (C) in cortical synaptosomal extracts from 19-20 month old Tg2576 and wild-type mice. n = 16 ketamine- and 15 vehicle-treated Tg2576 mice and 16 ketamine- and 14 vehicle-treated wild-type mice. IC = internal control.

5.4.2.4 Cortical opioid receptors

Inspection of panel A of Figure 5.13 indicates that, while cortical mu opioid receptor expression did not appear altered by ketamine treatment, there was a suggestion of an increased expression in Tg2576 mice overall. ANOVA results were somewhat consistent with this impression, revealing non-significant effects of genotype ($F(1,57) = 3.320$, $p = .074$, MSE = 0.244, $\eta^2_p = 0.055$) and drug ($F(1,57) = 2.080$, $p = .155$, MSE = 0.153, $\eta^2_p = 0.035$), and a non-significant genotype × drug interaction ($F(1,57) = 0.089$, $p = .767$, MSE = 0.007, $\eta^2_p = 0.002$). Bayesian ANOVA results provided unclear evidence regarding null effects of ketamine ($BF_{10} = 0.638$) and genotype ($BF_{10} = 1.052$), and suggested the absence of a genotype × drug interaction ($BF_{10} = 0.365$). Inspection of panel B of Figure 5.13 indicates that cortical kappa opioid receptor expression was not meaningfully altered by ketamine treatment across genotypes, but was increased in Tg2576 mice overall compared with wild-type mice. ANOVA results were consistent with this impression, revealing a significant effect of genotype ($F(1,57) = 6.110$, $p = .016$, MSE = 1.035, $\eta^2_p = 0.097$), a non-significant effect of drug ($F(1,57) = 1.674$, $p = .201$, MSE = 0.284, $\eta^2_p = 0.029$), and a non-significant genotype × drug interaction ($F(1,57) = 0.098$, $p = .755$, MSE = 0.017, $\eta^2_p = 0.002$). Bayesian ANOVA results were

somewhat consistent with this interpretation; while less clear regarding the absence of a drug effect ($BF_{10} = 0.484$), they were suggestive of a null genotype × drug interaction ($BF_{10} = 0.364$).
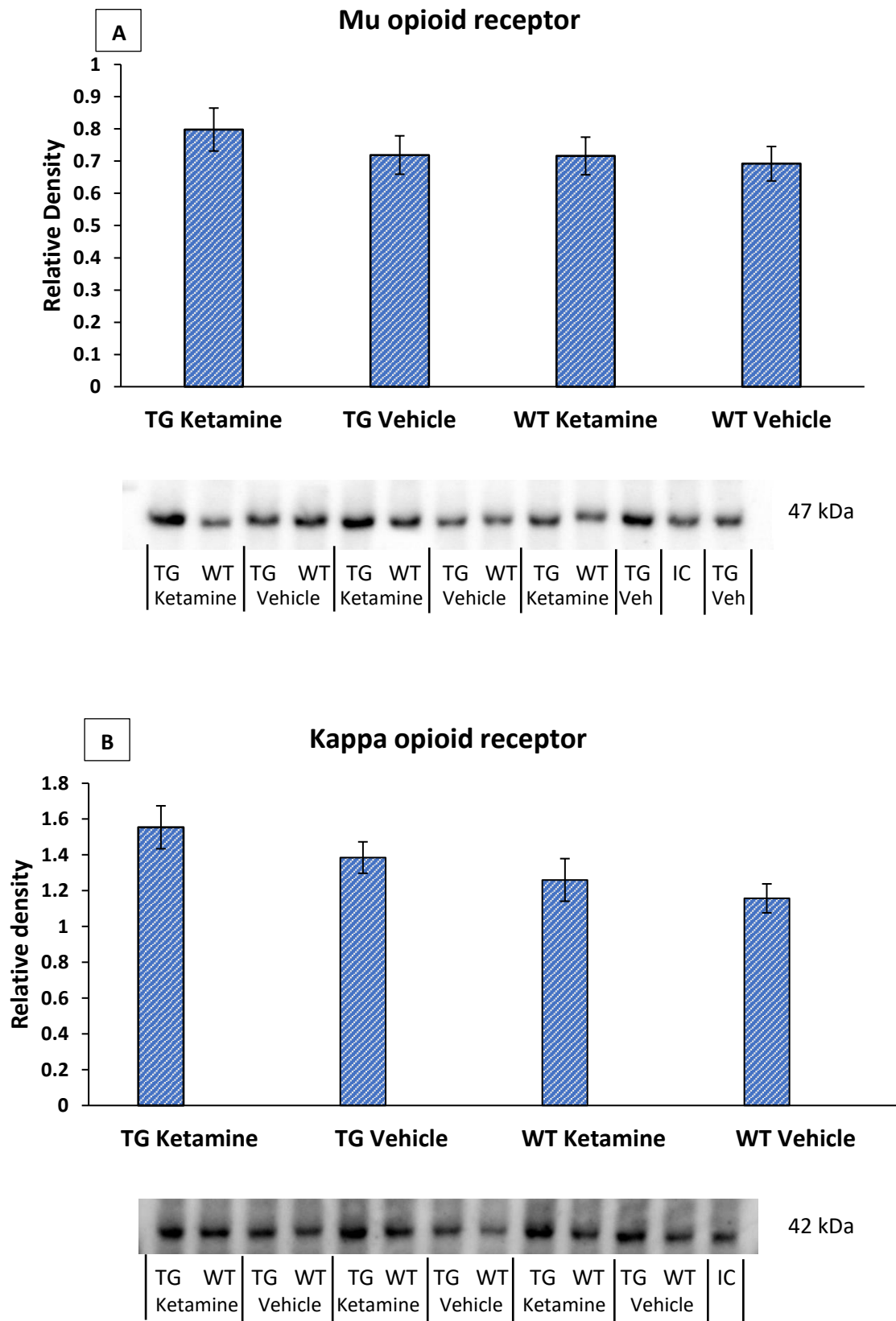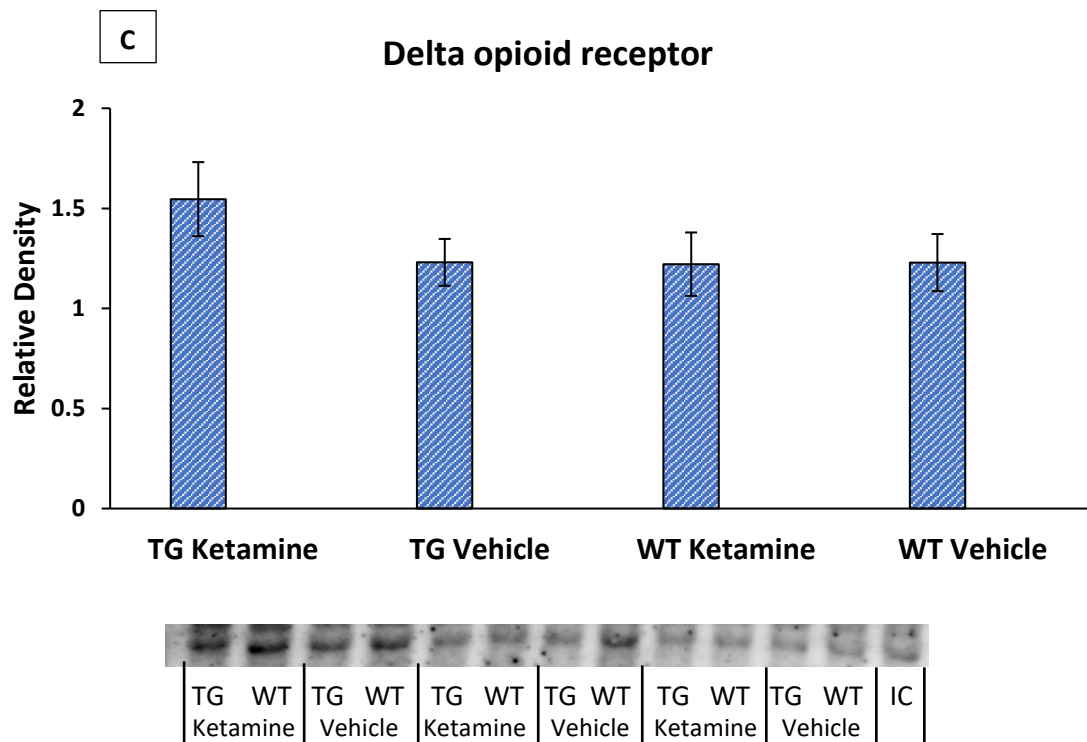
Figure 5.13. Mean relative densities and representative Western blot images of mu (A), kappa (B), and delta (C) opioid receptors in cortical synaptosomal extracts from 19-20 month old Tg2576 and wild-type mice. n = 16 ketamine- and 15 vehicle-treated Tg2576 mice and 16 ketamine- and 14 vehicle-treated wild-type mice. IC = internal control.

Inspection of panel C of Figure 5.13 indicates that cortical delta opioid receptor expression was not notably different between genotypes or treatment groups. ANOVA results were consistent with this impression, revealing non-significant effects of genotype ($F(1,57) = 1.103$, $p = .298$, MSE = 0.403, $\eta^2_p = 0.019$) and drug ($F(1,57) = 0.984$, $p = .325$, MSE = 0.360, $\eta^2_p = 0.017$), and a non-significant genotype × drug interaction ($F(1,57) = 1.092$, $p = .301$, MSE = 0.399, $\eta^2_p = 0.019$). Bayesian ANOVA results were somewhat consistent with this interpretation, though not conclusively so, providing unclear evidence of null genotype ($BF_{10} = 0.427$) and drug ($BF_{10} = 0.394$) effects, and less informative evidence regarding a genotype × drug interaction ($BF_{10} = 0.529$).

5.4.3 Summary

Experiment 8 revealed that cortical SERT expression was raised in 19-20 month-old Tg2576 compared with wild-type mice. However, in both the hippocampus and the cortex, the majority of serotonergic-related proteins did not differ between Tg2576 and wild-type mice. This absence of genotype difference was observed for $5HT_{1B}$ and $5HT_4$ receptors in both the hippocampus and the cortex, and for SERT in the hippocampus. Bayesian analyses generally suggested these were genuine

null differences between Tg2576 and wild-type mice, though with the caveat that the hippocampal 5HT$_4$ wild-type vehicle group contained a low number of mice. Ketamine treatment had no discernible effect on 5HT$_{1B}$, 5HT$_4$ or SERT expression in either the hippocampus or the cortex, and Bayesian analyses generally supported these being null effects of ketamine. Importantly, kappa opioid receptor expression was increased in Tg2576 mouse cortex, as was one isoform of the mu opioid receptor in the hippocampus within a subset of Tg2576 and wild-type mice. In addition, there was a suggestion of increased mu opioid receptor expression in Tg2576 mouse cortex, though this was a non-significant finding, whereas cortical delta opioid receptor expression did not differ between genotypes. A Bayesian analysis was somewhat consistent with delta opioid receptor expression genuinely not differing between genotypes. Ketamine treatment had no notable effects on the expression of any opioid receptor, and this too was generally supported by Bayesian analyses.

5.5 Experiment 9

5.5.1 Subjects, apparatus, procedure & data analysis

Experiment 9 investigates whether the genotype differences revealed in Experiments 7 and 8 are present at 4-5 months of age, in a separate cohort of Tg2576 and wild-type mice. Apparatus, procedure and data collection are as previously described. Synaptosomal hippocampal and cortical extracts were examined in 10 Tg2576 and 12 wild-type mice.

