Investigating the Role of Normal and Abnormal Beta-Amyloid Processing in Cognition

Thomas James Freeman

A thesis submitted to Cardiff University for the degree of Doctor of Philosophy

September 2019

In loving memory of

John Freeman

“Chief”

1934 – 2017

Table of Contents

Acknowledgements

There are so many people I would like to thank for their help and support throughout my PhD that I am not sure where to start, but chronologically seems most apt. Therefore, firstly I wish to thank my supervisors, personal tutors and fellow students at the University of Southampton for supporting me through my master's degree and inspiring me to have enough confidence to attempt a PhD. Thank you to Joanna Gould for your effervescent help with the multiple applications during the period in which I was applying for laboratory assistant roles and other PhDs, I most likely would not have made it to Cardiff without your editing of my cover letter!

Thank you to Professors John Aggleton, Anthony Isles and Frank Sengpiel who granted me an interview and, subsequently, a place on the Integrative Neuroscience course at Cardiff, I am incredibly grateful to you and the Wellcome Trust for funding the project. Everyone associated with this course has nurtured a great community which has been a wonderful source of moral support throughout my three & a half years. I hope that I have returned the favour! I'd especially like to thank Matt, Frankie, Jasmine and Laura as my year-mates. We have been together every step of the way and I wish you the best of luck with your submissions. I'd also like to thank Aurelien, who regularly provided much welcomed, sports-based distractions. My rotation project with Anthony and Professor Ros John provided my first experience of behavioural experiments that has served me well throughout my main PhD project. Gráinne McNamara was absolutely pivotal in this respect, particularly for her never-ending patience as I fumbled my way through the project one catastrophe at a time.

Gráinne continued to provide advice even after she thought she had escaped by relocating to London, and also recommended working with Mark for my full project and I am very glad she did. Mark, thank you so much for providing endless advice and support whenever I knocked on your office door, you have been a fantastic mentor. Every time I leave your office it is with more motivation as you pushed me to achieve as much as possible. The same is equally true for you, Emma, and I wish my project had involved more biochemistry and so I would have had more excuses to hear your pearls of wisdom. Both of your advice has stretched beyond the technical and into career and general life advice for which I am very grateful.

I must have thanked Charlie, my post-doc, multiple times a week for the last three years and so it almost feels hollow at this stage. Charlie, you are the biochemistry guru, the mouse whisperer and everything would have taken me ten times as long if I did not have you there and happy (99% of the time) to answer my copious questions. You have taught me everything from multi-channel pipetting technique to stereotaxic brain surgery to the selection of objects that are just the right level of interesting for a mouse. I'd also like to thank the other members of #TeamGood, Tara and Adam, who were there at the start to offer advice about mouse breeding and behaviour testing and more recently about thesis writing. Finally, a huge thank you to Chiara Favero for your help with the biochemistry assays in Chapters 6 and 7 in the last few months, without which I would not be in a position to submit. Thank you also to Jawza and Lucy for providing assistance to Chiara while I wrote this thesis in the office next door.

I would also like to thank all the people working in the BNL through both my rotation and full project. The basement labyrinth can be an unforgiving place to work and I have been very lucky to have such vibrant people to share the experience with. Rhys Perry and Helen Read have provided great help and advice as the NACWO's throughout my project, as well as the lab managers and technicians: Claudia, Moria, Eman and Harriet. A special mention to Jackie and Andrew, who were often the only faces I saw throughout the day. I don't know how you were always so positive but I'm very grateful that you were!

Finally, I would like to thank everyone who has supported me behind the scenes. To my parents and grandparents, who provided me with every opportunity possible to succeed, particularly endless love and support, for which I am eternally grateful. To my friends back in Poole, I am sorry I have missed birthdays, Christmases and everything in between, and yet you have still always been there. I'd also like to everyone in the six different houses I have inhabited in Cardiff for putting up with me despite regularly coming home late in an awful mood. Speaking of which, the biggest thank you of all goes to my wonderful girlfriend Nic, who has somehow put up with me throughout the literal blood, sweat and tears shed in the last three years that has gone into this thesis. Your patience has been unwavering, and I will never be able to make it up to you.

To whomever has taken the time to read this thesis, thank you. I hope it is as interesting to read as it was to write, but not as challenging!

Thesis Abstract

Alzheimer's Disease (AD) affects over 30 million people worldwide, however no disease modifying therapies have been approved to date. The majority of failed therapeutic intervention targeted the accumulation of beta-amyloid (Aβ) species. While these interventions were effective in preclinical mouse models of early stage amyloid pathology they failed to translate into the clinic. While several explanations may underlie this apparent lack of translation, one commonly held view is that first generation mouse models possess artefacts that contribute to brain and cognitive phenotypes. Another factor that has not received detailed consideration is the extent to which reduction of endogenous amyloid production influences cognition and synaptic processes in cognitively normal mice. This thesis aimed to elucidate the role of Aβ in cognitive function in both wild-type (WT) mice and a novel single knock-in mouse *APP*$^{NL-F}$ mouse model of pre-symptomatic amyloid pathology.

Selective inhibition of Aβ production was achieved by using the 2B3 antibody, which binds to the β-cleavage site of the amyloid precursor protein (APP), sterically inhibiting metabolism. 2B3 administration significantly reduced Aβ and altered glutamate receptor dynamics in the hippocampus of 5-month old WT mice following icv (intracerebroventricular) infusion. These mice failed to detect changes in object-in-place (OiP) associations, confirming that hippocampal Aβ was required for this cognitive task.

APP^{NL-F} knock-in mice express a humanised Aβ sequence with the Swedish (KM670/671NL) and Iberian (I716F) mutations within the endogenous murine APP gene. These mice underwent behavioural characterisation at 8 and 17 months of age and this revealed a pattern of selective memory deficits that were age-dependent. This included an age-dependent deficit in a foraging-based spatial working memory task and deficits in both associative OiP and temporal order recognition memory. 2B3 infusion in aged WT and *APP*$^{NL-F}$ mice reduced Aβ production but resulted in dissociable effects on memory. A deficit in OiP performance was rescued by 2B3 administration in *APP*$^{NL-F}$ mice but performance was impaired in healthy WT controls. These data provide novel evidence for the importance and dynamic role of Aβ in both normal and early stage AD pathology. One implication of these findings is that, should anti-amyloid therapies gain traction in the treatment of pre-symptomatic AD, the benefits must be weighed against the potential adverse effect of disrupting normal amyloid function.

Abbreviations

α7-NAChR – alpha-7 Nicotinic Acetylcholine Receptors

Aβ – beta-amyloid peptide

Ach – Acetylcholine

AD – Alzheimer's Disease

AICD – Amyloid Intracellular Domain

AMPA – α-amino-3-hydroxy-5-methy 4-isoxazolepropionic acid

APLP – Alzheimer-Like Precursor Protein

APO-E – Apolipoprotein E

APP – Alzheimer Precursor Protein

APP-Tg – Transgenic APP Mice

ARIA – Amyloid Related Imaging Abnormalities

BACE – β-Site APP CleavingEnzyme

BBB – Blood Brain Barrier

CTF – C-Terminal Fragment

CAA – Cerebral Amyloid Angiopathy

CSF – Cerebral Spinal Fluid

CNS – Central Nervous System

CR1 – Complement Receptor 1

DS – Down Syndrome

DR – Discrimination Ratio

EC – Entorhinal Cortex

ELISA – Enzyme-Linked Immunosorbent Assay

EPM – Elevated Plus Maze

FAD – Familial Alzheimer's Disease

fMRI – Functional Magnetic Resonance Imaging

GSK – Glycogen Synthase Kinase

Icv – Intracerebroventricular

IL-1β – Interleukin-1**β**

HPC – Hippocampus

KI – Knock-In

KO – Knock-Out

LTD – Long Term Depression

LTP – Long Term Potentiation

mAb – Monoclonal Antibody

MAPT – Microtubule Associated Protein Tau (Gene Encoding Tau Protein)

MCI – Mild Cognitive Impairment

mEPSPs – Miniature Excitatory Post-Synaptic Potentials

mGluRs – Metabotropic Glutamate Receptors

MMSE – Mini-Mental State Examination

mPFC – Medial Prefrontal Cortex

MRI – Magnetic Resonance Imaging

MTL – Medial Temporal Lobe

MWM – Morris Water Maze

NFT – Neurofibrillary Tangles

NMDA – N-methyl D-aspartate

OF – Open Field

OiP – Object-in-Place

ON – Object Novelty

PCR – Polymerase Chain Reaction

PET – Positron Emission Topography

PRC – Perirhinal Cortex

PS1 – Presenillin

PSD95 – Post-Synaptic Density Scaffolding Protein

RAM – Radial Arm Maze

sAPPα – Secreted Amyloid Precursor Protein alpha

SEM – Standard Error of the Mean

SWM – Spatial Working Memory

TBI – Traumatic Brain Injury

TNF-α – Tumour Necrosis Factor alpha

TO – Temporal Order

WM – Working Memory

WT – Wild-Type Mice

List of Figures

List of Tables

Chapter 1 –

General Introduction

1.1 Chapter Overview

This introductory chapter will initially describe the physiology of APP, detailing its major metabolic pathways and the enzymes involved within them. The evidence underpinning the current understanding of how the protein influences neuronal function will be presented as well as the roles of individual APP fragments with a particular focus on the beta-amyloid (Aβ) peptide. Section 1.3 describes some pathological features of APP fragments and introduces the argument detailing how the accumulation of Aβ triggers pathogenesis leading to neurodegeneration, "The Amyloid Cascade Hypothesis". This has become the principal hypothesis guiding the understanding of mechanisms of Alzheimer's Disease alongside research aiming to produce therapeutic treatments. Section 1.4 will outline the aetiology and clinicopathology of the disease and present examples of current treatments as well as the therapeutic strategies employed by anti-amyloid drugs currently in clinical development. Despite copious treatments demonstrating promising results in preclinical models of AD, none have successfully progressed through clinical trials, bringing the design of the models into disrepute. Section 1.5 will summarise how the use of first generation, transgenic mouse models has guided our understanding of the pathogenic mechanisms of Aβ, before presenting how potential artefactual influences of APP overexpression has necessitated more physiological models. Knock-in models do not overexpress APP and thus represent a more faithful recapitulation of the human disease. The design and development of these models is introduced, with a detailed description of the $APP^{NL\text{-}F}$ mouse, which will be the focus of much of this thesis. Finally, the aims and hypotheses of this thesis will be presented in section 1.6.