5.5.2 Results

5.5.2.1 Hippocampal NR2B-related measures

Inspection of panels A, B and C of Figure 5.14 indicates that at 4-5 months of age Tg2576 mice display levels of NR2B, p-Y1472 NR2B and the p-Y1472 NR2B:total NR2B ratio, respectively, that are not markedly lower than in wild-type mice. Student's t-tests were consistent with this impression, revealing non-significant genotype differences in NR2B ($t(19) = 0.541$, $p = .595$, $d = 0.236$), p-Y1472 NR2B ($t(19) = 0.913$, $p = .373$, $d = 0.399$), and the p-Y1472 NR2B:total NR2B ratio ($t(19) = 0.371$, $p = .714$, $d = 0.162$). Bayesian independent samples t-tests were somewhat supportive of this interpretation, consistent, though not unambiguously so, with the absence of genotype differences for NR2B ($BF_{10} = 0.434$), p-Y1472 NR2B ($BF_{10} = 0.525$) and the p-Y1472 NR2B:total NR2B ratio ($BF_{10} = 0.411$).
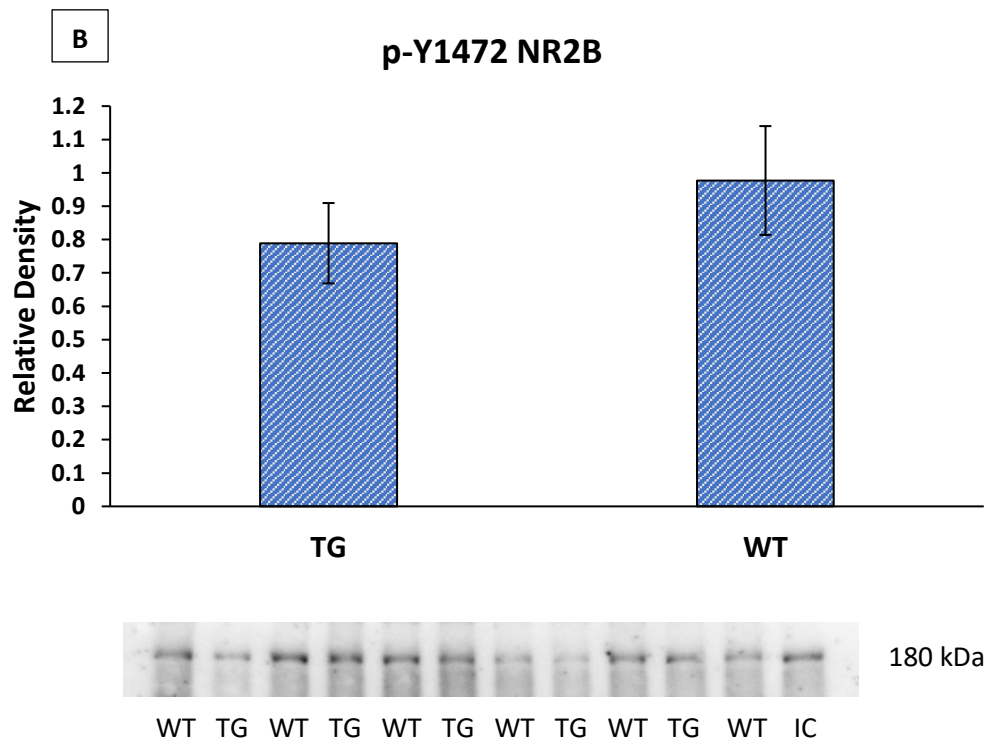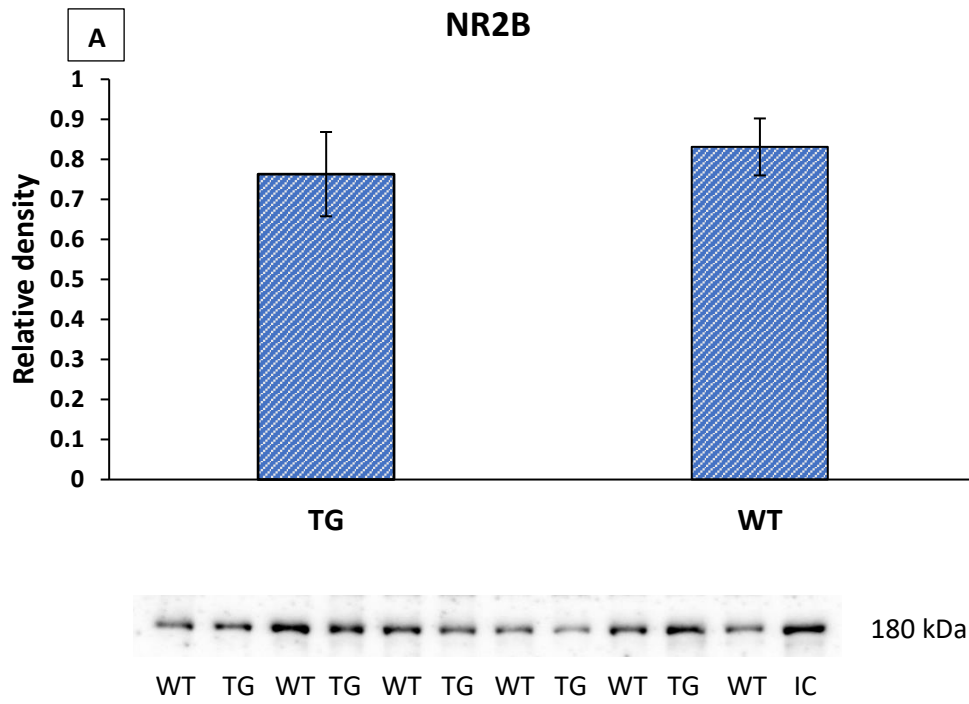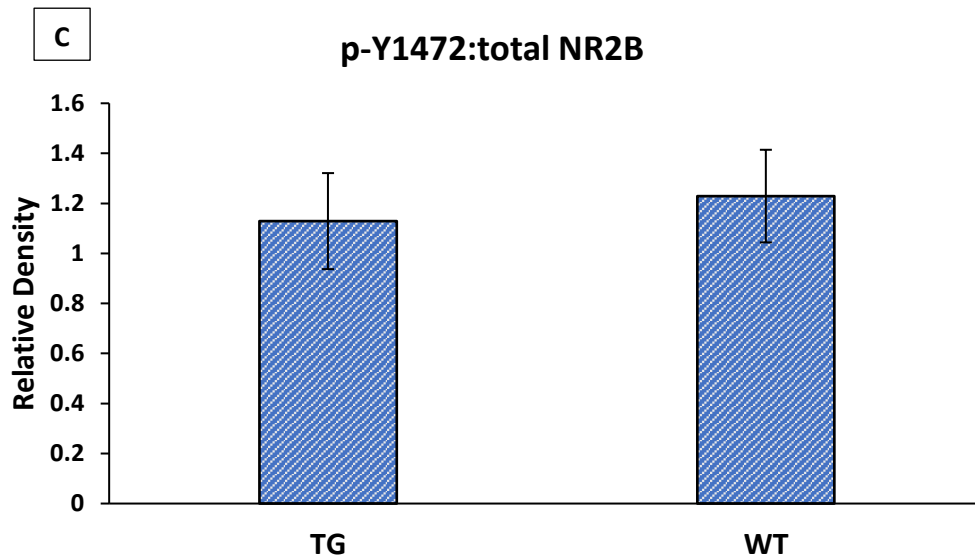
NR2B

p-Y1472 NR2B

Figure 5.14. Mean relative densities and representative Western blot images of NR2B (A), p-Y1472 NR2B (B), and the p-Y1472 NR2B:total NR2B ratio (C) in hippocampal synaptosomal extracts from 4-5 month old Tg2576 and wild-type mice. n = 10 Tg2576 and 11 wild-type mice. IC = internal control.

5.5.2.2 Cortical SERT expression

Inspection of Figure 5.15 indicates that Tg2576 mice display a slightly raised cortical SERT expression compared with wild-type mice at 4-5 months of age. A Student's t-test revealed this genotype difference to be non-significant ($t(19) = 1.536$, $p = .141$, $d = 0.671$), while a Bayesian independent samples t-test was not especially informative regarding the absence of a genotype difference ($BF_{10} = 0.882$).
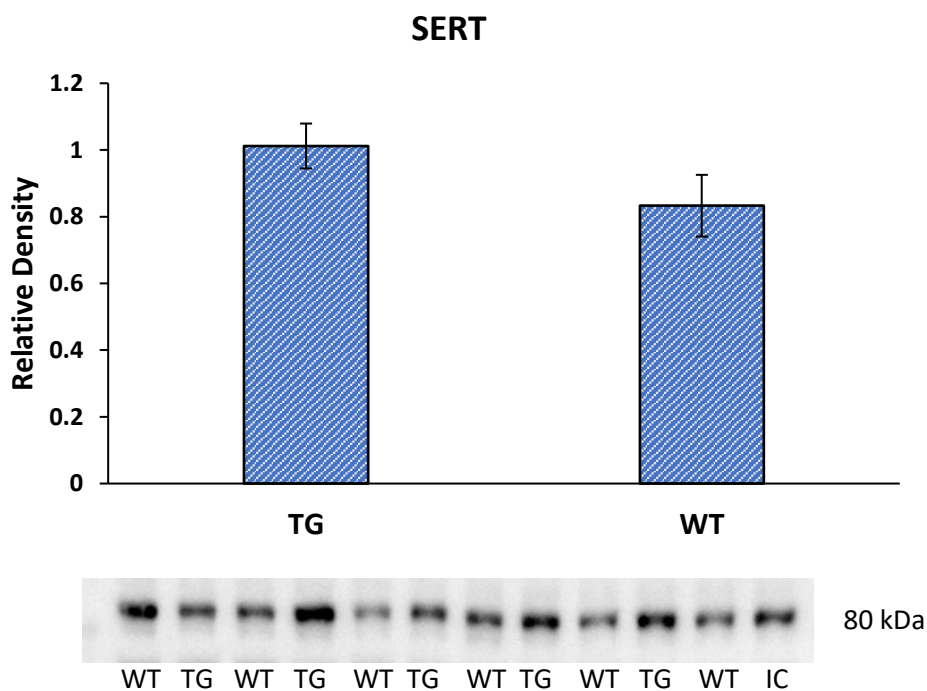
Figure 5.15. Mean relative density and representative Western blot image of SERT in cortical synaptosomal extracts from 4-5 month old Tg2576 and wild-type mice. n = 10 Tg2576 and 11 wild-type mice. IC = internal control.

5.5.2.3 Opioid receptors

Inspection of panel A of Figure 5.16 indicates that Tg2576 mice display a numerically higher, but not notably so, hippocampal expression of mu opioid receptor than wild-type mice at 4-5 months of age. A Student's t-test was consistent with this impression, revealing a non-significant difference between genotypes (t(19) = 1.340, p = .196, *d* = 0.586). A Bayesian independent samples t-test provided no clear evidence regarding the absence of a genotype difference (BF$_{10}$ = 0.731). Inspection of panel B of Figure 5.16 indicates that cortical mu opioid receptor expression displays a similar pattern of results as in the hippocampus, with numerically greater expression in 4-5 month old Tg2576 mice. A Student's t-test revealed this to be a non-significant genotype difference (t(19) = 1.941, p = .067, *d* = 0.848), while a Bayesian independent samples t-test provided inconclusive evidence regarding the presence of a genuine genotype difference (BF$_{10}$ = 1.392).

A Mu opioid receptor (hippocampus)

TG WT TG WT TG WT TG WT TG WT IC

47 kDa

B Mu opioid receptor (cortex)

WT TG WT TG WT TG WT TG WT TG WT IC

47 kDa

148
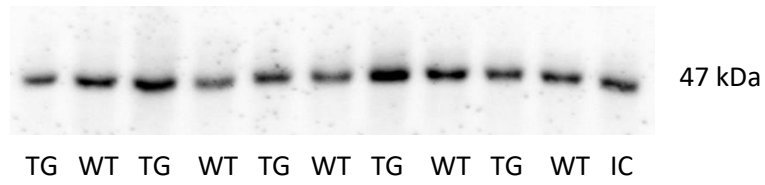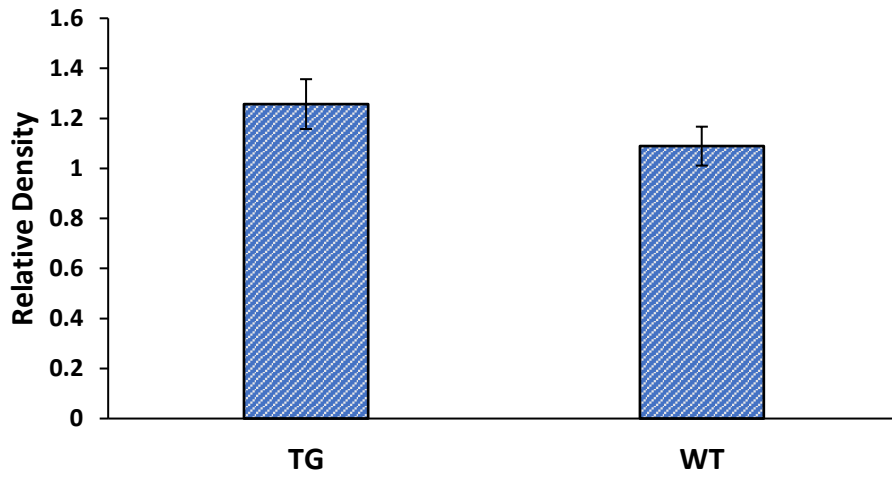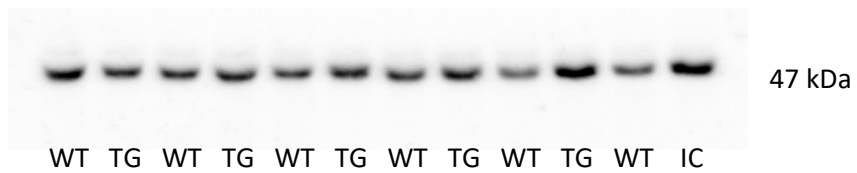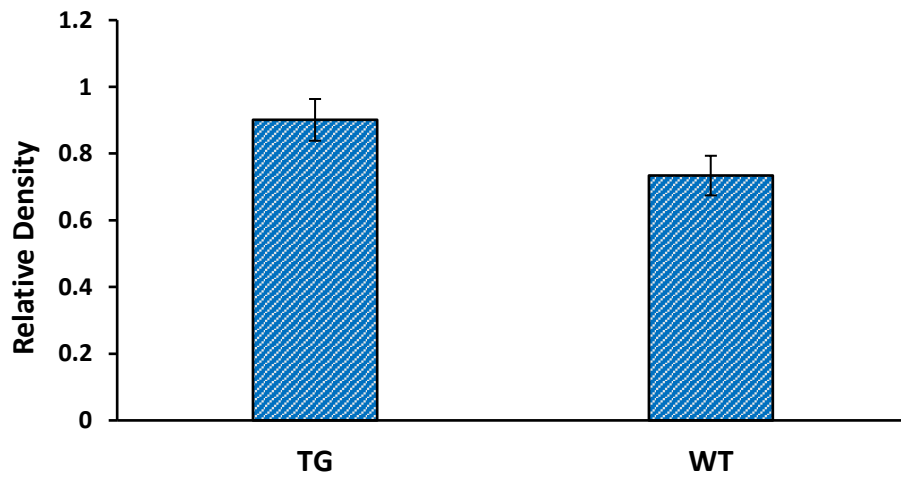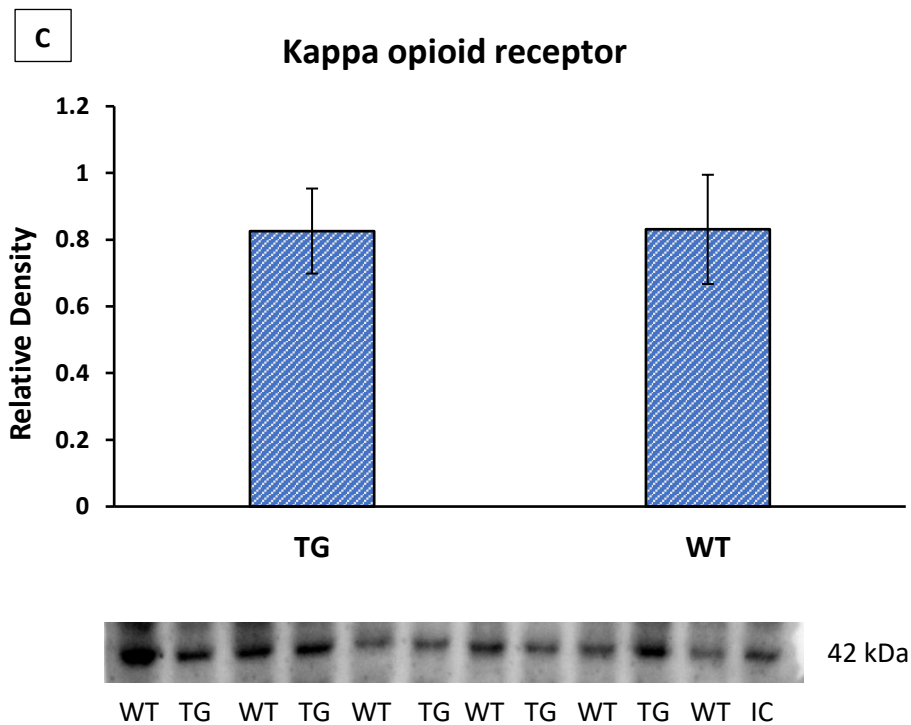
Figure 5.16. Mean relative densities and representative Western blot images of hippocampal (A) and cortical (B) mu, and cortical kappa (C), opioid receptor expression in hippocampal and cortical synaptosomal extracts from 4-5 month old Tg2576 and wild-type mice. n = 10 Tg2576 and 11 wild-type mice. IC = internal control.

Inspection of panel C of Figure 5.16 indicates that cortical kappa opioid receptor expression did not materially differ between Tg2576 and wild-type mice, at 4-5 months of age. A Student's t-test was consistent with this impression, revealing a non-significant genotype difference ($t(19)$ = 0.024, p = .981, d = 0.011), and a Bayesian independent samples t-test provided evidence consistent with an absence of a genotype difference (BF_{10} = 0.391).

5.5.3 Summary

Experiment 9 revealed that at 4-5 months of age, Tg2576 mice showed no significant reduction in hippocampal NR2B, p-Y1472 NR2B or the p-Y1472 NR2B:total NR2B ratio compared with wild-type mice. In addition, young Tg2576 mice displayed no significant increase in cortical SERT, hippocampal or cortical mu opioid receptor, and cortical kappa opioid receptor expression, compared with wild-type mice. The strongest evidence provided by Bayesian analyses for an absence of genotype difference was in cortical kappa opioid receptor expression and hippocampal p-Y1472 NR2B:total NR2B expression. These results stand in contrast with those from Experiments 7 and 8, in which older Tg2576 mice displayed a reduction in the hippocampal p-Y1472 NR2B:total

NR2B ratio, and an increase in hippocampal mu opioid receptor expression (in a subset of mice), and cortical kappa opioid receptor and SERT expression (Table 5.1). In the case of the kappa opioid receptor, there is a distinct difference between aged and young Tg2576 mice, in that there was a significantly raised expression in older Tg2576 mice and the absence of a genotype difference in younger mice. A distinct difference was also seen between aged and young Tg2576 mice regarding the hippocampal phosphorylated:total NR2B ratio, albeit with slightly less conclusive evidence regarding a null genotype effect in young mice. For cortical SERT there was a significant increase in older Tg2576 mice, with less informative evidence supporting a null genotype effect in younger mice. For hippocampal mu opioid receptor expression, the Bayesian analysis provided less clear evidence of a null genotype effect in young Tg2576 mice. In the case of cortical mu opioid receptor expression, the Bayesian analysis was not particularly informative in either age group, and was not only in the direction of a genotype difference in both Tg2576 age groups, but was slightly stronger in the younger Tg2576 mice.

Aged Tg2576 biochemical difference	Young Tg2576 biochemical difference
Reduced phospho:total NR2B ratio (HPC).	Null.
Increased mu opioid receptor (in vehicle-treated mice) (HPC).	Null.
Increased mu opioid receptor (trend) (CTX).	Increased mu opioid receptor (trend).
Increased SERT (CTX).	Null.
Increased kappa opioid receptor (CTX).	Null.

Table 5.1. Comparison of significant biochemical results from aged (19-20 month-old) Tg2576 mice (Experiments 7 and 8) against young (4-5 month-old) Tg2576 mice (Experiment 9), based on the conventional statistical analysis. CTX = cortex, HPC = hippocampus.

5.5.4 Chapter summary

Experiment 6 revealed that sub-anaesthetic ketamine treatment lowered soluble Aβ42 in the cortex and hippocampus of Tg2576 mice, while insoluble Aβ42, and both Aβ40 forms, were unaffected by this treatment in both regions. Experiment 7 demonstrated that directly following the T-maze test (Chapter 4, Experiment 5), 19-20 month-old Tg2576 mice showed a reduction in the hippocampal p-Y1472 NR2B:total NR2B ratio compared with wild-type mice. While GluR1, p-Ser845

GluR1 and the p-GluR1:total GluR1 ratio did not generally differ between Tg2576 and wild-type mice in the hippocampus and cortex, ketamine treatment did increase all three GluR1 measures across both genotypes in the hippocampus, with a near-significant increase in the p-GluR1:total GluR1 ratio in the cortex. PSD-95 expression did not differ between genotypes or drug treatment groups. Experiment 8 demonstrated that $5HT_{1B}$, $5HT_4$ and SERT expression were unaffected by ketamine treatment in mouse hippocampus and cortex, and did not differ between Tg2576 and wild-type mice, with the exception of increased SERT expression in Tg2576 cortex. Mu opioid receptor expression was increased in Tg2576 mouse hippocampus (when examining only vehicle-treated mice), with a near-significant mu receptor increase in Tg2576 cortex. Kappa opioid receptor expression was increased in Tg2576 cortex, while delta opioid receptor expression was not different to that of wild-type mice. Ketamine treatment had no apparent effect on expression of any of the opioid receptors. A summary of the significant and non-significant genotype differences found in aged Tg2576 and wild-type mice is presented in Table 5.2. As an additional note, Bonferroni corrections were also applied to the statistically significant results of Experiments 7 and 8, in order to examine whether results remained significant after having been corrected for multiple comparisons. While it was not immediately clear how best to organise different receptors investigated in different brain regions into sensible groupings to which corrections would be applied, an approach was taken as follows: corrected groups comprised receptors for a particular neurotransmitter within one brain region, for example NMDAR-related measures within the hippocampus, AMPAR-related measures within the cortex, 5HTergic-related measures within the hippocampus, opioidergic-related measures within the cortex, etc. After applying Bonferroni corrections in this way, the hippocampal p:total NR2B genotype difference, hippocampal mu receptor (within vehicle-treated mice) genotype difference and all hippocampal GluR1-related ketamine effects no longer reached statistical significance, while the cortical SERT and kappa receptor genotype differences remained statistically significant.

Aged Tg2576 biochemical difference (relative to wild-type mice)	Statistical significance
NR1 (HPC).	Non-significant.
NR2B (HPC).	Non-significant.
p-NR2B Y1472 (HPC).	Non-significant.
Reduced phospho:total NR2B ratio (HPC).	**Significant.**
GluR1 (HPC and CTX).	Non-significant.
p-GluR1 Ser845 (HPC and CTX).	Non-significant.
Phospho:total GluR1 ratio (HPC and CTX).	Non-significant.
$5HT_{1B}$ receptor (HPC and CTX).	Non-significant.
$5HT_4$ receptor (HPC and CTX).	Non-significant.
SERT HPC.	Non-significant.
Increased SERT (CTX).	**Significant.**
49 kDa mu opioid receptor (HPC).	Non-significant.
Increased 47 kDa mu opioid receptor (in vehicle-treated mice) (HPC).	**Significant.**
Increased mu opioid receptor (trend) (CTX).	**Significant (trend).**
Increased kappa opioid receptor (CTX).	**Significant.**
Delta opioid receptor (CTX).	Non-significant.

Table 5.2. Description of significant (bolded) and non-significant (unbolded) findings from aged Tg2576 and wild-type mice (Experiments 7 and 8), based on conventional significance testing.

Experiment 9 served as a control test to determine whether APP overexpression per se could produce the biochemical differences observed between aged Tg2576 and wild-type mice, by examining both genotypes at a younger age (4-5 months old). While conventional significance testing revealed non-significant genotype differences in young mice for all receptors of interest, the Bayesian analysis indicated differences in the strength of evidence for null genotype effects. Hippocampal p-Y1472 NR2B:total NR2B ratio and the cortical kappa opioid receptor had the strongest Bayesian evidence for not differing between Tg2576 and wild-type mice at this age, while the Bayesian evidence supporting no genotype difference was less conclusive for hippocampal and cortical mu opioid receptor, and for cortical SERT expression. The major findings of importance for

this thesis are the hippocampal p-Y1472 NR2B:total NR2B ratio decrease, and the cortical SERT and kappa opioid receptor increases, in Tg2576 mice, discussed below. How the overall biochemical results relate to the hedonic and cognitive deficits displayed by Tg2576 mice in Chapters 3 and 4, respectively, as well as the effects and non-effects of ketamine treatment, will now be discussed.

5.6 Discussion

Experiment 1 in Chapter 3 gave a longitudinal profile of the hedonic behaviour of Tg2576 mice, in which they showed a hedonic deficit which emerged between 4-5 and 8 months of age. Experiment 2 in the same chapter revealed that sub-anaesthetic ketamine treatment did not improve this hedonic deficit in Tg2576 mice. Chapter 5 has examined two neurotransmitter systems (serotonergic and opioidergic) in order to investigate whether they showed any alterations which might relate to either anhedonia or the lack of a ketamine effect on anhedonia in Tg2576 mice. In addition, gauging the state of these transmitter systems in Tg2576 mice may suggest further avenues for exploration in the treatment of depression in early Alzheimer's disease.