1.2 Amyloid Precursor Protein

Amyloid precursor protein (APP) is a highly conserved transmembrane protein that is expressed both in neuronal and non-neuronal tissue (Tharp and Sarkar 2013). It undergoes a complex metabolism producing various proteolytic fragments and so has been implicated in copious cellular functions including neuronal development, cell signalling and synaptic function. The gene encoding App is located on chromosome 21 in humans and alternative splicing produces 8 isoforms ranging from 639 to 770 amino acid residues in length, with APP_{695} identified as the major isoform expressed in the central nervous system (CNS)

(Sisodia et al. 1993). Two other isoforms, APP751 and APP770 are also common but are predominantly expressed in non-neuronal tissue. APP belongs to a family of homologous proteins related by structure and function. The amyloid precursor-like proteins 1 and 2 (APLP1 & APLP2) are both also expressed in mammals and they, like APP, have a single transmembrane region and large extracellular N-terminal domains (Bayer et al. 1999). While APP and APLP2 are ubiquitously expressed, APLP1 mRNA is exclusively detected in the CNS (Lorent et al. 1995). Phylogenetic analysis reveals that the genes encoding APP-like proteins emerged in insects such as the fruit fly (*Drosophila melanogaster*) and roundworm (*Caenorhabditis elegans*), organisms with simple nervous systems and functional synapses (Shariati and De Strooper 2013). The APP family genes seems to have undergone multiple duplication and contraction events throughout evolution, for example while insects have one gene encoding an APP protein, fish and amphibians have four, birds have two and mammals have three (Zheng and Koo 2011). The complex trajectory and highly conserved sequences of these genes indicates that they have been consistently subjected to stringent evolutionary forces, suggesting that they serve important cellular functions. However, despite evidence implicating APP involvement in many different signalling pathways, thorough understanding of its physiological function remains limited.

Genetic deletion of the APP gene results in viable offspring displaying a mild phenotype involving reduced grip strength and age-dependent cognitive deficits, suggesting it plays a subtle role in neuronal function (Dawson et al. 1999, Senechal et al. 2007). However, duplication of genes affords genetic robustness, increased gene dosage and the possibility of redundancy, potentially precipitating compensatory activity of the APLP proteins. In fact, double knock-out of both APP and APLP2 results in a lethal phenotype, as mice die shortly after birth (von Koch et al. 1997). Whether this severe phenotype is produced due to abolition of redundant function or interruption of various independent pathways is unclear. The expression of 181 genes is affected in both APP and APLP2 single knock-out mice compared to the total of 1061 that are altered following combined deletion (Aydin et al. 2011). However, the severity induced following loss of both genes further indicates that together they may play important roles in physiology.

▪ 1.2.1 – APP Metabolism

APP, APLP1 and APLP2 are all metabolised in a similar way, producing large N-terminal ectodomains as well as intracellular regions. They have all been found aggregated

in the neuritic plaques that are a hallmark of brain pathology in Alzheimer Disease (AD) patients (Bayer et al. 1997). However, only the metabolism of APP generates an amyloidogenic fragment, due to a divergent sequence around the beta-amyloid section (O'Brien and Wong 2011). APP is heavily expressed in the CNS and is rapidly metabolised, undergoing sequential cleavage along two (amyloidogenic versus non-amyloidogenic) pathways, (Lee et al. 2008) as depicted in figure 1.1. In reality, metabolism of this protein is far more complex and many more secretase enzymes and proteolytic fragments have recently been identified. These non-canonical pathways include δ- and η-secretases which cleave the ectodomain of APP and have been reported to increase amyloidogenic processing of APP. Gene deletion of δ-secretase resulted in less Aβ generation in vitro and ameliorated biochemical, synaptic and cognitive pathology in transgenic APP mouse models (Zhang, Song, et al. 2015). η-secretase generates alternative proteolytic fragments linked to synaptic dysfunction (Willem et al. 2015, Baranger et al. 2016). The existence of these alternative APP processing pathways is of particular relevance when considering BACE1 inhibition as a therapeutic mechanism, as modulating its activity may lead to accumulation of other, potentially neurotoxic fragments. For a full review, see Andrew *et al* (2016) and Nhan *et al* (2015).

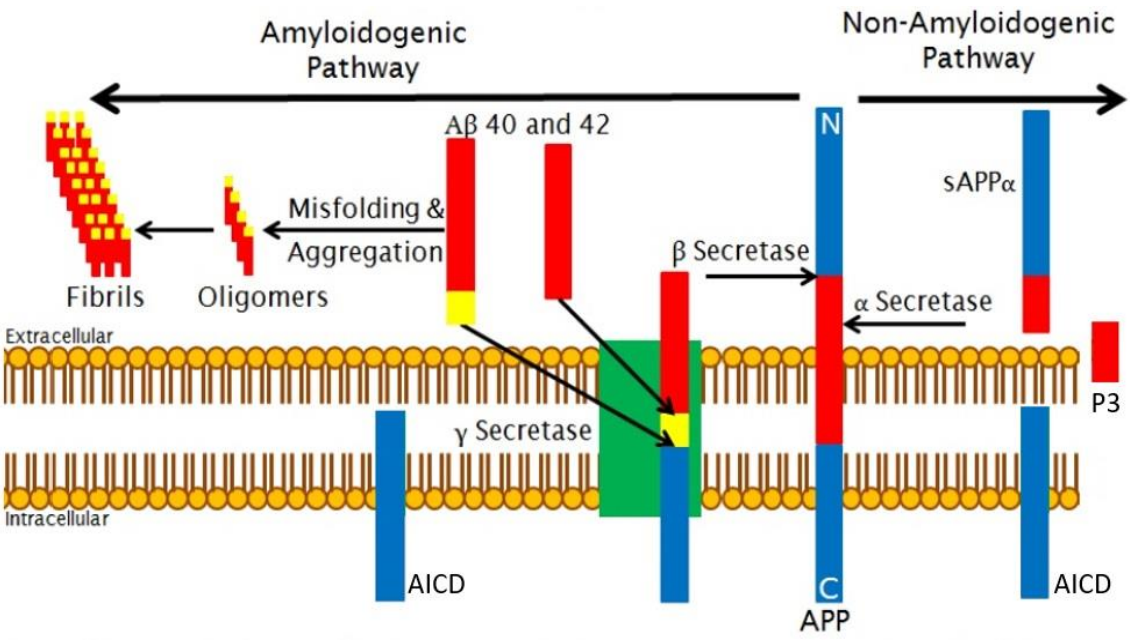


Figure 1.1: Alternative pathways of APP metabolism.
APP can be processed via amyloidogenic or non-amyloidogenic pathways. The former involves β-secretase cleavage followed by γ-secretase, which permits variation in the exact cleavage of the Aβ C terminus due to different composition of the enzyme complex. Therefore, different lengths of Aβ are possible, predominantly 40 or 42 amino acids long. In the non-amyloidogenic pathway, APP is cleaved by α-secretase, preventing Aβ formation.

Following translation in the endoplasmic reticulum, APP is delivered down the axon to synaptic terminals and inserted into either cell surface or cell body endosomal compartments (Koo et al. 1990). The direction of metabolism depends on the cellular location and the enzyme complexes involved. The "non-amyloidogenic pathway" comprises cleavage by α- and γ-secretases at the cell surface (Parvathy et al. 1999), while the "amyloidogenic pathway" occurs in cell body endosomal compartments containing β- and γ-secretase (Zhang et al. 2011). Following proteolysis, the metabolic products of the latter pathway are secreted during cell surface endosome recycling.

The "non-amyloidogenic pathway is the predominant pathway for APP processing (~90%) (Zhang et al. 2011). α-secretase cleavage is mediated by the membrane bound ADAM (a disintegrin and metalloproteinase) family of enzymes, of which three have been proposed as the putative enzyme, ADAM9, ADAM10 and AMAM17 (Asai et al. 2003). This enzyme cleaves at a point on the APP sequence between the β- and γ-secretase cleavage sites, therefore preventing release of the amyloidogenic peptide. Instead, the large, soluble, N-terminal ectodomain sAPPα is produced along with the membrane bound C-terminal fragment CTF83 (CTFα). While the ectodomain is released into the extracellular space, γ-secretase cleavage of the CTF83 fragment generates two more peptides: P3 and the APP intracellular domain (AICD) (Haass et al. 1993, Kojro and Fahrenholz 2005). γ-secretase is a large complex of enzymes including presenillin-1 or 2 (PS1, PS2), nicastrin, anterior phalanx defective and presenillin enhancer 2 (Francis et al. 2002, Haass and Steiner 2002). Although the individual roles of these constitutive proteins are not yet fully elucidated, selective deletion of two transmembrane aspartate residues in PS1 prevented APP cleavage by γ-secretase and significantly decreased production of Aβ, while βCTF levels increased (Wolfe et al. 1999). γ-secretase has numerous alternative substrates which are involved in vital signalling functions, for example the Notch family of proteins which are crucial during stem cell differentiation and nervous system development (Louvi and Artavanis-Tsakonas 2006).