5.6.1 $5HT_{1B}$ receptor expression

Previous research had found a decrease in hippocampal $5HT_{1B}$ expression in Tg2576 mice (Tajeddinn et al., 2015), which this project did not replicate in either Tg2576 hippocampus or cortex. The study of Tajeddinn et al reported that Tg2576 mice at 2 years of age showed a reduced hippocampal $5HT_{1B}$ expression compared with wild-type mice, however some differences between this earlier study and the present results of this chapter should be commented on. Firstly, Tajeddinn et al used only female Tg2576 mice, whereas only male Tg2576 mice were used in the experiments of this thesis. This is highly likely to produce differences in biochemical measurements, as the 4-5 day oestrus cycle of rodents includes fluctuating levels of sex hormones such as oestradiol, progesterone and luteinising hormone (Caligioni, 2009; Goldman, Murr, & Cooper, 2007). Such hormonal changes could cause differences in $5HT_{1B}$ receptor expression; oestradiol treatment in ovariectomised rats decreased $5HT_{1B}$ mRNA in a subregion of the dorsal raphe nuclei, for example (Hiroi & Neumaier, 2009). In addition, Tajeddinn et al appear to have examined the $5HT_{1B}$ receptor in hippocampal lysates, whereas Experiment 8 examined $5HT_{1B}$ receptor expression within the narrower pool of synaptosomes in order to investigate synaptically-related changes. A large proportion of $5HT_{1B}$ receptors are stored in intraneuronal vesicles (Liebmann et al., 2012), so divergences in findings could be due to the fact that different cellular fractions were scrutinised. Lastly, Tajeddinn et al appear to have used behaviourally naïve Tg2576 mice, while the Tg2576 mice

in this thesis experienced multiple hedonic and cognitive behavioural tests before being sacrificed for biochemistry. Experience-dependent alterations to $5HT_{1B}$ receptor expression therefore cannot be ruled out in this thesis. In sum, while the $5HT_{1B}$ receptor expression results shown in Experiment 8 were not in agreement with a previous report, this could be due to a combination of sex differences, the precise cellular fraction being investigated and the prior experience of Tg2576 mice.

The $5HT_{1B}$ receptor was investigated due to its presence in the nucleus accumbens and ventral pallidum being implicated in the antidepressant mechanism of action of ketamine (Yamanaka et al., 2014). As ketamine did not improve the hedonic behaviour of Tg2576 mice, whom in a previous report showed a reduction in hippocampal $5HT_{1B}$ expression, it was thought that such a reduction could provide indirect evidence explaining why ketamine failed to relieve the Tg2576 mouse hedonic deficit. As no $5HT_{1B}$ reduction was observed in Tg2576 hippocampus or cortex in Experiment 8, the failure of ketamine may lie elsewhere biochemically, though this has not conclusively ruled out localised $5HT_{1B}$ receptor changes in discrete Tg2576 brain structures, such as the nucleus accumbens or ventral pallidum.

5.6.2 $5HT_4$ receptor expression

$5HT_4$ receptor expression was investigated in Tg2576 mouse hippocampus and cortex due to it potentially being altered by the presence of Aβ, as well as possibly contributing to Tg2576 cognitive and/or hedonic deficits. The $5HT_4$ receptor has a complex relationship with Aβ, as cell culture and animal studies have reported that $5HT_4$ receptor agonism stimulates sAPPα secretion and lowers Aβ release (Cachard-Chastel et al., 2007; Cho & Hu, 2007), including reducing Aβ in Alzheimer's disease model mice (Baranger et al., 2017; Giannoni et al., 2013; Tesseur et al., 2013). One possibility, therefore, is that $5HT_4$ receptor activation may shift APP processing towards the non-amyloidogenic pathway. However, chronic $5HT_4$ receptor activation has also been shown to increase sAPPβ production (Tesseur et al., 2013), meaning that its effect on APP may not be simply shifting APP processing away from the amyloidogenic pathway. Regardless, the fact that insoluble Aβ-burdened Alzheimer's disease patients show increased brain $5HT_4$ receptor expression (Madsen et al., 2011), and that $5HT_4$ receptor activation decreases Aβ levels in Alzheimer's disease model mice, suggested that this receptor may respond to the presence of Aβ and thus may be altered in Tg2576 mice. In addition, the $5HT_4$ receptor has been implicated in cognition and hedonics by pharmacological and genetic knockout rodent studies (Amigó et al., 2016; Hagena & Manahan-Vaughan, 2017; Lucas et al., 2007), and thus could contribute to the deficits displayed by Tg2576 mice in Chapters 3 and 4.

Experiment 8 revealed no difference in 5HT$_4$ receptor expression between Tg2576 and wild-type mice, in either the hippocampus or cortex. While 5HT$_4$ receptor agonists have been used in Alzheimer's disease model mice, this is the first report of expression of the receptor itself in such a mouse model, and suggests that the 5HT$_4$ receptor may not be synaptically altered in mouse models of Alzheimer's disease. This result differs from a study of humans in an earlier stage of Alzheimer's disease in which 5HT$_4$ receptor expression was increased (Madsen et al., 2011), though in principle other pathological events absent in Tg2576 mice, such as tau NFTs, could also cause biochemical changes. Investigating 5HT$_4$ receptor expression in Alzheimer's disease patients with and without anhedonic symptoms could clarify whether a relationship exists between 5HT$_4$-mediated signalling and anhedonia. Further, the 5HT$_4$ receptor can exist in several splice variants with different C-terminal amino acid sequences, some of which differ between mice and humans in the same splice variant (Claeysen, Sebben, Becamel, Bockaert, & Dumuis, 1999). If the 5HT$_4$ receptor does react, whether directly or indirectly, to Aβ then species differences in C-termini or in variant expression could account for different 5HT$_4$ receptor levels seen across species in which Aβ has accumulated (for example, if the C-termini of 5HT$_4$ receptors react to intracellular Aβ). As 5HT$_4$ receptor expression was not altered in synaptosomal extracts from 19-20 month-old Tg2576 mice in Experiment 8, this thesis provides no evidence suggesting this receptor may be involved in the hedonic and cognitive deficits revealed in Chapters 3 and 4, respectively. However, targeting this receptor could still prove useful as an approach for lowering Aβ levels and, given that 5HT$_4$ receptor agonists have improved cognition and hedonic behaviour in mice (Hagena & Manahan-Vaughan, 2017; Lucas et al., 2007), it would be interesting to see whether such drugs could improve the Tg2576 behavioural deficits seen in Chapters 3 and 4. Importantly, prior studies assessing the impact of 5HT$_4$ receptor knockout and agonism on mouse hedonic behaviour have used raw sucrose solution intake as a hedonic index (Amigó et al., 2016; Lucas et al., 2007). Gauging the impact of activating 5HT$_4$ receptors using a more appropriate technique such as lick cluster analysis would provide a more informative measure of hedonic responsiveness. Lastly, ketamine treatment had no observable effect on hippocampal or cortical 5HT$_4$ receptor expression in either Tg2576 or wild-type mice. While some component of the antidepressant effect of ketamine may depend upon serotonergic signalling (du Jardin et al., 2016; Gigliucci et al., 2013; Pham et al., 2017; Yamanaka et al., 2014), the treatment itself does not appear to alter 5HT$_4$ receptor expression.

5.6.3 SERT expression

Experiment 8 also revealed that synaptic SERT expression was not altered in 19-20 month-old Tg2576 hippocampus, but was elevated in Tg2576 cortex compared with wild-type mice. This

differs from a previous report showing reduced SERT expression in Tg2576 hippocampus (Tajeddinn et al., 2015), however, the same caveats mentioned above regarding differing 5HT$_{1B}$ receptor results apply. That is, the Tajeddinn et al study used all female (behaviourally naïve) Tg2576 mice, and appeared to examine hippocampal lysate rather than synaptosomal extracts, whereas this thesis used all male Tg2576 mice which had undergone multiple behavioural tests, and examined synaptosomal SERT expression, which could account for differences in observed hippocampal results. The Tg2576 elevated cortical SERT expression seen in Experiment 8 is contrary to studies in APP/PS1 mice, in which SERT expression is reduced in the dorsal raphe (Metaxas et al., 2018), and frontal and parietal cortex (Metaxas et al., 2019), however, again these were studies using exclusively or overwhelmingly female mice. In addition, APP/PS1 mice also display a loss of serotonergic axons with age in parts of the hippocampal formation, as well as in motor and barrel cortex (Liu et al., 2008). However, this latter study did not report the sex of mice used, and also examined mice only harbouring the Swedish APP mutation, expressed in Tg2576 mice, which did not display any abnormalities in serotonergic axonal density (Liu et al., 2008). Further, SERT expression has also been studied in male 3xTgAD mice, which show an increase in SERT-immunoreactive fibre density in the stratum molecular layer of CA1 (Noristani, Meadows, Olabarria, Verkhratsky, & Rodríguez, 2011; Noristani, Olabarria, Verkhratsky, & Rodríguez, 2010). The present literature on whether SERT expression is altered in Alzheimer's disease mouse models is thus unclear, and comparisons across studies are difficult due to differences in the sex of mice used, specific mutations and pathology present (i.e. PS1 and tau mutations, tau or accelerated Aβ pathology), the cellular fraction investigated, and the technique being used (e.g. Western blotting or immunohistochemistry). Experiments comparing SERT expression in both Alzheimer's disease mouse models and human patients, of both sexes, may resolve the apparently contradictory findings.

It is not immediately obvious what explains the elevated Tg2576 cortical SERT expression seen in this chapter. While the report of Metaxas et al using APP/PS1 mice did not report an increase in cortical SERT expression, their study did reveal that Aβ40 diminishes the activity of SERT in terms of serotonin uptake (Metaxas et al., 2019). Given that ketamine treatment in this thesis only reduced soluble Aβ42, not Aβ40, in Tg2576 mice, then the increased Tg2576 cortical SERT expression across treatment groups could represent a compensatory response to lowered SERT activity. Other possibilities could include a response to changes in serotonin availability or the activity of other 5HT receptors not investigated, though this would need to be confirmed by further study. Whether this SERT alteration in Tg2576 mice could relate to the hedonic deficit documented in Chapter 3 is unclear. There is some limited preclinical evidence potentially linking SERT with anhedonia, in that hippocampal SERT reduction has co-occurred with anhedonic behaviour (Tang et al., 2014, 2013),

and fluoxetine treatment both increased SERT and improved hedonic behaviour (measured by sucrose preference test) (Tang et al., 2013). However, this does not directly link SERT with anhedonia and involves a SERT reduction rather than elevation. Further investigations of SERT in Tg2576 mice, such as the functionality of SERT in this mouse model, and whether specifically targeting SERT improves hedonic behaviour as measured by lick cluster analysis, would be useful.

5.6.4 Opioid receptor expression

The three major opioid receptors (mu, kappa, and delta) were also investigated in Tg2576 mice as potential mediators of the hedonic deficit detailed in Chapter 3. While delta opioid receptor expression was not altered in Tg2576 mouse cortex, hippocampal mu expression was increased in vehicle-treated Tg2576 mice, and cortical mu expression was non-significantly elevated in Tg2576 mice overall. Importantly, kappa opioid receptor expression was greater in Tg2576 mouse cortex. In general, studies of depressive behaviour in rodents have revealed that agonism of mu and delta opioid receptors has an anti-depressant effect, while kappa opioid receptor agonism has a pro-depressive effect (Lutz & Kieffer, 2013). In addition, several behavioural studies have suggested that the kappa receptor could mediate anhedonia specifically. For example, kappa opioid receptor agonists increase the intra-cranial self-stimulation threshold in rats (Carlezon et al., 2006; Todtenkopf, Marcus, Portoghese, & Carlezon, 2004), which is taken as an indicator of anhedonia (Der-Avakian & Markou, 2012). Kappa receptor agonists also decrease mouse self-grooming in the splash test (Butelman, McElroy, Prisinzano, & Kreek, 2019), something also used as an indicator of anhedonia. Such behavioural changes can be prevented or diminished by the use of kappa opioid receptor antagonists (Butelman et al., 2019; Chartoff et al., 2012), and there is interest in the use of kappa opioid receptor antagonists as antidepressant and anti-anhedonic agents (Carlezon & Krystal, 2016; Peciña et al., 2019). While these behavioural studies have not used direct measures of hedonic behaviour or response, there is speculation that hedonic tone is balanced on the one (positive) side by mu and delta opioid receptors, and on the other (negative) side by kappa opioid receptors (Bailey & Husbands, 2018). If this is indeed the case, then the increase in kappa opioid receptors in Tg2576 cortex could decrease the hedonic response of Tg2576 mice, and the non-significant mu opioid receptor increase could potentially represent a compensatory process. Importantly, this Tg2576 cortical kappa opioid receptor increase was not present at 4-5 months of age, an age when Tg2576 mice show normal hedonic functioning. This is not direct proof that the elevated kappa opioid receptor expression seen in Tg2576 cortex mediates the hedonic deficit seen in these mice. However, given the linkage of the kappa opioid receptor with other, less direct, measures of

anhedonia, the results of this chapter are certainly suggestive that the kappa opioid receptor could underlie the hedonic deficit seen in Tg2576 mice.

Opioid receptors have not previously been investigated in Alzheimer's disease mouse models, and so this is not only the first report of an altered opioid receptor system in such a mouse model, but also pairs the kappa receptor elevation with a hedonic deficit which it may mediate. However, the functional status of kappa opioid receptors in Tg2576 mice is not known, and future studies should measure not only receptor expression and functionality, but also the levels of the endogenous ligands which activate opioid receptors, such as dynorphins, enkephalins and β-endorphin, in order to provide a full picture of the opioid system in Tg2576 mice. Investigating the potential mechanism underlying this kappa receptor elevation is also important, whether it be a direct result of Aβ accumulation, or a response to the consequences of Aβ, such as excitotoxicity or inflammation. A critical follow-up to this finding of increased kappa opioid receptor expression in Tg2576 mice would be trialling a kappa receptor antagonist in these mice, in order to ameliorate their hedonic deficit. It is noteworthy in this context that ketamine treatment failed to improve the hedonic behaviour of Tg2576 mice, and also failed to reduce kappa opioid receptor expression. However further work is needed to establish whether these two phenomena are directly related.

5.6.5 Glutamate receptor subunits

The NMDAR and AMPAR profile revealed in Experiment 7 suggests that the glutamatergic system in these 19-20 month-old Tg2576 mice is selectively rather than generally altered. That is, Tg2576 mice show a reduction in the hippocampal p-Y1472 NR2B:total NR2B subunit ratio within the NMDAR, while the NR1 subunit, along with AMPAR subunits GluR1 and p-Ser845 GluR1, did not differ between genotypes. Importantly, this relative reduction in NR2B phosphorylation was not seen in younger (4-5 month-old) Tg2576 mice. In addition, PSD-95 expression was not disturbed in Tg2576 mice. Some of these findings are in keeping with previous studies while others are distinct from prior reports. For example, cultured cortical Tg2576 neurons have shown lowered surface NR1 availability (Snyder et al., 2005), whereas a change in synaptic NR1 expression was not seen in Tg2576 hippocampal tissue in this chapter. The same study which revealed this NR1 internalisation also demonstrated that NR2B endocytosis and dephosphorylation at Y1472 are induced by Aβ in cultured neurons (Snyder et al., 2005), which is consistent with the relatively reduced NR2B phosphorylation observed in Tg2576 hippocampus in Experiment 7. Reductions in both NR2B and p-Y1472 NR2B have also been observed in cortical synaptosomal fractions from 9 month-old and 12 month-old, but not 3 month-old, Tg2576 mice (Kurup et al., 2010; Zhang et al., 2010), which

reinforces the finding in this chapter that synaptic NR2B (Y1472) dephosphorylation appears to be an age-related occurrence in Tg2576 mice. This could suggest that changes in the phosphorylation state of the NR2B subunit in Tg2576 mice occur gradually as Aβ accumulates, either due to Aβ acting on NMDARs or as a compensatory response to Aβ-induced changes.

These glutamatergic receptor components were primarily investigated in relation to the Tg2576 object-in-place deficit documented in Chapter 4. In this context it is notable that PDAPP mice, which develop an object-in-place deficit with age, have shown a strikingly similar profile of reduced p-Y1472 NR2B:total NR2B ratio in hippocampal synaptosomes, while NR1, NR2B and PSD-95 were unaltered (Evans et al., 2019). Importantly, administration of an antibody which lowered soluble Aβ levels in the hippocampus, and increased NR2B phosphorylation at the Y1472 residue, abolished the object-in-place task deficit in PDAPP mice. The importance of the NR2B subunit in object-in-place task performance was confirmed in this study by a further experiment, in which a selective NR2B antagonist administered to wild-type mice impaired their performance in the object-in-place task (Evans et al., 2019). The reduction in the ratio of hippocampal phosphorylated to total NR2B, at the tyrosine 1472 residue, could therefore account for the Tg2576 object-in-place deficit. NMDARs in general are thought to allow for the encoding of memories (Morris, 2013), and inhibition of NMDARs in the hippocampus prevents acquisition but not retrieval of object-in-place memory (Barker & Warburton, 2015). The NR2B subunit of the NMDAR is at least partially responsible for LTP induction, and is particularly important in mediating synaptic plasticity and information acquisition by interacting with CamKII (Shipton & Paulsen, 2014). The tyrosine 1472 residue is the major phosphorylation site on the NR2B subunit, and its phosphorylation promotes synaptic retention while dephosphorylation encourages endocytosis of the NMDAR (Prybylowski et al., 2005; Zhang, Edelmann, Liu, Crandall, & Morabito, 2008). A relative reduction in the availability of NMDARs, caused by dephosphorylation, would thus presumably mean diminished NMDAR currents, and a compromised facility to encode object-in-place information in Tg2576 mice. As synaptosomal preparations can contain endocytotic vesicles (Bai & Witzmann, 2007), the reduced hippocampal NR2B phosphorylation ratio in Tg2576 mice may indicate that there are less NR2B subunit-containing NMDARs present at the membrane surface, which would be consonant with a failure of information encoding in Tg2576 mice. This altered NR2B phosphorylation status could be a response to the accumulation of Aβ in Tg2576 mice, as Aβ can activate NMDARs both directly (Texidó et al., 2011), and indirectly by increasing glutamate availability (Li et al., 2009; Talantova et al., 2013). Such direct and indirect NMDAR activation could lead to NR2B dephosphorylation as a method of decreasing excitotoxicity (Liu et al., 2007).

While a selective NMDAR subunit disturbance which could contribute to the Tg2576 object-in-place deficit was found in this chapter, no reductions in synaptic GluR1, p-Ser845 GluR1 or PSD-95 were found in Tg2576 mice. This is in contrast to other studies in which Tg2576 cultured neurons show reductions in GluR1 and PSD-95 (Almeida et al., 2005), and Tg2576 mouse hippocampus shows trafficking of GluR1 away from the synapse and an overall reduction in p-Ser845 GluR1 (D'Amelio et al., 2011). The fact that these changes were not found in the present results could be explained by the fact that the GluR1 and p-Ser845 GluR1 changes were found in young Tg2576 mice, meaning that GluR1 levels could potentially readjust over time. Another difference is the extensive behavioural testing schedule all aged mice went through in this thesis, which could in and of itself produce biochemical changes. For example, Tg2576 mice exposed to only 6 days of water maze training show an increased presence of GluR1 and PSD-95 in the hippocampus, while NR2B remained unaffected (Jiang et al., 2015). Therefore there could potentially be some contribution of GluR1 and/or PSD-95 to the Tg2576 object-in-place deficit under 'typical' conditions, and the use of repeated cognitive testing may obscure their impact.

While the use of sub-anaesthetic ketamine treatment reduced soluble Aβ42 levels, it did not alter NR2B phosphorylation and failed to improve Tg2576 performance in the object-in-place task. As the current, broadly accepted hypothesis of Alzheimer's disease is that Aβ is the beginning of a pathogenic cascade (Selkoe & Hardy, 2016), one interpretation of these possible incongruities is simply that by 19-20 months of age the pathological cascade in Tg2576 mice is well established, with a number of neuronal insults being long-present and unaffected by Aβ reduction by this point. If this is the case, an earlier intervention with ketamine, coupled with a longer treatment duration, could be a more sensible intervention. Other possible interpretations include there being an Aβ threshold for cognitive (and hedonic) impairments (both in terms of behaviour and underlying biochemistry), below which ketamine treatment did not push the Aβ concentration. There could also possibly be roles for Aβ40, which was not reduced by ketamine, and Aβ plaques (as insoluble Aβ was not reduced) which can act as reservoirs of soluble Aβ. It is possible that in older Alzheimer's disease model mice, which have already accrued a number of behavioural deficits, treatments that either specifically target precise neurotransmitter systems, or have this targeted effect alongside an Aβ-lowering approach, may be the most effective. For example, the study of Evans et al found that an antibody aimed at lowering Aβ production reduced both soluble Aβ40 and increased NR2B phosphorylation in PDAPP mouse hippocampus, and rescued performance in the object-in-place task (Evans et al., 2019). This distinction in terms of affecting or not affecting NR2B phosphorylation may explain the difference in behavioural effects of these two Aβ-lowering agents. Potentially Aβ was for some reason more sensitive to the effects of ketamine than was the NR2B subunit, or a higher

ketamine dose may have been needed to alter NR2B phosphorylation. While ketamine treatment did, however, generally increase both GluR1 and p-Ser845 GluR1 in Tg2576 (and wild-type) mice, it is not surprising that this did not improve object-in-place performance, as the Tg2576 deficit may be a specific result of an NR2B-containing NMDAR deficiency in the hippocampus.

In sum, the results of this chapter reveal specific disturbances to the glutamatergic, opioidergic and serotonergic neurotransmitter systems in 19-20 month-old Tg2576 mice. Namely, these are a relative decrease in hippocampal NR2B Y1472 phosphorylation, and increased expression of cortical kappa opioid receptor, hippocampal mu opioid receptor (in a subset of mice), and cortical SERT. The kappa and mu opioid receptor changes may indicate an unbalanced hedonic tone which is weighted towards anhedonia, and the NR2B dephosphorylation may relate to a failure to encode object-location associations. The effect of the cortical SERT increase, however, is unclear. While these changes may be the result of a protracted period of Aβ accumulation, reducing Aβ levels with ketamine did not relieve these behavioural problems. This suggests either more targeted treatments or alternative ketamine regimens should be trialled in future behavioural studies. Further interpretations of the results of this chapter, along with the broader implications of this thesis as a whole, will be considered next.

Chapter 6: General Discussion

6.1 Summary of results

The aims of this thesis were to profile hedonic and cognitive behaviour in Tg2576 mice, using lick cluster analysis and the object-in-place task, respectively. A longitudinal approach was used in these tasks, as age-related deteriorations would be consistent with chronic Aβ accumulation rather than APP overexpression per se. Further, ketamine treatment and the biochemistry potentially underlying the behavioural deficits were investigated, including a young cohort analysed to control for the simple presence of APP overexpression. In Chapter 3, Experiment 1 revealed an age-dependent decrease in lick cluster size in Tg2576 mice, consistent with a hedonic deficit, although the age by genotype interaction was only found to be significant in a secondary statistical analysis. The absence of this hedonic deficit in Tg2576 mice at 4-5 months of age was consistent with it being an Aβ-related impairment, rather than being attributable to APP overexpression per se. Experiment 2 in Chapter 3 revealed that sub-anaesthetic ketamine, at a dose generally beneficial in alleviating depressive behaviour in other rodent studies, failed to improve hedonic responsiveness in Tg2576 mice at 19 months of age.

The cognitive ability of Tg2576 mice was examined longitudinally in Chapter 4, using the object-in-place task. Experiment 3 did not directly establish an age-dependent cognitive deficit in Tg2576 mice using this task, which may have been due to lower object contact times with repeated testing interfering with task performance. Nonetheless, a cognitive deficit in this task was present in Tg2576 mice at 16-17 months of age, but not at any previous time points. In Experiment 4 of Chapter 4, sub-anaesthetic ketamine treatment also had no impact on object-in-place task performance at 19-20 months of age, which remained deficient in Tg2576 mice. A T-maze task examining spatial novelty preference (Experiment 5) was introduced at this final time point, in order to investigate cognition in a task not reliant upon object contact, which found that Tg2576 mice were not impaired in spatial novelty preference, suggesting a profound spatial memory deficit was not present. As was discussed in Chapters 2 and 4, studies of object recognition in Tg2576 mice have revealed what appears to be a selective deficit in object-in-place memory, in that memory for other object manipulations, such as object location novelty, remains intact (Good & Hale, 2007). It therefore seems reasonable to infer that this dissociation reflects the fact that binding specific object-location configurations and recognising objects in novel locations depend upon different memory processes. The T-maze data from Experiment 5, in which spatial novelty detection appears intact, or at the very least not obviously impaired, in Tg2576 mice with an object-in-place memory deficit, would seem to

be in keeping with this observation. However, object location novelty and T-maze spatial novelty testing are not perfectly matched tasks, and so a simple difference between the two in task sensitivity cannot be ruled out.