The alternative mechanism for APP processing is the amyloidogenic pathway, in which the protein is first cleaved by beta-site APP cleaving enzyme (BACE, or β-secretase). BACE is a membrane-associated aspartic protease-2 (memapsin 2) and has attracted considerable attention as a potential therapeutic target. It was reported by five independent groups in 1999 who all agreed on a consistent sequence of 501 amino acids

(Sinha et al. 1999, Vassar et al. 1999, Yan et al. 1999, Hussain et al. 1999, Lin et al. 2000). Secretion of Aβ was abolished in BACE1-null neuronal cultures, confirming BACE1 over BACE2 as the neuronal β-secretase and the rate limiting enzyme for production of the peptide (Cai et al. 2001). Although most cell types produce Aβ, implying that both APP and BACE1 are widely expressed (Haass, Schlossmacher, et al. 1992); β-secretase activity seems to be greatest in neurons than other cells (Zhao et al. 1996). BACE1 exhibits optimal enzymatic activity in acidic environments such as endosomes in which Aβ is thought to occur (Huse et al. 2000). Indeed, immunofluorescence experiments showed BACE1 naturally colocalising with APP in endosomes, and blocking surface endocytosis was shown to inhibit Aβ secretion by 80% while impairing direct trafficking of APP to the cell surface increased it (Kinoshita et al. 2003, Haass, Koo, et al. 1992). In contrast, increased trafficking to the cell surface significantly reduced Aβ production (Cataldo et al. 1997). γ-secretase may be localised to both the cell surface and endosomal compartments, enabling it to complement both α- and β-secretase processing of APP (Fukumori et al. 2006).

BACE1 cleavage of APP releases sAPPβ, and a 99 residue long C terminal fragment (β-CTF) containing Aβ which remains attached to the membrane until γ-secretase cuts APP at multiple sites (Sinha et al. 1999). AICD is released into the cytosol from the ε-site before cleavage at the γ-site releases the Aβ peptide (Glenner and Wong 1984, Zhao, Tan, et al. 2007). This is a short, ~4.2 kDa peptide of 38-43 amino acids (predominantly 40 or 42), depending on the exact position at which it is cleaved. The 40-residue peptide is most common, particularly in healthy people. Approximately 10% of human Aβ is the 42 residue species, which is more prone to aggregation and associated with neurotoxicity and plaque formation (Jarrett, Berger, and Lansbury 1993, Walsh and Selkoe 2007).

▪ 1.2.2 – Physiological Role of APP

Evidence concerning the function of APP is controversial, in part due to the many metabolites of the protein and the vast range of different signalling pathways and protein interactions with which they have been associated. The protein interacts with over 200 molecules and is able to act as both a receptor or a ligand to influence both intra- and extracellular signalling (Müller, Deller, and Korte 2017). The evolutionary emergence of APP family genes in the first organisms with functional central nervous systems and synapses immediately hints that the proteins may play a role in synapse formation and/or function. Furthermore, the maximal expression of the protein occurs during post-natal

development, during which a high rate of synaptogenesis occurs (Wang et al. 2009). Beyond this circumstantial evidence, APP-KO mice show subtle phenotypes including reduced brain and body weight, locomotor activity and grip strength (Zheng et al. 1995, Ring et al. 2007). They also exhibited hypersensitivity to seizures and brain injury as well as impairments in neuronal morphology, synaptic function and cognition that developed from 10 months of age (Steinbach et al. 1998, Corrigan et al. 2012, Dawson et al. 1999, Seabrook et al. 1999).

APP exhibits characteristics of cell adhesion molecules which are critical for the development of the synaptic junction in early metazoan eukaryotes (Emes and Grant 2012, Soba et al. 2005). APP has also been implicated in the guidance and growth of neurites in hippocampal cell cultures (Qiu et al. 1995). Germline APP deletion resulted in neuromuscular junction deficits, and this was replicated following conditional KO of APP in either the pre-synaptic motor neuron or post-synaptic muscle tissue, indicating the protein is necessary in both synaptic compartments (Wang et al. 2009). The same paper also reported that, when overexpressed in HEK cells, APP induced synaptogenesis in cocultured primary hippocampal neurons. Furthermore, overexpression of APP increased spine density in hippocampal neurons and genetic deletion reduced it in both the aforementioned cultures and transgenic mice (Lee et al. 2010, Tyan et al. 2012, Weyer et al. 2014). Another study reported that while overall spine density was not significantly reduced in APP-KO mice, spine turnover (both formation and pruning) was decreased, indicating a role of the protein in spine dynamics (Zou et al. 2016). The mechanism for synapse formation and stabilisation may involve dimerization across the synapse where two APP molecules interact via the heparin binding domains in their extracellular N termini to produce cross-linked dimers (Soba et al. 2005, Coburger, Hoefgen, and Than 2014). Immunofluorescence and live cell-imaging experiments revealed that APP localises to lamellipodia (motile, actin-rich protrusions in cellular growth cones) both *in vitro* and *in vivo* and influences actin-based membrane motility (Sabo et al. 2003, Cheung et al. 2014). Together this evidence promotes APP as a putative mediator of both axonal outgrowth and synapse formation.

Further to its role in synapse formation, APP has been extensively implicated in neuronal function. The metabolism of APP by α- and β-secretases is dependent on neuronal activity, suggesting that it occurs with a specific purpose (Nitsch et al. 1993, Fazeli et al. 1994, Kamenetz et al. 2003). However, studies concerning the role of APP often generate

conflicting results, for example: overexpression of APP in neuronal cultures was shown to depress synaptic activity in one study, yet enhance synaptic plasticity and memory in another (Kamenetz et al. 2003, Ma et al. 2007). Interactome analysis revealed that APP associates with vesicle release machinery at hippocampal synapses, is involved in vesicle turnover and facilitates neurotransmitter release (Fanutza et al. 2015, Kohli et al. 2012). Meanwhile, APP deletion in mice decreased the expression of the synaptic vesicle proteins synaptophysin and synaptotagmin-1 at the pre-synaptic membrane (Lassek et al. 2014, Dawson et al. 1999). Unsurprisingly, these mice exhibited decreased frequency of miniature excitatory post synaptic potentials (mEPSPs), which are an indicator of vesicle release probability. These results suggest a presynaptic function for APP. Furthermore, conditional APP-KO mice lacking expression of the protein in excitatory forebrain neurons exhibited impairment of paired-pulse facilitation and post-tetanic potentiation (Hick et al. 2015). These processes represent short term plasticity which is dependent on pre-synaptic release mechanisms such as vesicle release probability, recycling rate and neurotransmitter content (Regehr 2012). These studies therefore validate the protein as a potential mediator of presynaptic plasticity mechanisms. However, as previously mentioned, APP is also localised to post-synaptic compartments, where there is also evidence of its impact on synaptic function.

The contribution of APP to pre- or post- synaptic mechanisms may depend on individual synapse function. The post-synaptic role may involve interaction with N-methyl D-aspartate (NMDA) glutamate receptors, which are ligand-gated cation channels. Immunoprecipitation experiments demonstrated that APP, as well as APLP1 and APLP2, associate with the GluN1 subunit of NMDA receptors and enhance their expression at the postsynaptic membrane (Cousins, Dai, and Stephenson 2015). Together, the described putative roles of APP at both the pre- and post-synaptic terminals would result in enhanced transmission and plasticity due to the augmented release of neurotransmitters and increased availability of NMDA receptors. Indeed, APP-KO mice do exhibit deficits in transmission and LTP induction (Ring et al. 2007, Dawson et al. 1999, Seabrook et al. 1999), while conditional KO of APP in the forebrain of young adult mice induced pronounced impairment of LTP induction and maintenance (Hick et al. 2015). As plasticity of neuronal communication is thought to underpin memory processes, it is unsurprising that APP-KO mice also exhibit deficits in tests of cognitive performance. While young (3-4 months old) mice were cognitively normal, 10 month old APP-KO mice demonstrated deficits in spatial learning in the Morris water maze (Phinney et al. 1999, Dawson et al. 1999, Ring et al.

2007), passive avoidance memory (Senechal et al. 2007) and fear conditioning (Dawson et al. 1999). The interaction of the observed synaptic and cognitive deficits with age is particularly interesting in the context of APP involvement in AD, another age-related pathology. Together this suggests that the function of the protein becomes more important in aged neurons.

Current understanding of APP function mainly stems from genetic deletion in addition to overexpression studies. However, conclusions from these studies must be tempered by the understanding that these mice lack APP from conception and therefore any phenotype is potentially the result of loss of its function during development. APP is expressed as early as embryonic day 7.5 and so its deletion is likely to have a wide-ranging impact. Alternatively, compensatory activity of other genes, particularly by APLP1 and APLP2, may mask the effect of APP deletion on the resulting phenotype. Indeed, constitutive deletion of multiple APP family proteins results in more severe abnormalities in brain development (Herms et al. 2004, Weyer et al. 2014). The fact that cognitive phenotypes of APP-KO mice become apparent in aged mice suggests that these compensatory mechanisms may fail with age. Another major issue with investigating the function of APP is the difficulty in independently manipulating different fragments as inhibition techniques such as knock-out or siRNA cannot discriminate specific loss of function effects of each peptide. Understanding of individual functions has mainly come from isolation of the peptides and administering them into neuronal cultures or mouse models. Further discussion concerning the physiological function of APP will be divided into separate reviews of individual fragments.