Despite not improving Tg2576 mouse behavioural performance, ketamine treatment was associated with reduced soluble Aβ42 in both hippocampus and cortex, in Experiment 6 of Chapter 5. Insoluble Aβ42, and both forms of Aβ40, however, were not decreased by ketamine treatment. Experiment 7 (Chapter 5) revealed that many of the glutamate receptor subunits examined (NR1, NR2B, p-NR2B, GluR1, p-GluR1 and phosphorylated:total GluR1) were generally unaltered in synaptosomal extracts from Tg2576 mice, while phosphorylated:total NR2B (Y1472) expression was reduced in Tg2576 hippocampus, consistent with a failure of NMDAR-enabled encoding in the object-in-place task. Despite not improving task performance, ketamine treatment did increase expression of all GluR1 measures in Tg2576 mouse hippocampus.

When transmitter systems related to depression were investigated, Experiment 8 in Chapter 5 demonstrated that the hippocampal and cortical synaptosomal profile of $5HT_{1B}$, $5HT_4$ and SERT expression was generally similar between Tg2576 and wild-type mice, with the exception of Tg2576 mice possessing elevated cortical SERT expression. Further, examination of the opioidergic system in Experiment 8 revealed that Tg2576 mice displayed elevated kappa receptor expression in the cortex, and an elevation of one mu receptor isoform in the hippocampus. While there was a suggestion of elevated cortical mu in Tg2576 mouse cortex, delta receptor expression was not meaningfully different between Tg2576 and wild-type mouse cortex. While the relevance of elevated cortical SERT in Tg2576 mice was uncertain, increased kappa receptor expression was proposed to mediate a negative hedonic tone, accounting for the Tg2576 hedonic dysfunction seen in Chapter 3. Elevated hippocampal mu opioid receptor in a subset of Tg2576 mice, and the suggestion of a mu increase in Tg2576 cortex, could represent a compensatory process. However, there was also the suggestion of elevated cortical mu opioid receptor expression in young Tg2576 mice, and therefore this particular observation may be unrelated to aging and Aβ. Ketamine treatment had no observable impact on any of the serotonergic or opioidergic receptors investigated. Importantly, Experiment 9 in Chapter 5 revealed that at 4-5 months of age, when no Tg2576 hedonic or cognitive deficit was present, none of the biochemical measures in which aged Tg2576 mice differed from wild-type mice (hippocampal phosphorylated:total NR2B ratio, cortical kappa, hippocampal mu, cortical SERT) were significantly different between genotypes. This supported the view that these biochemical changes were a result of, or downstream from, Aβ accumulation, rather than being caused by APP overexpression per se.

Taken together, the results of these experiments provide tentative answers to several questions posed by this thesis. Firstly, the fact that an age-related hedonic deficit was profiled in Tg2576 mice suggests that Aβ pathology is sufficient to produce anhedonic behaviour, though the precise mechanisms by which this could occur, and the brain regions this may take place within, require further investigation. Importantly, this does not discount tau species or neurodegeneration as other salient pathological events which could disrupt hedonic functioning in the course of Alzheimer's disease. This does, however, suggest that depressive symptoms such as anhedonia could be an early (that is, pre-clinical) manifestation of Alzheimer's disease. In addition, the biochemical results of this thesis indicate that the opioid system in Tg2576 mice may be altered as a result of Aβ accumulation, and that elevated kappa opioid receptor expression may represent an imbalance in hedonic tone which skews reward processing towards anhedonia. However, the route by which Aβ might be causing this disturbance is not known, though some speculation will be offered in Section 6.2. In addition, though a clear and fully informative cognitive profile in aging Tg2576 mice could not be obtained due to the effects of repeated testing, this thesis did confirm the presence of a Tg2576 object-in-place deficit from 16-17 months of age onwards. This memory impairment being present in aged Tg2576 mice, but absent in younger mice, is consistent with it being due to Aβ pathology. Examination of synaptic receptor-driven mechanisms which could account for this cognitive deficit suggested that this is an NMDAR-related dysfunction, consistent with a failure to encode specific object-location memories. However, owing to the longitudinal experimental design, impairments in AMPAR function that may disrupt object-in-place performance in Tg2576 mice could have been obscured, and therefore cannot be entirely ruled out. Lastly, one aim of the thesis was to investigate the use of a novel treatment for alleviating these hedonic and cognitive deficits, to which end sub-anaesthetic ketamine was trialled. While the particular dose and regimen used did not improve either of these impairments in Tg2576 mice, broad and firm conclusions should not be drawn from these null results, and this will be commented on further in Section 6.5.

6.2 Outstanding considerations relating to Tg2576 behavioural deficits

The view presented in this thesis thus far is that the hedonic deficit which develops in Tg2576 mice could be attributed to an altered opioidergic system, represented by an increase in cortical kappa receptor expression. In addition, the cognitive deficit that eventually manifested in Tg2576 mice has been understood in terms of a disturbed synaptic NMDAR function. However, there are other biological systems or events which could, individually or through interplay with one another, also contribute to the Tg2576 behavioural deficits documented in this thesis. In particular,

inflammation, the glutamatergic system and reduced synaptic or functional connectivity could also underlie hedonic dysfunction, while the opioidergic and cholinergic systems could contribute to impaired cognition, all of which may be caused by Aβ accumulation. These additional processes, and how they might relate to the results reported in this thesis, will be considered in turn.

6.2.1 Inflammation and anhedonia

Studies in both rodents and humans demonstrate that anhedonia may feature an inflammatory component. In otherwise healthy individuals who were administered an inflammation-inducing endotoxin, left ventral striatum activity when anticipating a monetary reward was reduced, which also mediated the relationship between endotoxin challenge and an increase in observer-rated depressed mood (Eisenberger et al., 2010). In rodents, inflammatory mediators such as IL-1β and TNF-α, and the inflammation-inducing agent lipopolysaccharide (LPS), diminish the consumption of, or preference for, palatable solutions in rodents (Brebner, Hayley, Zacharko, Merali, & Anisman, 2000; Henry et al., 2008). Taken together, such studies suggest that inflammation can be sufficient to disrupt hedonic functioning. Of note here is that Aβ activates multiple aspects of the innate immune system (Salminen, Ojala, Kauppinen, Kaarniranta, & Suuronen, 2009), and several studies have reported that Tg2576 mice exhibit neuroinflammation and a profile of elevated pro-inflammatory cytokines (Abbas et al., 2002; Apelt & Schliebs, 2001; Benzing et al., 1999; Sly et al., 2001). Therefore the hedonic deficit which develops in Tg2576 mice could contain an inflammatory component, resulting from gradual Aβ accumulation.

One specific consequence of neuroinflammation in Tg2576 mice which could produce anhedonia is activation of the kynurenine pathway. Pro-inflammatory cytokines induce the expression of indoleamine 2,3-dioxygenase (IDO), which is the rate-limiting enzyme in the conversion of tryptophan to kynurenine (Swardfager, Rosenblat, Benlamri, & McIntyre, 2016). Following the metabolism of tryptophan to kynurenine by IDO and other enzymes, kynurenine is then further metabolised to damaging compounds such as 3-hydroxy-kynurenine (3-HK), 3-hydroxy-anthranilic acid (3-HAA) and quinolinic acid (Schwarcz, Bruno, Muchowski, & Wu, 2012). While 3-HK and 3-HAA induce oxidative stress by generating free radicals, quinolinic acid is an NMDAR agonist and excitotoxin, revealing an overlap between inflammation and NMDARs (Schwarcz et al., 2012; Swardfager et al., 2016). The kynurenine pathway, specifically IDO activity, has been correlated with anhedonia scores in both major depressive disorder and combined depressed and non-depressed adolescents (Gabbay, Ely, Babb, & Liebes, 2012). Further, the ratio of serum kynurenic acid to quinolinic acid negatively correlates with anhedonia severity in patients with major depressive

disorder (Savitz et al., 2015). This suggests that redirection of the kynurenine pathway away from kynurenic acid and towards quinolinic acid production may be important in inflammation-induced anhedonia. These studies thus reveal a route by which Aβ-provoked inflammation could theoretically disrupt hedonic functioning in Tg2576 mice, with the stimulation of pro-inflammatory cytokines inducing IDO expression, activating the kynurenine pathway and then increasing production of quinolinic acid relative to kynurenic acid. In fact, studies using Alzheimer's disease brain, transgenic mice, and cultured cells lend support to the notion that Aβ could have such an effect.

Post mortem investigation of Alzheimer's disease brain has revealed that in the hippocampus, microglia and astrocytes express the greatest amount of IDO and quinolinic acid in the vicinity of Aβ senile plaques (Guillemin, Brew, Noonan, Takikawa, & Cullen, 2005), suggesting a stimulatory effect of Aβ on the kynurenine pathway, mediated by neuroinflammation. Further, 3xTgAD mice display an increase in hippocampal quinolinic acid production with age (Wu et al., 2013), consistent with, though not direct proof of, a role of Aβ. The role of Aβ has, however, been directly demonstrated in a cell culture study, which revealed that application of Aβ induces IDO expression and quinolinic acid production in human primary macrophages and microglia (Guillemin, Smythe, Veas, Takikawa, & Brew, 2003). The kynurenine system has been indirectly examined in Tg2576 mice, in which IDO expression was measured (Akimoto, Yamada, & Takikawa, 2007). While this study found no difference between 8-11 month old Tg2576 and wild-type mice in brain IDO expression, this used a relatively small number of mice and only investigated whole brain activity rather than activity in discrete brain regions. However, even with these limitations, this study did reveal that once Tg2576 mice have been exposed to a pro-inflammatory insult (LPS), IDO activity was greatly increased, suggesting to the authors that microglia in Tg2576 mice are primed to 'overreact' to an inflammatory challenge. While nothing conclusive can be stated from this one result, taken together with the broader literature, this does suggest that pro-inflammatory cytokines activating the kynurenine pathway could potentially bear upon hedonic dysfunction in Tg2576 mice, as a result of Aβ activity.

A broader related point about inflammation and anhedonia in the context of Alzheimer's disease is that inflammation itself could represent a common pathway partially accounting for anhedonia occurring alongside Alzheimer's disease. For example, variants of CD33 and TREM2, both of which genes are associated with inflammatory activity, have been identified as genetic risk factors for Alzheimer's disease (Jonsson et al., 2013, Naj et al., 2011). As inflammation may represent one risk factor underlying Alzheimer's disease, and because there may also be an inflammatory

component of anhedonia, it is possible that one mechanism by which anhedonia occurs in Alzheimer's disease is through inflammatory activity which contributes to both phenomena.

6.2.2 Glutamate and anhedonia

In addition to inflammation, altered glutamate signalling or availability also appears to contribute to anhedonia. In major depressive disorder patients, both glutamate concentrations and negative blood oxygenation level-dependent (BOLD) responses to emotional stimulation in the pregenual ACC correlated with emotional intensity ratings, which was used as a surrogate measure of anhedonia (Walter et al., 2009). A more recent study has reported that glutamate concentration in the basal ganglia positively correlated with self-reported anhedonia subscale score (Haroon et al., 2016), suggesting that altered glutamate levels or glutamatergic signalling may be a component of anhedonia. Similarly, preventing the uptake of glutamate by astrocytes, thus increasing its availability, results in increased ICSS thresholds in rodents (Bechtholt-Gompf et al., 2010; John et al., 2012), a behaviour typically interpreted as anhedonic. Cell culture experimentation has shown that Aβ stimulates the release of glutamate from both rat and human astrocytes (Talantova et al., 2013), and that Aβ42 prolongs the availability of extracellular glutamate by reducing surface expression of the glutamate transporter GLT-1 in mouse astrocytes (Scimemi et al., 2013). While this thesis did not examine glutamate concentration, an Aβ-induced increase in either glutamate concentration or time available at the synapse could be a contributor to hedonic dysfunction in Tg2576 mice. This possibility is supported by the fact that in Tg2576 hippocampal slices, transient astrocytic internal $Ca2^+$ elevations occurred with greater frequency, and NMDAR-mediated slow inward currents were enhanced in CA1 neurons, when compared with slices from wild-type littermates (Pirttimaki et al., 2013). These findings are consistent with enhanced astrocytic glutamate release due to Aβ (Pirttimaki et al., 2013). Whether this does in fact pertain to hedonic behaviour in Tg2576 mice, however, requires investigation.