β-*Amyloid*

The Aβ peptide sequence is conserved throughout vertebrates with over 90% homology between humans, birds, reptiles and amphibians, suggesting it conveys an evolutionary advantage (Tharp and Sarkar 2013). Ever since its identification in the neuritic plaques of AD patients by Glenner and Wong (1984), the main focus of research into Aβ has been its neurotoxic properties. However, hints at possible neurotrophic functions were suggested after the peptide was first described. While high concentrations of Aβ led to dendritic retraction and neuronal death in differentiated neurons, low concentrations were neurotrophic to undifferentiated cells and a recombinant peptide corresponding to the first 28 residues of the Aβ sequence enhanced survival of hippocampal pyramidal neuronal

cultures (Yankner, Duffy, and Kirschner 1990, Whitson, Selkoe, and Cotman 1989). Despite this indication of beneficial activity at physiological concentrations, the field has focused on the toxic effects of high (>nanomolar) Aβ. More recently, attention has returned to the physiological role of the peptide, and the concentration dependent reversal of activity has gained traction as an hormetic effect (Puzzo et al. 2008). Hormesis is a pharmacological phenomenon whereby a substance can be beneficial at low concentrations but toxic when increased and seems to be a consistent rule for memory enhancers, first described by Karl Lashley in 1917 while documenting the effects of strychnine and caffeine (Lashley 1917).

The hypothesis that physiological Aβ facilitates synaptic function and memory has gained support through experiments showing that addition of picomolar concentrations to hippocampal slices enhanced synaptic plasticity, while nanomolar concentrations impaired it (Puzzo et al. 2008, Garcia-Osta and Alberini 2009, Morley et al. 2010). The electrophysiology results were supported evidence that injection of low concentrations of the peptide into the hippocampus augmented memory performance. Furthermore, anti-Aβ antibodies injected in the same manner inhibited memory, as well as LTP induction in hippocampal slices (Puzzo et al. 2011). The facilitatory effect of Aβ may only require the N-terminal domain which is shared with sAPPα. Concurrent α- and β-secretase cleavage of APP releases a short, 15 residue peptide which exhibited similar enhancement of LTP synaptic plasticity, similar to that induced by full length Aβ and sAPPα (Lawrence et al. 2014, Portelius et al. 2011). Meanwhile, another group reported that the Tg5469 mouse model, which overexpresses human APP at ~6 times greater than the endogenous protein, exhibit enhanced spatial memory which is blocked by BACE1 inhibition, indicating it was mediated by Aβ and not sAPPα (Ma et al. 2007).

Further to the evidence that the synaptic role of Aβ activity is concentration dependent, (Koppensteiner et al. 2016) reported that increased duration of incubation also switched its impact from facilitatory to dysfunctional. Neurons incubated with picomolar $A\beta_{42}$ for minutes exhibited increased plasticity, while incubation for hours reduced it. These findings were replicated *in vivo*. This change in effect of the peptide may be due to an increasing aggregation state, whereby the three-dimensional structure of the peptide is altered sufficiently until it is not recognised by receptors (Puzzo et al. 2015). For example, picomolar Aβ modulates presynaptic vesicle release via activation and potentiation of α7 nicotinic acetylcholine receptors (α7-NAChR), while higher concentrations antagonise

them (Mura et al. 2012). In fact, a specific tyrosine residue within the agonist binding pocket of α7-NAChR receptors was critical to permit Aβ-mediated potentiation, although this study did not investigate multiple concentrations of the peptide (Tong et al. 2011). Activation of α7-NAChR can modulate NMDA receptor internalisation, which may represent a mechanism for Aβ-mediated enhancement of LTP (Snyder et al. 2005). Furthermore, application of the peptide also enhanced LTD in the dentate gyrus of rat hippocampal slices and this was mediated through metabotropic glutamate receptors (Chen et al. 2013). However, it is often difficult to evaluate the impact of the peptide as many studies do not specify the concentration of Aβ used in their experiments.

While a physiological effect of Aβ on synaptic activity is now widely accepted, its overall role in neuronal activity remains poorly understood. Production of the peptide is sensitive to excitatory synaptic activity, raising the possibility of it being involved in a regulatory feedback mechanism (Cirrito et al. 2005, Kamenetz et al. 2003, Lesné et al. 2005). This hypothesis was supported by experiments in which β- and γ-secretase inhibitors were incubated with neuronal cell cultures for 24 hours, leading to increased cell death (Plant et al. 2003). The same group later reported that this effect was dependent upon increased excitotoxicity following the loss of regulation of potassium K_v4 subunit expression by Aβ (Plant et al. 2006). The loss of this protective action may explain why APP and BACE1 knock out mice exhibit a hypersensitivity to seizure (Steinbach et al. 1998, Hitt et al. 2010). Aβ has also been implicated in various other protective physiological mechanisms including antimicrobial activity, tumour suppression, blood brain barrier (BBB) integrity and brain injury recovery (Brothers, Gosztyla, and Robinson 2018). The intriguing proposal of the peptide as protective against infection and cancer is the beyond the scope of this thesis. However, Aβ interacts with multiple species involved in vascular function, including endothelial cells, microglia, red blood cells and haemoglobin and metal ions (Fe and Cu), allowing it to act as an anti-coagulant and mediate the sealing of damaged blood vessels (Ravi et al. 2004). In fact, cerebrovascular insults such as TBI have been shown to trigger Aβ production and localisation to the site of injury within minutes (Roberts et al. 1991). Aβ also accumulates around cerebral blood vessels following hypertension, ischemia or haemorrhages caused by needle stick lesions (Carnevale et al. 2016, Hung et al. 2015, Purushothuman et al. 2013). Two studies reported that a greater concentration of the peptide in brain interstitial fluid following acute brain injury corresponded to better neurological outcome in patients (Brody et al. 2008, Magnoni

et al. 2012). Meanwhile, controlled cortical impact and stroke induced greater severity of outcome in mice with deletion of APP or BACE1, further indicating that Aβ exerts a protective function (Mannix et al. 2011, Koike et al. 2012). Although plaques have been identified in children following head injury, the brains of long-term survivors do not show greater plaque deposition than age matched controls despite Aβ deposits remaining in injured axons (Graham et al. 1995, Chen et al. 2009). Collectively this suggests that the recruitment of Aβ following injury is an immediate, protective and transient effect.

Breakdown of the BBB is a well-characterised effect of aging and is highly associated with AD (Montagne et al. 2015). Amyloid plaques contain vascular and inflammatory proteins and are associated with microhaemorrhages (Cullen, Kocsi, and Stone 2006). Therefore, it is tempting to suggest that plaque accumulation is initiated by the recruitment of Aβ to disruption of BBB integrity. Amyloid involvement in the development of AD will be discussed in detail in the upcoming section. However, the interaction of Aβ and the BBB is especially relevant in the context of its physiological functions following recent evidence from multiple clinical trials of anti-amyloid therapies have reported adverse side-effects including brain oedema and/or microhaemorrhages (Sperling et al. 2012, Sevigny et al. 2016). These effects are termed Amyloid related imaging abnormalities (ARIA) because they are identified following brain magnetic resonance imaging (MRI) analysis. Collectively, this evidence indicates that Aβ plays a role in protecting BBB integrity which may become more important during the age-related increase in permeability. It therefore hints at a physiological role for the development of amyloid plaques independent of their cardinal role in signalling of AD pathology.

sAPPα

sAPPα is widely considered to have a neuroprotective function and enhances synaptic plasticity and memory (Turner et al. 2003, Meziane et al. 1998). This evidence mainly comes from studies reporting that the presence of the sAPPα ectodomain rescued the morphological abnormalities in dendrites, synaptic plasticity deficits and cognitive impairments observed in mice and cell cultures following deletion of the APP gene (Ring et al. 2007, Tyan et al. 2012, Hick et al. 2015). The soluble ectodomain may directly associate with BACE1 to modulate APP metabolism, promoting the non-amyloidogenic pathway (Obregon et al. 2012). Expression of sAPPα naturally declines in aged WT rats

but addition of recombinant sAPPα facilitated LTP in hippocampal slices and enhanced memory in an object location test (Anderson et al. 1999, Moreno et al. 2015, Xiong et al. 2017). Furthermore, infusion of sAPPα enhanced LTP during *in vivo* recording of the perforant path in anaesthetised rats (Taylor et al. 2008). The Taylor group also demonstrated that both infusion of antibodies targeted to sAPPα and pharmacological inhibition of α-secretase impaired LTP and spatial memory, while both effects were prevented by co-administration of the peptide (Taylor et al. 2008). Importantly, despite being only 16 residues shorter than sAPPα, addition of sAPPβ did not reproduce the plasticity enhancing properties either *in vitro* or *in vivo*, indicating that those effects were specific to the α-secretase cleavage product (Hick et al. 2015, Taylor et al. 2008, Tyan et al. 2012). The synaptic mechanism for the action of sAPPα is not fully elucidated, but it appears to facilitate postsynaptic NMDA mediated currents, activate signalling cascades and upregulate protein synthesis in the synaptosome, leading to neurotrophic and excitoprotective effects (Claasen et al. 2009, Taylor et al. 2008, Cheng et al. 2002, Mattson et al. 1993). sAPPα may exert its excitoprotective effect by activation of potassium ion channels leading to hyperpolarisation of the neuron and reduction of intracellular calcium levels (Furukawa et al. 1996). The loss of this excitoprotective function may have been a major factor in the epileptic phenotype exhibited by mice with conditional KO of the major α-secretase ADAM10 (Prox et al. 2013). However, it must be noted that inhibition of α-secretase cleavage of APP increases β-secretase activity and therefore the effects may be mediated by a toxic gain of function of amyloidogenic pathway fragments (Wang et al. 2012). While the overall role of sAPPα at the synapse is becoming clearer, the specific receptor activating the effect of the peptide at the postsynaptic membrane has yet to be elucidated.