Alongside disturbances in synaptic concentration or availability of glutamate itself, specific alterations in glutamate receptor subunits may also disrupt hedonic functioning. For example, mice in which the GluR1 serine 831 phosphorylation site has been mutated to alanine, preventing its phosphorylation by CamKII, display a reduced sucrose preference (Cai et al., 2013), suggesting that impaired glutamate signalling and synaptic plasticity may lead to anhedonia in rodents. Such a view seems to be further validated by the fact that GluR1 knockout mice display anhedonic behaviour when lick cluster size in response to palatable fluids is investigated (Austen et al., 2017). Taken together, such studies may suggest that the glutamate transmitter system is involved in hedonic

behaviour or experience, with normal hedonic functioning mediated at least in part by tightly regulated glutamate activity. Hedonic dysfunction may arise if glutamatergic signalling becomes deregulated, either by being dampened down (as in loss of GluR1-containing AMPARs) or excessively activated (e.g. an overabundance of glutamate). Interestingly, this thesis has documented hedonic dysfunction in Tg2576 mice in which GluR1 was not diminished in synaptosomal extracts. One possibility may be that there are multiple biochemical pathways which can diminish or interrupt hedonic processing. Within such a view, GluR1 absence may lead to anhedonia, but so equally might an imbalance in hedonic tone caused by increased kappa opioid receptor expression, as might an increase in pro-inflammatory cytokines and subsequent activation of the kynurenine pathway. Therefore the fact that this thesis reported a hedonic deficit whilst not revealing a GluR1 deficiency is not necessarily surprising. In addition, while this thesis found that phosphorylation of the Ser845 GluR1 site in Tg2576 mice was generally similar to that of wild-type mice, it did not investigate the Ser831 site, and so cannot speak to the possibility that this site could be involved in a Tg2576 hedonic deficit. Further, while this thesis did not find a GluR1 deficiency in Tg2576 mice, it did reveal a reduction in the hippocampal phosphorylated:total NR2B ratio at the Y1472 residue. Therefore another possibility is that glutamate receptor dysfunction is an important facet of anhedonia, but that multiple receptors within the glutamate system can mediate hedonic dysfunction, something which will be revisited in the next section.

6.2.3 Reduced synaptic and/or functional connectivity and anhedonia

One further consideration in how the effects of Aβ, either directly by synapse loss or indirectly by other means, could produce anhedonia is in terms of loss of synaptic or functional connectivity. Impaired connectivity in the brain's reward network has been linked to anhedonia, in adolescent depressive patients for example (Gabbay et al., 2013). In these patients, anhedonia severity negatively correlated with functional connectivity between the left nucleus accumbens and subgenual ACC and caudate, and right nucleus accumbens and occipital fusiform cortex (Gabbay et al., 2013). In addition, disturbed corticostriatal functional connectivity has been reported in people with social anhedonia (Wang et al., 2016). In patients with major depression, C-reactive protein (a general inflammatory marker) positively correlates with decreased ventral striatum and ventromedial PFC connectivity, and this decreased connectivity correlates with greater anhedonia (Felger et al., 2016). Such losses of connectivity and potential subsequent anhedonia could be produced by Aβ, as in both clinically normal and mild cognitive impairment patients, the presence of Aβ is associated with degraded functional connectivity between cortical structures (Drzezga et al., 2011; Hedden et al., 2009). The fact that these connective interruptions can be seen in clinically

normal and MCI patients suggests that they may be an early Aβ-related event in Alzheimer's disease, and could plausibly contribute to hedonic dysfunction in Tg2576 mice.

A recent hypothesis which may explain the link between connectivity and hedonic function is the 'excitatory synapse hypothesis' of depression (Thompson et al., 2015). This posits that when specific subsets of excitatory (that is, glutamatergic) synapses within the brain's reward network become weakened, the resulting diminished activity in reward circuitry, for example, can lead to depressive symptoms such as anhedonia. In such a light, antidepressant treatments such as SSRIs, electroconvulsive therapy and ketamine are thought to owe their effectiveness to their ability to strengthen excitatory synapses. As Aβ is known to reversibly reduce synapse number by interacting with NMDARs, which are expressed at excitatory synapses (Shankar et al., 2007), as well as disrupt synapse function (Mucke & Selkoe, 2012), then the degradative effects of Aβ at excitatory synapses could possibly disrupt connectivity between nodes in the reward system. Such an effect could be sufficient to produce anhedonia in Tg2576 mice. The finding in this thesis that aged Tg2576 mice show a reduction in hippocampal NR2B Y1472 phosphorylation, relative to total NR2B expression, may indicate a diminished availability of active NMDARs and thus impaired excitatory synaptic transmission. If this state exists in reward-related brain regions in Tg2576 mice, then this impaired system may disrupt hedonic processing, consistent with the 'excitatory synapse hypothesis'.

6.2.4 Summary of Tg2576 hedonic deficit interpretations

The above remarks make the point that hedonic dysfunction in Tg2576 mice could potentially be the result of multiple aberrations, all of which may be caused by Aβ accumulation. While possibly the most compelling explanation found in this thesis is an increase in kappa opioid receptor expression in Tg2576 mice, and possible resultant imbalance in hedonic tone, a role of NMDARs, glutamate more broadly, and inflammation as concomitant insults is also possible. Interestingly, a subtype of depression characterised by inflammation, elevated glutamate, anhedonia and loss of network integrity has been proposed (Haroon et al., 2018). Given that Aβ can activate multiple aspects of the innate immune system (Salminen et al., 2009), increase glutamate availability and/or neuronal sensitivity to glutamate (Li et al., 2009; Mattson et al., 1992; Talantova et al., 2013), and reduce synapse function and number (Mucke & Selkoe, 2012; Shankar et al., 2007), then it could lie at the centre of such a syndrome. The fact that Tg2576 mice have shown in this thesis a behavioural state consistent with anhedonia, while other studies have reported Tg2576 mice showing elevated pro-inflammatory cytokines (Abbas et al., 2002; Apelt & Schliebs, 2001; Benzing et al., 1999; Sly et al., 2001), and neuronal activity consistent with increased astrocytic glutamate

release (Pirttimaki et al., 2013), suggests that such a depressive phenotype may be present in this mouse model, though this requires demonstrating. A further possibility is that the elevated kappa opioid receptor expression in Tg2576 mice shown in this thesis could be provoked by elevated glutamate levels, or the prolonged synaptic presence of glutamate. Kappa opioid receptors inhibit glutamate release in the nucleus accumbens (Hjelmstad & Fields, 2001, 2003), one site in the brain's reward network, for example. Therefore changes in the opioidergic system in Tg2576 mice could represent a compensatory response to excitotoxicity, whether due to glutamate directly or to other molecules such as quinolinic acid. Taken together, these possibilities reveal that there is a rich and possibly interconnected repertoire of signalling events which may interact to produce anhedonia in Tg2576 mice, or alternatively may simply represent additive insults. Whichever of these possibilities may be the case, there would seem to be ample biological targets which future investigations of anhedonia and its treatment in Tg2576 mice could investigate. Such investigations could both reveal additional mechanisms underlying this hedonic deficit, and suggest further therapeutic approaches.

6.2.5 Opioid receptors and cognition

While the Tg2576 object-in-place memory deficit has largely been discussed thus far as representing an NMDAR-mediated dysfunction, there are other transmitter systems which could also be considered. For example, while the opioidergic transmitter system has primarily been discussed in this thesis as a regulator of hedonic tone, opioid signalling also contributes to memory performance in a variety of tasks. Antagonising mu opioid receptors in the CA3 region of the hippocampus impairs spatial learning in the Morris water maze, and mu receptor knockout mice show impaired learning in both the Morris water maze and radial maze while kappa receptor knockout mice were unimpaired (Daumas et al., 2007; Jamot, Matthes, Simonin, Kieffer, & Roder, 2003). The mu receptor increase seen in Tg2576 hippocampus (and possibly the suggestion of the same in the cortex) could therefore potentially be a compensatory process linked to both rebalancing hedonic tone and improving mnemonic ability. In addition, in mice exposed to forced swim stress, kappa opioid receptor stimulation by dynorphin appears to contribute to the stress-induced impairment in novel object recognition (Carey, Lyons, Shay, Dunton, & McLaughlin, 2009). However, procedural issues with how the authors ran the object novelty experiments meant that object novelty was combined with relative location unfamiliarity, thus it is currently unclear how kappa receptors relate to either object or location processing. While no experiments in this thesis involved exposing mice to forced swim stress, and did not examine novel object recognition memory in Tg2576 mice, the possibility of a disrupted opioidergic system contributing to the Tg2576 object-in-place memory deficit cannot be ruled out. The precise brain region and the manner in which

opioid receptors or their neurotransmitters could perturb object-in-place memory would require investigating. This thesis, for example, did not examine kappa opioid receptor expression in the hippocampus, and the perirhinal cortex and medial prefrontal cortex are other regions which it would be sensible to scrutinise, all of which contribute to object-in-place performance.

6.2.6 Cholinergic receptors and cognition

The importance of the cholinergic system in cognition has been recognised for some time (Hasselmo, 2006; Warburton et al., 2003), including in studies of recognition memory in rodents (Barker & Warburton, 2009; Winters & Bussey, 2005), though cholinergic transmission was not examined in this thesis. Interrupted cholinergic signalling due to Aβ accumulation, in particular disturbances to nicotinic cholinergic receptors, may be an additional component of the Tg2576 object-in-place memory deficit. Nicotinic cholinergic receptors are present on some percentage of hippocampal glutamatergic neurons, and increase the likelihood of LTP induction via both membrane depolarisation and providing a supplementary calcium influx (Dani & Bertrand, 2007), thus contributing to learning and memory processes. There are a number of nicotinic cholinergic receptors, and Aβ is known to bind to the α7nAchR with high affinity (Cecon et al., 2019; Wang, Lee, D'Andrea, et al., 2000; Wang, Lee, Davis, & Shank, 2000), and this binding appears to lead to the neuronal internalisation of both Aβ and α7nAchRs (Nagele, D'Andrea, Anderson, & Wang, 2002). The α7nAchR may be neuroprotective, at least earlier in the course of Alzheimer's disease, as crossing Tg2576 mice with α7nAchR knockout mice results in a number of changes at 5 months of age, including a more severe cognitive impairment and a reduction of cholinergic markers and functionality in the hippocampus (Hernandez, Kayed, Zheng, Sweatt, & Dineley, 2010). This possible neuroprotective ability may explain why the α7nAchR is upregulated in Tg2576 mouse brain from a relatively young through to older age, including in the hippocampus (Bednar et al., 2002; Dineley et al., 2001), representing a potential compensatory response to Aβ. However, in both APP/PS1 mouse and human Alzheimer's disease brain the α7nAchR appears to lose functionality in certain brain regions (Søderman et al., 2008; Wang et al., 2009), and because dissociating Aβ-α7nAchR complexes improves their functioning (Wang et al., 2009), this seems to be due to the binding of Aβ. Despite the observed upregulation of α7nAchR in Tg2576 mouse brain, therefore, the functionality of this receptor in Tg2576 mice may be compromised. The importance of this is that α7nAchRs in the medial prefrontal cortex are required for successful encoding by rodents in the object-in-place task (Sabec et al., 2018), and as such they could be another means by which task performance is impaired in Tg2576 mice. However, this has only been demonstrated at the longer delay period of 24 hours, whereas a short delay of 2 minutes was used in this thesis. Given the importance of the α7nAchR in

object-in-place task performance, and the fact that its functionality may be diminished in Tg2576 mice, the cholinergic system may also contribute to cognitive impairment in Tg2576 mice.

6.2.7 Summary of Tg2576 cognitive deficit interpretations

While this thesis has focused on hippocampal NMDARs and AMPARs as receptors of interest in the Tg2576 object-in-place deficit, a more nuanced understanding that multiple transmitter systems could feed into this deficit should be brought to bear upon future studies. There could potentially be a role for the opioidergic system in impaired object-in-place performance in Tg2576 mice, as well as the cholinergic system, in particular the α7nAchR. As with the understanding of the Tg2576 hedonic deficit, impaired cognition in Tg2576 mice could be the result of multiple and potentially interlinked disturbances, all of which may be directly or indirectly due to Aβ activity. For example, some degree of hippocampal NMDAR disruption by Aβ could be mediated through the α7nAchR; the α7nAchR can form a complex with NMDARs (Li, Li, Pei, Le, & Liu, 2012), interruption of which interferes with the ability of cholinergic transmission to strengthen NMDAR currents and enhance LTP, and impairs novel object recognition (Li, Nai, Lipina, Roder, & Liu, 2013). Importantly, disruption of the α7nAchR-NMDAR complex can be caused by Aβ42 oligomers (Elnagar et al., 2017), and if Aβ oligomers have such an effect in Tg2576 mice, then this could interfere with object-in-place task performance, depending on the brain region such an effect occurred in. In addition, changes in functional connectivity, mentioned in Section 6.2.3, could be another event which degrades cognitive ability in Tg2576 mice, and Aβ-driven neuroinflammation may contribute to this disconnectivity. For instance, in patients with MCI or probable Alzheimer's disease, higher neuroinflammation was associated with an altered functional connectivity profile which included diminished connectivity within the default mode network, though connectivity within important structures such as the hippocampus was not examined (Passamonti et al., 2019). Further, the stronger the association between inflammation and connectivity, the worse the cognition of patients, suggesting that the relationship between inflammation and altered connectivity could impair memory performance (Passamonti et al., 2019). While the relationship between neuroinflammation and connectivity does not appear to have been investigated in Tg2576 mice, impaired resting-state connectivity in this mouse model has been reported in the hippocampal network (Shah et al., 2016), and default mode network (Belloy et al., 2018). Given the documented neuroinflammation in Tg2576 mice and the ability of Aβ to provoke neuroinflammation, detailed in Section 6.2.1, an inflammatory response to Aβ could damage functional connectivity and thereby impair cognition in Tg2576 mice. In sum, the object-in-place memory deficit displayed by Tg2576 mice could be the result of multiple events, including impaired NMDAR signalling, loss of α7nAchR

function, neuroinflammation and degraded connectivity, as well as potentially altered opioid signalling. Disentangling the relevance and timing of these multiple derangements in relation to Tg2576 object-in-place performance will be an important goal of future studies.

6.3 Caveats concerning APP overexpressing transgenic mice

While APP overexpressing transgenic mice have historically been the most commonly used mouse models of Alzheimer's disease, their nature confers certain limitations upon them. APP overexpression itself could induce behavioural or biochemical changes not directly related to Aβ accumulation; expressing APP at supra-physiological levels and thus over-producing other APP fragments, for example, can potentially induce artefactual phenotypes (Saito et al., 2016). This thesis has attempted to rule out the overexpression of APP per se underlying behavioural or biochemical changes, by both longitudinally testing mice behaviourally, and including a young cohort for biochemical analysis. In Tg2576 mice, levels of the APP protein itself appear to be consistently elevated from 2 months of age onwards (Hsiao et al., 1996; Kawarabayashi et al., 2001). The fact that the Tg2576 behavioural deficits profiled in Chapters 3 and 4, and the major biochemical changes in Chapter 5, were both absent in younger mice (4-5 months of age) but present in older mice is consistent with their being due to the accumulation of Aβ. However, the involvement of other APP fragments in these changes cannot be conclusively ruled out in this thesis. In addition to APP overexpression itself, other limitations of these mouse models include the use of different promoters, APP constructs, and specific APP mutations, which complicates the comparison of results across different models (Sasaguri et al., 2017).

A more sophisticated approach to modelling Alzheimer's disease in mice has recently been established, based on the cross-breeding of mouse lines harbouring different human APP mutations, and the humanising of the murine Aβ sequence (Saito et al., 2014). The benefit of this approach is that it avoids the need for expressing APP at supra-physiological levels, thus reducing the likelihood of creating certain artefactual phenotypes (Saito et al., 2016). This technique has produced models such as NL-F and NL-G-F mice, which display Aβ accumulation, Aβ plaque deposition with associated inflammatory response, a reduction in synaptic markers, and cognitive deficits (Saito et al., 2014). This 'knock-in' approach thus allows for a 'cleaner' mouse model of early or pre-clinical Alzheimer's disease, which should provide results which are more straightforward to interpret, though these models still have limitations of their own (Sasaguri et al., 2017). While more sophisticated, this approach to modelling Alzheimer's disease is relatively new. The various neuropathological features and behavioural deficits these mice display require further investigation, and validation across

multiple laboratories, which is now beginning to occur (Masuda et al., 2016; Mehla et al., 2019). Whilst the future of modelling Alzheimer's disease in mice may lie in APP knock-in mice, at the current juncture they remain relatively new and are still being validated; the Tg2576 mouse was therefore an appropriate choice of model for this thesis, due to it being much more established at the beginning of this thesis, more widely used, and more well-characterised in terms of neuropathology and behaviour.

6.4 Do Alzheimer's disease mouse models capture neuropsychiatric symptoms?

Putting the caveats surrounding APP overexpressing mice to one side, the fact that a hedonic deficit was discovered in Tg2576 mice, and that anhedonia is present in patients with mild cognitive impairment and early Alzheimer's disease (Kumar, Jorm, Parslow, & Sachdev, 2006; Lopez et al., 2003), suggests that Alzheimer's disease mouse models could capture the neuropsychiatric, as well as cognitive, symptoms of the disease. This collection of neuropsychiatric symptoms, also known as BPSD, includes depression, anxiety, agitation and aggression, and disturbed sleep and appetite (Haupt, Kurz, & Jänner, 2000; Mirakhur, Craig, Hart, McLlroy, & Passmore, 2004; Petrovic et al., 2007). Notably, many of these non-cognitive disturbances seen in Alzheimer's disease are also observed in Alzheimer's disease mouse models. As has been mentioned in Chapter 1, depression is a fairly common symptom of Alzheimer's disease (Chi et al., 2015), and many Alzheimer's disease mouse models display some type of depressive behaviour, (Abdel-Hafiz et al., 2018; Filali et al., 2009; Iascone et al., 2013; Romano et al., 2015), although there are certain issues with the behavioural tests generally used (discussed in Chapters 2 and 3). Outside of depressive behaviour, certain disturbances in sleep and/or circadian activity have been reported in Tg2576 (Wisor et al., 2005), and APP/PS1 knock-in (Duncan et al., 2012), mice. Further, male Tg2576 mice display more aggressive behaviour than their wild-type littermates in the resident-intruder test (Alexander et al., 2011). 3xTgAD mice consistently display an elevated food intake from an early age, and in later life also have a reduced body weight compared against their wild-type counterparts (Knight, Verkhratsky, Luckman, Allan, & Lawrence, 2012). After a 12 hour fast, 3xTgAD mice also consume a greater amount of food than wild-type mice, and spend more time feeding (Adebakin, Bradley, Gümüsgöz, Waters, & Lawrence, 2012). This profile of elevated consumption alongside eventual reduced body weight is similar to the consumption and body weight profile of Tg2576 mice shown in Chapter 3, both of which are similar to reports of weight loss and hyperphagia in Alzheimer's disease (Aziz et al., 2008; Ikeda, Brown, Holland, Fukuhara, & Hodges, 2002; Morris, Hope, & Fairburn, 1989). Taken together, such results suggest that Alzheimer's disease mouse models could capture a range

of BPSD, although care should be taken when interpreting results, again due to differences in mutations, promoters, genetic backgrounds, and the use of APP overexpression. Nonetheless, if the presence of such non-cognitive symptoms of Alzheimer's disease is validated through multiple studies, including in APP knock-in mice, then it would suggest a basis for investigating the relationship between Aβ and BPSD more broadly, and for using such mouse models as a platform for testing therapies which target BPSD.

6.5 Future directions

The novel findings of this thesis largely related to the Tg2576 hedonic deficit profiled in Chapter 3, and the possible biochemical underpinnings of it (e.g. an elevated kappa opioid receptor expression). Therefore the future directions which could follow will place more weight on this aspect of the empirical data, though consideration will also be given to following up on other results from this thesis. These latter points will be covered first, followed by an exploration of how the more theoretically consequential findings could be further expanded upon.

Profiling cognitive decline in Tg2576 mice, using the object-in-place task, was in practice complicated by decreasing object contact times after the first testing period, particularly for Tg2576 mice. In addition, the repeated cognitive testing in and of itself could also influence biochemical changes that may ordinarily occur with age in Tg2576 mice, as well as potentially delay the cognitive deficit itself. This effectively means that comparing the emergence of the hedonic and cognitive deficits in Tg2576 mice in this thesis is unlikely to be informative. In hindsight the longitudinal within-subjects use of an object exploration-dependent test in Tg2576 mice was not the most appropriate methodological choice. Future experiments investigating cognition and age in Tg2576 mice may benefit from a cross-sectional rather than longitudinal design, especially if using tasks which rely upon object exploration. Further, a re-examination of the glutamate system in Tg2576 mice using such a cross-sectional approach should provide biochemical results unaltered by repeated testing, and may suggest further therapeutic approaches to improving cognition. As it stands, based on the results of this thesis, agents which precisely modulate NMDAR function, in particular the NR2B subunit, in such a way as to alleviate memory impairment under pathological conditions but not interfere with functionality under 'typical' physiological conditions, may merit investigation. However, other changes to the glutamate system, such as in scaffolding proteins such as PSD-95 or AMPAR subunits cannot be definitively ruled out in Tg2576 mice, owing to the nature of repeated testing that was employed in this thesis (Jiang et al., 2015). This will be a matter for future studies to confirm or disconfirm.

Chapters 3 and 5 demonstrated that the particular sub-anaesthetic ketamine dosage regime selected failed to improve both the Tg2576 hedonic deficit, and the receptor-based changes which could underlie the deficit (e.g. elevated kappa opioid receptor expression). However, because this thesis was both aiming to capture an age profile and test a therapeutic agent in the same cohorts of mice, this necessarily involved trialling an antidepressant agent once the hedonic deficit had been present for some time (roughly 11-12 months from its first clear emergence). Therefore future experiments going beyond this work could include initiating ketamine treatment at a much earlier time (e.g. prior to the deficit appearing), or using a longer treatment period, as well as selecting a different dose. In addition, the utility of other potential anti-depressant agents, such as kappa opioid receptor antagonists, would be useful to investigate in Tg2576 mice. Importantly, given that inflammation and the kynurenine pathway may be non-opioid related routes by which anhedonia arises in Tg2576 mice, anti-inflammatory drugs or agents which modulate the kynurenine system also merit investigation.

Chapter 3 detailed the age-related hedonic deficit displayed by Tg2576 mice, the first major finding of consequence in this thesis. While an important finding, the profiling of this deficit is the first time lick cluster size has been examined over time in an Alzheimer's disease mouse model, and thus this can be followed up in a number of ways. This hedonic deficit was seen when 4% and 16% sucrose solutions were used; a basic expansion upon these experiments would be to replicate the Tg2576 hedonic deficit, but across a larger and more comprehensive range of sucrose solutions, in order to fully capture the hedonic capacity of Tg2576 mice. In addition, the age-dependency of the Tg2576 hedonic dysfunction was only confirmed when grouping the results into 4-5 months of age compared against the lick cluster size values averaged across all later time points. Thus the precise age of emergence and trajectory could be better profiled in future experiments, by a more judicious choice of time points at which to examine lick cluster size. It would also be of interest to compare the Tg2576 lick cluster size deficit against other common mouse models of depression, e.g. the chronic unpredictable mild stress or olfactory bulbectomy models (Song & Leonard, 2005; Willner et al., 1987). Such a comparison would allow for a qualitative evaluation of the Tg2576 hedonic dysfunction relative to other 'standard' rodent depression models; that is, the questions of how similar they might look in terms of hedonic behaviour, and how treatable Tg2576 mice are compared to these other models, could be answered. Answering these questions would provide more information about the precise nature of Aβ-induced anhedonic behaviour. Given the caveats about APP overexpressing mouse models, it would be useful to examine hedonic behaviour in other Alzheimer's disease mouse models, both different APP overexpressing lines and APP knock-in mice, in order to better assess the view that this anhedonia is an Aβ-caused event. While the age-related

Tg2576 hedonic deficit is consistent with a causative role for Aβ, whether any particular Aβ species is the most salient requires further examination. Soluble Aβ dimers, trimers and dodecamers have all emerged as important pathological species in disrupting cognitive processes (Klyubin et al., 2008; Lesné et al., 2006; Shankar et al., 2008; Townsend et al., 2006), but whether these are particularly important in mediating anhedonia remains unaddressed. As a final suggestion for further investigations into the nature of hedonic dysfunction in Tg2576 mice, it should be noted that this thesis has only examined lick cluster size in direct response to the consumption of palatable solutions. That is, what has been reported is a deficit of consummatory anhedonia. However, anhedonia can be fractionated into distinct aspects, including anticipatory anhedonia, motivational anhedonia, and reward-related learning (Berridge, Robinson, & Aldridge, 2009; Rømer Thomsen, Whybrow, & Kringelbach, 2015; Treadway & Zald, 2011). This thesis was in part a preliminary investigation into hedonic behaviour in Tg2576 mice, and as such focused on one aspect of anhedonia, direct (consummatory) hedonic response. What would be especially informative is if future studies investigated the full repertoire of hedonic processes, in both Tg2576 mice specifically, and other Alzheimer's disease mouse models. Such a set of experiments could reveal whether Aβ pathology interacts with multiple components of the hedonic system in mice.

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