AICD

Evidence concerning the function of the APP C-terminal Intracellular Domain (ACID) is less defined than that of the sAPPα ectodomain. However, studies have shown that this region may convey some features of APP function, particularly through a highly conserved YENPTY sequence located within the final 15 amino acid residues of the C terminus. APP knock-in mice have been generated which lack these final 15 residues, bred onto an APLP2-null background to circumventing compensatory activity by the APLP2. These mice displayed morphological defects at the neuromuscular junction and

impairments of synaptic plasticity, spatial learning and memory (Klevanski et al. 2015). To more specifically determine the role of this domain, the tyrosine residue in the conserved sequence was selectively mutated to glycine (Matrone et al. 2012). These mice exhibited age-dependent cognitive decline and reduction in both dendritic spine density and neuromuscular function, while combining the mutation with deletion of APLP2 led to postnatal lethality. These loss of function experiments along with the nature of evolutionarily conserved sequences, suggest that AICD performs a physiological function. This may involve regulation of transcription, as the fragment translocates to the nucleus and forms a complex with the nuclear adapter protein FE65 and the histone acetyltransferase Tip60 following liberation by γ-secretase cleavage (Cao and Sudhof 2001, Schettini et al. 2010). This influence on gene transcription may be responsible for the implicated role of AICD in cellular functions, such as calcium signalling, cytoskeletal dynamics of dendritic spines and apoptosis (Chang and Suh 2010, Muller et al. 2008). The association of AICD with the FE65 adaptor protein in the endoplasmic reticulum is also significant because it was shown to promote translocation of APP to the cell surface, as well as both sAPPα and Aβ secretion (Sabo et al. 2003) Deletion of the conserved YENPTY region also strongly decreased amyloidogenic processing of APP (Klevanski et al. 2015). However despite this, the impact of overexpression of the AICD on amyloid pathology is controversial, as demonstrated by one study reporting increased hippocampal degeneration, tau pathology and working memory deficits and another showing no such effects (Ghosal et al. 2009, Giliberto et al. 2010). This difference may have arisen due to the methodology used. The former study co-expressed AICD with FE65 resulting in 1.5 to 2-fold increased AICD levels and the amyloidosis-like pathology. However, the latter paper overexpressed AICD in mice with human tau protein without manipulating FE65, indicating the interaction of these two species is critical for increasing amyloid pathology.

1.3 Pathological Role of APP in AD

This section evaluates the involvement of APP cleavage products in neuronal disfunction and in particular how, according to the amyloid cascade hypothesis (Hardy and Higgins 1992), the accumulation of Aβ leads to AD.

β-*CTF*

In contrast to the AICD, the C-terminal fragment produced by β-secretase, is regarded as neurotoxic and detrimental to synaptic function. Overexpression of this fragment leads to age-dependent deficits in spatial and reversal learning measured in the Morris water maze, as well as in the maintenance of LTP (Nalbantoglu et al. 1997). Overexpression of β-CTF increased reactive gliosis and hippocampal neurodegeneration - two hallmarks of amyloid pathology (Berger-Sweeney et al. 1999). Whether these effects are specifically caused by β-CTF or following its metabolism to Aβ is unclear. γ-secretase is membrane-bound, meaning that the C-terminus construct would need to be translocated to the membrane in these transgenic mice in a similar manner to APP for this cleavage to take place. Recombinant β-CTF injected into the brain is unlikely to localise onto cell membranes, so any effects would be mediated by the full 99 residue sequence. In fact, intracerebroventricular (icv) injection of β-CTF did indeed induce similar effects as the overexpression models, with increased gliosis in both the hippocampus and neocortex and neurodegeneration identified in the latter region (Song et al. 1998). BACE1 expression is increased in the brains of AD patients, meaning that this fragment is also upregulated (Zhao, Fu, et al. 2007). Meanwhile, transgenic APP mice overexpress this fragment compared to WT controls, meaning the observed pathology cannot be specifically attributed to the accumulation of Aβ (Sasaguri et al. 2017). Therefore, the detrimental effect of β-CTF must be considered as a potential contributor to pathology in both AD patients and transgenic mouse models. Reducing Aβ may not impact β-CTF, allowing it to continue to impair neuronal function, and this effect may be partly responsible for the failure of anti-Aβ therapies to improve cognition in clinical trials.

β-*Amyloid*

The plethora of studies describing the neurotoxic functions of Aβ generally describe impaired synaptic plasticity and/or memory performance following application of high (>nanomolar) concentrations or oligomeric forms of the peptide (Flood et al. 1994, Lesne et al. 2006, Townsend et al. 2006). However, the specific mechanisms by which this occurs

are not yet fully understood. Considering the involvement of Aβ in the synaptic mechanisms described above, any deregulation of these functions may contribute to neuronal dysfunction. Increased aggregation of the peptide can influence its interaction with receptors such as the α7-NAChR, leading to internalisation of NMDA receptors and impaired LTP (Mura et al. 2012, Snyder et al. 2005, Dewachter et al. 2009). Chronic Aβ exposure is generally associated with synaptic depression, through NMDA or metabotropic glutamate receptor (mGluR1) dependent mechanisms, resulting in the internalisation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Hsieh et al. 2006, Chang et al. 2006). However, the biochemical dysfunction induced by Aβ accumulation extends beyond synaptic impairment and includes mitochondrial dysfunction leading to oxidative stress, excitotoxicity and neurodegeneration (Devi et al. 2006).

Mitochondrial Dysfunction

Despite the aforementioned potential of Aβ as a key part of a protective, negative feedback loop in neuronal excitation, the ability of pathological levels of Aβ to induce excitotoxicity has been proposed to involve blocking the reuptake of glutamate by astrocytes (Harkany et al. 2000). Increased extracellular accumulation of glutamate will cause greater NMDA receptor activation and calcium overload that ultimately induces cell death. Aβ accumulation and disrupted calcium homeostasis is also associated with mitochondrial dysfunction, which is an early event in AD pathogenesis (Swerdlow and Khan 2004, Reddy 2009). *Post-mortem* analysis of patients revealed an accumulation of APP in the protein import channels of mitochondria, particularly within AD-vulnerable brain regions, that was not present in age-matched control brains (Devi et al. 2006). Aβ itself displays the ability to cross mitochondrial membranes by binding to the translocase of the outer mitochondria membrane homolog (TOMM40) (Tillement et al. 2006). Altered expression of this transport protein has been reported in AD patients, while polymorphisms in the gene have been reported as risk factors for AD by genome-wide association studies, strengthening the evidence that this protein is involved in Aβ-mediated mitochondrial dysfunction (Goh et al. 2015, Roses et al. 2010). Accumulation of the peptide inhibits the function of TOMM40 and other components within the organelle, limiting mitochondrial function resulting in decreased ATP production and generation of oxidative stress that has been associated with hyperphosphorylation of tau and neuronal damage (Reddy 2011, Melov et al. 2007). Importantly, these mechanisms generate a positive feedback loop

whereby the increased oxidative stress induces greater Aβ production (Leuner et al. 2012). A mitochondria-targeted anti-oxidant drug treatment arrested progression of oxidative stress, neuroinflammation and synapse loss in an APP transgenic mouse model and inhibited development of spatial memory deficits (McManus, Murphy, and Franklin 2011). Accordingly, this evidence adds further support to the hypothesis that AD pathogenesis involves a cascade of mitochondrial dysfunction (Swerdlow and Khan 2004).

Inflammation

While mitochondrial dysfunction can stimulate neuroinflammation due to the release of ROS (Kaur et al. 2015), amyloid accumulation alone can induce activation of the immune system in both humans and animal models (Heneka et al. 2010). In fact, Trem2 and complement receptor type 1 (CR1) are two immune-associated risk genes for AD, further suggesting a pathogenic involvement of the inflammation pathway (Carmona et al. 2018). Microglia cells are the resident innate immune cells of the brain, and they are activated by Aβ accumulation due to their role in phagocytosis and degradation of aggregated proteins (Liu et al. 2012, Simard et al. 2006). Inflammatory cytokines such as IL-1β, IL-6 and TNF-α have been shown to ameliorate amyloid pathology (Shaftel et al. 2007). However, similar to other mechanisms of Aβ-mediated neuronal dysfunction, neuroinflammation forms part of a positive feedback loop, generating more peptide and exacerbating biochemical dysfunction (Goldgaber et al. 1989). Chronic inflammation and prolonged activation of microglia disrupt their ability to clear Aβ, possibly due to the downregulation of Aβ-binding scavenger receptors, leading to further immune activation and neurotoxicity (Hickman, Allison, and El Khoury 2008, Meda et al. 1995). Chronic neuroinflammation has also been proposed as the putative link between increased amyloid deposition and tau pathology, following reports that administration of lipopolysaccharide, an inducer of neuroinflammation, significantly induced tau hyperphosphorylation in young 3xTg-AD mice (Kitazawa, Yamasaki, and LaFerla 2004). Furthermore, co-culture of astrocytes with cortical neurons exacerbated Aβ-mediated tau phosphorylation, caspase-3 activation and neuronal damage (Garwood et al. 2011). Epidemiological studies have hinted that anti-inflammatory drugs may exhibit a protective role in AD (Breitner et al. 1994), this has been replicated in mouse models supporting the hypothesis that Aβ-mediated neuroinflammation plays a malicious role in the pathogenesis of the disease (McGeer, Schulzer, and McGeer 1996, Olmos-Alonso et al. 2016).

1.3.1 – The Amyloid Cascade Hypothesis

Amyloid metabolism and accumulation have been a cornerstone of AD research for over 25 years. Some of the initial findings related to APP came from studies of patients with genetic abnormalities. Individuals with Down Syndrome who possess an extra copy of chromosome 21, which encodes APP (Delabar et al. 1987, Goldgaber et al. 1987) and miss-sense mutations in families with an increased incidence of AD (Goate et al. 1991, Mullan et al. 1992, Sherrington et al. 1996, Chartier-Harlin et al. 1991, Murrell et al. 1991) provided considerable support for the cardinal role of amyloid in AD onset. Biochemical studies connected amyloid pathology to the other hallmarks of the diseases including neurofibrillary tangles (NFT) and neurodegeneration (Pike et al. 1991, Koh, Yang, and Cotman 1990, Mattson et al. 1992, Baudier and Cole 1987). Collectively, this evidence led to the formation of the "Amyloid Cascade Hypothesis" which presented accumulation of the peptide as the central event in the pathogenesis of AD (Hardy and Higgins 1992). The hypothesis stated: "that deposition of amyloid β protein, the main component of the plaques, is the causative agent of Alzheimer's pathology and that the neurofibrillary tangles, cell loss, vascular damage, and dementia follow as a direct result of this deposition". The recent discovery of an APP mutation that reduces β cleavage and lowers the incidence of AD further strengthens the hypothesis (Jonsson et al. 2012). However, despite the Amyloid cascade hypothesis guiding research for 25 years, numerous therapies reducing Aβ burden have failed in clinical trials (Cummings, Morstorf, and Zhong 2014). The ensuing challenges to the hypothesis have resulted in recent updates published by the original authors (Selkoe and Hardy 2016).

The development of MRI and positron emission topography (PET) scanning of patients has permitted detailed assessment of amyloid plaques alongside neuronal damage and cognitive performance. Multiple studies have reported that the severity of NFT pathology, not plaque deposition correlates with clinical measurements of disease progression (Braak and Braak 1991, Arriagada et al. 1992). In further challenges to the amyloid cascade hypothesis, prominent plaque deposition has been identified in brains of non-demented individuals, meanwhile up to 30% of the elderly population are positive for amyloid imaging signals, despite appearing cognitively normal (Jellinger 1995, Jack et al. 2009). However, recent evidence suggests that these individuals may exhibit subtle deficits in the AD cooperative Study-Preclinical Alzheimer Cognitive Composite (ADCS-PACC, a comprehensive assessment of multiple cognitive domains) as well as accelerated cortical

atrophy. This could be considered a pre-symptomatic or prodromal stage of AD with more severe cognitive decline yet to develop (Donohue et al. 2014, Chételat et al. 2012). Despite these challenges to the amyloid cascade hypothesis, it still remains the prevailing viewpoint for the pathogenesis of AD and has been updated to reflect the more recent findings (figure 1.2) (Selkoe and Hardy 2016). Analysis of the timepoints of pathophysiological processes in AD reveals that measurement of rising Aβ levels in the CSF and in amyloid-PET scans precedes detectable changes in tau, while cognitive deficits occur much later (Jack et al. 2013), supporting the theory that amyloid pathology is the initiating mechanism. Furthermore, a recent study of 300 cognitively normal individuals reported that while Aβ pathology and hippocampal atrophy were both related to memory capability, there was no association between the two variables (Svenningsson et al. 2019). Furthermore, Aβ pathology was associated with memory only in relatively younger individuals (65 to 73 years of age); meanwhile hippocampal volume exhibited the opposite effect and was associated with memory function in participants of an older age range (73 to 88). Therefore, this study further suggests that the influence of Aβ on cognition occurs during early aging.

In the last two decades, research into the aggregation mechanisms of Aβ has revealed a more specific understanding of its neurotoxicity. While the peptide is relatively benign in its monomeric and insoluble plaque states, the intermediary oligomeric or protofibril state is proving to be the neurotoxic species (Martins et al. 2008, Haass and Selkoe 2007, Hefti et al. 2013, Shankar et al. 2008). For example, Aβ dimers extracted from the cortices of AD patients induced tau hyperphosphorylation and degradation of neurites in hippocampal neurons, and this effect was inhibited by co-administration of anti-Aβ immunotherapy (Jin et al. 2011). Shankar *et al* (2007) observed that application of a picomolar concentration of oligomers to rat organotypic slices led to a degeneration of synapses through an NMDA receptor dependent mechanism (Shankar et al. 2007). Oligomers also inhibit the induction of LTP as well as memory performance when infused into the brain of live rats (Townsend et al. 2006, Lesne et al. 2006, Reed et al. 2011). Concentration of oligomeric Aβ also correlates more effectively than that of plaques with the severity of clinical symptoms (Bao et al. 2012). Furthermore, while many clinically normal individuals exhibit prominent plaque pathology, Esparza and colleagues used oligomer-specific ELISAs to demonstrate that these individuals had a very low oligomer-to-plaque ratio compared to AD brains with a comparable plaque density (Esparza et al. 2013). In this way, presenting oligomeric Aβ as the neurotoxic species sustains the central arguments supporting the amyloid cascade hypothesis while counteracting its most salient

criticisms. It also relegates amyloid plaques, the classical hallmark of AD pathology, to an undetermined role in the disease. Recent hypotheses involve plaques acting as inert "sinks" that are able to sequester soluble Aβ in an equilibrium up to a physical saturation point, at which the oligomers diffuse onto the surrounding synapses (Hong et al. 2014, Benilova, Karran, and De Strooper 2012). In fact, observations of plaques in transgenic APP mice revealed a penumbra of soluble oligomers surrounded each plaque correlating with decreased synaptic density in that area which ameliorated with distance from the plaque core (Koffie et al. 2009). Meanwhile, Martins *et al* (2008) described the formation of amyloid protofibrils (oligomers) into fibrils (plaques) as reversible and that once formed, plaques may act as sources of the neurotoxic Aβ species. Multiple clinical trials of anti-amyloid therapies have reported clearance of plaques in absence of cognitive benefit for patients. Perhaps this clearance represents the recycling of plaques back into oligomers which would not alleviate neuronal impairment. Furthermore, if the accumulation of plaques is considered a downstream symptom of augmented soluble Aβ, patients presenting with extensive plaque pathology may represent a late stage of AD that is beyond the therapeutic window for remedial treatment. Having acknowledged these factors, current clinical trials now aim to reduce oligomeric or protofibril Aβ species in early-stage AD patients.

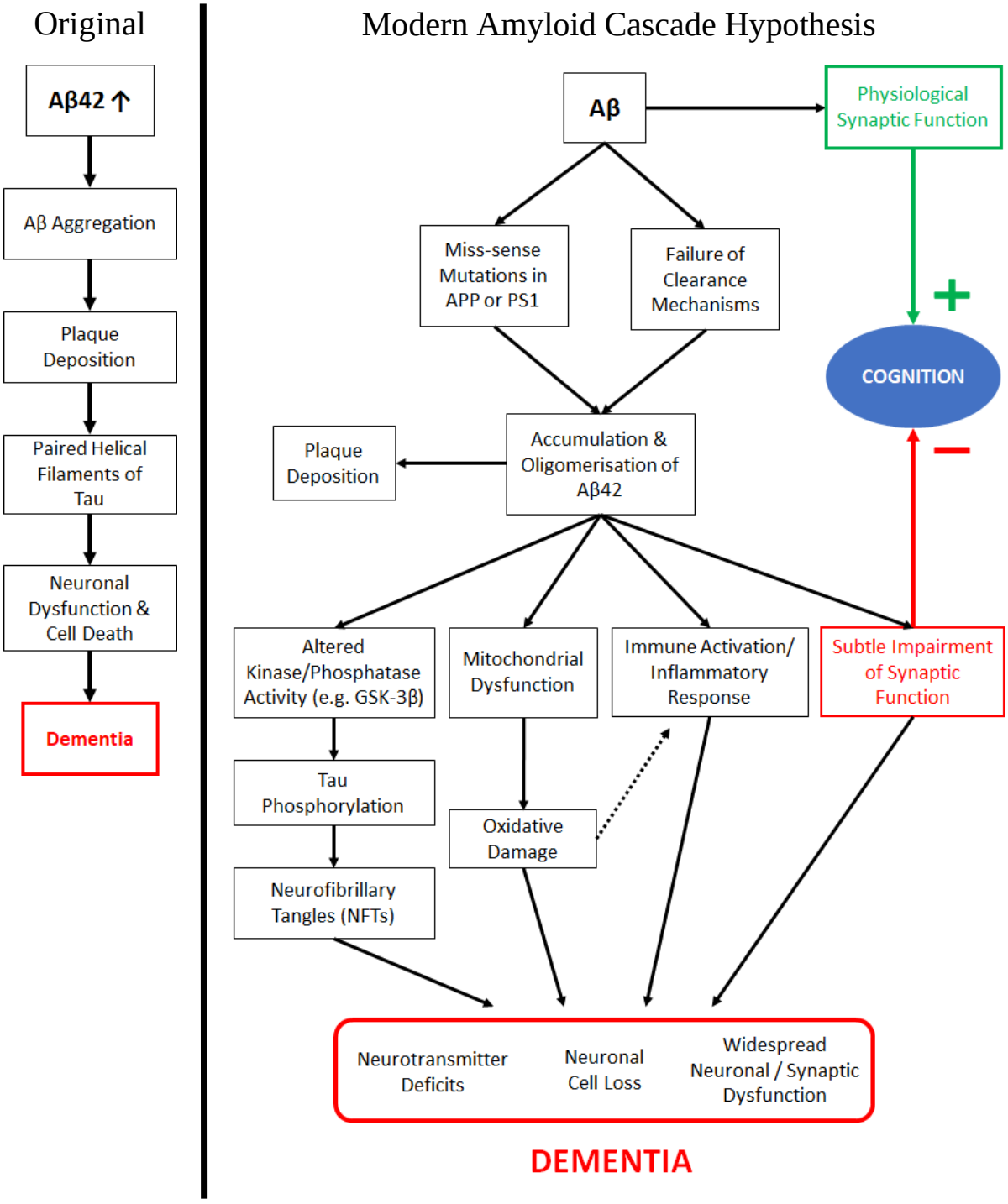


Figure 1.2: The original and revised amyloid cascade hypotheses.
The original amyloid cascade hypothesis, proposed by Higgins and Hardy in 1992, focused on amyloid deposition directly leading to the other forms of neuropathology associated with AD. However, in the decades since, the hypothesis has been embellished with additional details that have been elucidated by studies into amyloid, for example how plaque deposition is no longer considered as part of the pathogenic process. This diagram highlights the fact that Aβ performs a physiological, synaptic function. However, as the peptide accumulates, it exerts a detrimental effect on synaptic function, causes mitochondrial damage, activates the immune system and induces tau pathology. These processes culminate in the synaptic dysfunction and neuronal cell death that are hallmarks of AD.

1.4 Alzheimer Disease

▪ 1.4.1 – AD Introduction

First described in 1906 by Alois Alzheimer, Alzheimer's Disease (AD) is an age-related neurodegenerative condition and the leading form of dementia, responsible for around two-thirds of the over 50 million cases of dementia worldwide (World Alzheimer's Disease Report 2018). In the US the percentage of the population living with Alzheimer's dementia is 3% of 65-74-year olds and this statistic increases with age as it affects 17% of people aged 75-84 and 32% of people over 85 (Alzheimer's Association Facts and Figures, 2019). Due to the globally aging population, the number of people living with dementia will increase by 204% from 50 million in 2019 to 152 million in 2050 (Dementia fact sheet 2017, World Health Organisation). Therefore, while the cost of healthcare for dementia patients in the UK was £26.3 billion in 2014 (Alzheimer's Society: Dementia UK update, 2014) and $290 billion in the US in 2019 (Alzheimer's Association: Facts and Figures, 2019), this is expected to more than double by 2040. Meanwhile, over 18.5 billion hours of informal (unpaid) care was provided to US AD patients in 2018 at an estimated value of $234 billion, showing that the actual cost of dementia care far exceeds the original value. Moreover, the physical and emotional impact suffered by dementia care-givers leads to increased development of stress, anxiety and depression and resulted in an estimated further $11.8 billion in health care costs in the US (Ferrara et al. 2008). The financial and personal care burden will particularly affect the developing world, whose population is set for the steepest increase in age due to improved life expectancy (World Alzheimer's Disease Report 2015). There are currently no therapies able to prevent AD progression, and while symptomatic treatments are able to enhance cognition, they do not impede the rate of decline. Therefore, considering the expected increase in its impact, it is imperative that further research is undertaken in order that the mechanisms leading to AD pathogenesis are better understood.

▪ 1.4.2 – Aetiology of Alzheimer's Disease

Over 95% of AD cases are classified as sporadic, or late onset AD, in which pathogenesis is likely initiated by a combination of genetic and environmental factors (Bettens, Sleegers, and Van Broeckhoven 2013). In contrast, familial AD (FAD) represents the other 1-5% of cases and is an autosomal dominant inherited form involving mutations in three genes and resulting in augmented production of the Aβ peptide. Importantly, the

neuropathological cascade leading to clinical onset is common between the two classes of AD, resulting in the amyloid cascade hypothesis (Lippa et al. 1996), further detail on which is discussed in section 1.3.1. While sporadic AD does not have a discrete genetic cause, its heritability has been estimated to reach 79% by studies involving the Swedish Twin registry, indicating genetics still play a meaningful role (Gatz et al. 2006). The rapid development in sequencing techniques has led to genome-wide association studies that analyse genetic variants within a population and give a readout of any genes in which variants associate with a particular trait such as AD. These studies have revealed genes in which single nucleotide polymorphisms (SNP) increase the risk of developing AD. The gene with the greatest known effect on sporadic AD development is APOE, which has three genetic isoforms. The APOE ε3 allele is the most common, and the risk of developing AD increases by 3 or 15 times in individuals that are hetero- or homozygous for the APOE ε4 allele, respectively (Farrer et al. 1997). Meanwhile, the APOE ε2 variant reduces risk of developing the disease, but it is less common than the APOE ε4 (Corder et al. 1994). The APOE gene encodes the apolipoprotein E protein, and two decades of interest has implicated it in many pathways such as vascular function, neuroinflammation, glucose metabolism, synaptic plasticity and transcriptional regulation (Liao, Yoon, and Kim 2017). However, the prevailing view is that APOE function is involved in Aβ metabolism and clearance and that efficiency of this is reduced due to expression of the APOE ε4, leading to the increased deposition observed in these individuals (Kok et al. 2009). Other risk loci implicated by recent GWAS studies include PICLAM, CLU, BIN1, (Harold et al. 2009). Importantly, these risk genes appear to converge on amyloidogenic processing of APP as they have been implicated in clathrin-mediated endocytosis, giving further weight to the amyloid cascade hypothesis (Liu and Niu 2009, Thomas et al. 2016). Recently, CR1 and TREM2 have been reported as risk genes and their involvement in inflammatory pathways has galvanised investigation into how this process interacts with AD (Guerreiro et al. 2013, Carmona et al. 2018). While further identification and analysis of functional risk loci will enhance our understanding of sporadic AD pathogenesis, current GWAS data can be applied to individual genomes to generate polygenic risk scores, which can predict whether that individual will develop AD with a success rate over 80% (Chaudhury et al. 2019). Furthermore, the ability to predict the age-specific genetic risk of AD development would be invaluable for recruiting patients to clinical trials at an earlier stage of the disease, potentially opening access to the elusive therapeutic window for clinical efficacy of disease-modifying treatments.

1.4.3 – Symptoms & Diagnosis

AD is characterised by progressive decline in cognitive ability, such as memory loss and problem solving due to functional impairments in the brain regions underpinning cognition. Amyloid neuropathology initiates in the medial temporal lobe (MTL), which encompasses the entorhinal cortex and hippocampus, and progresses to the parietal and frontal lobes (Braak and Braak 1991). In terms of its clinical progression, the earliest symptoms involve a decline in MTL-dependent episodic and semantic memory, which can be detected during a "preclinical" stage of the disease (Hodges, Salmon, and Butters 1990, Bondi et al. 2008). Worsening cognitive condition may lead to the diagnosis of mild cognitive impairment (MCI), a transitional stage from typical cognitive function for a given age to AD (Petersen et al. 1999). However, whether patients are diagnosed with AD depends on both clinical and biomarker criteria (McKhann et al. 2011). More severe stages of the disease involve alterations in personality, difficulty with speech and the loss of everyday function, alongside a pronounced impairment of recent memory and the inability to form new memories (Forstl and Kurz 1999). The two biochemical hallmarks of the disease, first described by Alzheimer himself, are extracellular amyloid plaques and intracellular neurofibrillary tangles (NFT) of hyperphosphorylated tau.

The clinical criteria for diagnosis of different stages of Alzheimer's dementia were revised in 2011 from the initial guidelines set out in 1984 by the National Institute on Aging-Alzheimer's Association (NIA-AA) (Albert et al. 2011, Sperling et al. 2011, McKhann et al. 2011). The original criteria for definitive diagnosis required both clinical and histopathological assessment, meaning that it was not possible until *post mortem* examination of the patient (McKhann et al. 1984). The revision included the use of biomarker evidence (including imaging, serum and CSF analysis) alongside cognitive tests to distinguish patients with even the earliest stages of AD from those with other forms of dementia and MCI (McKhann et al. 2011). The ability to monitor cognitive and biochemical symptoms concurrently is proving to be particularly useful to the pharmaceutical industry. Current trials utilise biomarkers for both inclusion criteria and an outcome measurement (Sevigny et al. 2016). In fact, recent analysis estimated that up to 25% of patients enrolled in AD clinical trials did not exhibit amyloid pathology (Selkoe and Hardy 2016). This information is particularly important within the context of this thesis, as anti-amyloid therapies may have exacerbated cognitive decline in these patients.

In terms of cognitive assessment of potential AD patients, two of the most commonly used tests are the Mini-Mental Status Examination (MMSE) and the Cambridge Neuropsychological Tests Automated Battery (CANTAB) (Cockrell and Folstein 1988, Fray, Robbins, and Sahakian 1996). The MMSE (or Folstein test) is a 30-point questionnaire able to assess episodic and semantic memory, working memory, attention, language and spatiotemporal orientation within 10 minutes (Folstein, Folstein, and McHugh 1975). In contrast, the CANTAB is a non-verbal, computer-based examination of learning, working memory, semantic memory, executive function, attention and reaction time (Fray, Robbins, and Sahakian 1996). Similar frequently used cognitive assessments for AD patients include the General Practitioner Assessment of Cognition (GPAC) and the Montreal Cognitive Assessment (MCA) (Brodaty et al. 2002). In order to measure the impact of a drug on a patient's daily functions during a clinical trial, the Alzheimer Disease Cooperative Study – Activities of Daily Living (ADCS-ADL) records observations of their competence when performing basic tasks such as eating, walking and dressing (Fish 2019).

The criteria of AD diagnosis now require both a clinical deficit in episodic memory measured on one of the tests above as well as at least one abnormal AD relevant biomarker. This includes analysis of Aβ42, hyperphosphorylated tau and total tau in the cerebral spinal fluid (CSF), which delivers diagnostic accuracy of 85-90% (Palmqvist et al. 2015). However, CSF biopsy necessitates lumbar puncture, an invasive and sometimes inconvenient procedure, while results may take weeks to obtain (Zetterberg et al. 2010). Meanwhile, the development of amyloid specific PET ligands such as florbetapir, florbetaben and flutemetamol has permitted non-invasive, diagnostic imaging analysis (Yeo et al. 2015). For example, florbetapir PET scans accurately diagnosed AD in 59 patients with up to 92% sensitivity and 100% specificity, as proven by *post mortem* neuropathological analysis (Clark et al. 2012). However, the patients were in a late-stage of AD and therefore the sensitivity for detecting prodromal AD was not assessed. When examining prodromal patients, the same method measured significantly increased cortical uptake in patients versus age-matched controls, while global cortical amyloid load correlated with cognitive performance (Saint-Aubert et al. 2013). However due to the high expense, amyloid-PET imaging is mainly limited to analysis of patients within clinical trials. Development of less invasive diagnostic tests are under way and recently there have been promising results in blood biomarker assays that measure BACE1 activity or the Aβ42/Aβ40 ratio and are able to predict amyloid pathology with up to 94% success rate (Nakamura et al. 2018, Shen et al. 2018).

1.4.4 – Current Treatments

Despite a plethora of AD modifying therapies entering clinical trials with promising preclinical results, no new treatments have been approved for the disease since 2003. The five treatments currently available for patients are merely symptomatic: although they enhance cognition, they do not impede the rate of cognitive decline. Of these five, three are anticholinesterase inhibitors, one is an NMDA antagonist and one is a combinatorial therapy of two of these compounds. The anticholinesterase inhibitors donepezil (Aricept; Pfizer), rivastigmine (Exelon; Novartis) and galantamine (Reminyl; Janssen), are prescribed for mild to moderate stage AD and augment acetyl choline (Ach) activity by inhibiting the enzyme responsible for its breakdown, acetyl cholinesterase (Birks 2006). Cholinergic transmission has been shown to be decreased during AD pathology and ACh is an important neurotransmitter within learning and memory mechanisms (Davies and Maloney 1976). Therefore, enhancing the activity of ACh may compensate for the impairments in neuronal communication present in AD brains and partially restore efficient memory processing. Meanwhile, the NMDA receptor inhibitor memantine (Ebixa; Eli Lilly), is prescribed for moderate-to-severe stage AD patients (Tariot et al. 2004). Its method of action is to impede the NMDA-dependent excitotoxicity caused by surplus extracellular glutamate and disrupted calcium homeostasis (Danysz and Parsons 2003). Furthermore, combinatorial administration of memantine with donepezil resulted in significantly greater improvement of symptoms than donepezil alone (Tariot et al. 2004). However, although these treatments relieve some of the cognitive decline associated with AD, they do not halt the progression of the disease because they do not target the underlying pathology. Therefore, there has been significant investment to explore both the pathogenic mechanisms of the disease, and how these might be impeded. Disease modifying therapies aimed at manipulating the deposition/clearance of amyloid and/or ameliorating tau pathology have been extensively tested in preclinical models, but have consistently failed in human clinical trials.

1.4.5 – Developing Treatments

Putative therapeutic treatments for AD have mainly focused on two amyloid-related strategies: small molecule inhibitors of the enzymes responsible for APP metabolism and immunotherapies that target Aβ to augment its clearance by the immune system. Despite promising results in preclinical studies, these strategies have consistently failed in clinical

trials. While an overview of the proposed strategies and their outcomes will be highlighted in this section, a thorough review of clinical trial outcomes will be presented in chapter 7.

Although this section focuses on anti-amyloid strategies due to the themes of the thesis, a summary of the current state of tau-based therapy development provides context of the current clinical environment around AD. As tau pathology in AD involves aberrant phosphorylation, kinase and phosphatase enzymes have been proposed as therapeutic targets despite selectivity complications and potential off-target effects. Glycogen synthase kinase 3β (GSK-3β) has been proposed as one of the key enzymes responsible for the abnormal tau phosphorylation associated with AD (Lovestone et al. 1994). Tideglusib (Zeltia Group) is an orally available molecule that irreversibly inhibits GSK-3β and demonstrated the potential to reverse tau pathology, rescue neuronal death and alleviate memory deficits in a transgenic mouse model expressing mutated APP and tau (Sereno et al. 2009). However, a phase II trial did not demonstrate clinical benefit (Lovestone et al. 2015). Another strategy involves inhibition of tau aggregation with drug molecules such as LMTM (TauRx), however this also failed to achieve primary endpoints in phase II trials (Gauthier et al. 2016). As tau is a microtubule associated protein, its primary role in AD pathogenesis is widely considered to be loss of its microtubule-stabilising function, and so pharmacological stabilisation is a potential avenue for AD treatment (Ramser et al. 2013). However, despite demonstrating promising memory protective effects in tau transgenic mice, no drugs of this class have progressed beyond phase I clinical trials for AD (Matsuoka et al. 2008). The final anti-tau treatment described here involves using antibodies raised against abnormally phosphorylated tau to promote its clearance by the immune system, a concept which is detailed below within the context of anti-amyloid strategies. Some anti-tau immunotherapies have shown promising preclinical results and are currently in early stage clinical trials (Theunis et al. 2013, West et al. 2017). In summary, while tau-based strategies for the treatment of AD have demonstrated some potential, they are at an earlier stage of development compared to anti-amyloid therapies.

Small Molecule Inhibitors

One therapeutic strategy to relieve amyloid pathology is to inhibit the β– and γ–secretase enzymes responsible for amyloidogenic APP metabolism. Evidence supporting this strategy is available from genetic studies. For example, genetic deletion of the enzyme in KO mouse lines eradicated production of Aβ and βCTF (Luo et al. 2001, Kobayashi et al. 2008, McConlogue et al. 2007). BACE1 KO also rescued cognitive deficits exhibited

by transgenic APP mouse lines, e.g., Tg2576, PDAPP and 3xTg mice (Ohno et al. 2004, Ohno et al. 2007). First generation BACE1 inhibitor molecules were peptidomimetic analogues of the APP cleavage site and significantly reduced soluble Aβ *in vitro* and *in vivo*. Nevertheless, they suffered from poor oral bioavailability, poor BBB penetrability and a long serum half-life (Asai et al. 2006, Kimura et al. 2005, Ghosh, Brindisi, and Tang 2012, Yan and Vassar 2014). More recent iterations of inhibitor molecules have demonstrated improved pharmacokinetics leading to robust effects on Aβ clearance and rescued cognitive deficits in transgenic models (Hussain et al. 2007, Fukumoto et al. 2010). Several drugs such as LY2811376 (Eli Lilly) and MK-3931 (Verubecestat, Merck) have demonstrated the potential to reduce CSF Aβ levels in both animals and humans (May et al. 2011, Kennedy et al. 2016). However, these molecules have all been withdrawn from trials at varying stages due to either off-target effects (possibly due to the influence of BACE1 within other pathways) or a lack of effect on cognitive symptoms (see section 7.2).

A similar pattern has been observed during the development of γ-secretase inhibitors. Modulation of γ-secretase function is known to impact AD pathogenesis because mutations in PS1 cause FAD (Sherrington et al. 1995). γ-secretase inhibitors were able to reduce Aβ levels *in vitro* and in the brains of PDAPP transgenic mice (Dovey et al. 2001). However, similar to the BACE1 inhibitors, promising preclinical results have not translated to clinical trial success. Both avagacestat (Bristol-Myers Squibb) and semagacestat (Eli Lilly) were discontinued from their respective phase II and III trials due to lack of cognitive benefit as well as increased risk of adverse effects including skin cancer (Doody et al. 2013, Coric et al. 2012). Moreover, patients receiving the highest doses of both drugs were associated with a trend for worse cognitive decline compared to placebo-treated control patients, despite semagacestat having previously demonstrated a dose-dependent decrease in human plasma and CNS Aβ (Bateman et al. 2009, Fleisher et al. 2008). The detrimental effects of γ-secretase inhibition may be due to the impact on non-APP substrates (Haass 2004). For example, PS1 deficiency reduced the γ-secretase cleavage of both APP and Notch, which has been linked to oncogenesis (De Strooper et al. 1999). This potential for off-target effects has led to the development of γ-secretase modulators that have demonstrated selective inhibition of APP cleavage; reducing the amyloid plaque burden in the cortex and hippocampus while attenuating the spatial memory deficit of a transgenic AD mouse model, with no effect on Notch signalling in HEK239Swe cells (Imbimbo et al. 2009). Other groups have reported similar effects in Tg2576 mice using alternative novel

compounds, further indicating the preclinical efficacy of this mechanism (Rogers et al. 2012, Kounnas et al. 2010, Wagner et al. 2017). These modulator compounds were originally derived from non-steroidal anti-inflammatory drugs, which have been epidemiologically associated with a reduced risk of AD development (Breitner et al. 1994, Crump, Johnson, and Li 2013). Whether this more selective inhibition of γ-secretase cleavage successfully translates to clinical trials remains to be seen.

Anti-Amyloid Immunotherapies

Immunotherapy - the recruitment of immune molecules to perform a therapeutic effect – is a popular pharmacological approach due to the specificity granted by high affinity antibodies. Active immunotherapy exploits the patient's immune response, while passive immunotherapy involves injection of exogenous antibodies raised against the target protein. Aβ peptides and plaques were the most logical target, especially considering AD patients were found to have fewer endogenous anti-Aβ antibodies in their serum and CSF (Du et al. 2001). The Solomon group reported in 1996 that monoclonal antibodies (mAb) raised against Aβ inhibited aggregation of the peptide when incubated *in vitro,* suggesting mAbs as a potential therapeutic approach (Solomon et al. 1996). There are several mechanisms by which anti-Aβ antibodies might promote clearance of the peptide. Firstly, immunoglobulin molecules contain an Fc domain, which specifically interacts with the Fc receptors on microglia and stimulates phagocytosis of both the antibody and the Aβ peptide it is bound to (Bard et al. 2000). However, this may not be the exclusive mechanism as the effect of Aβ immunisation was maintained following deletion of the Fc receptor (Das et al. 2003, Bacskai et al. 2002). Anti-Aβ antibodies may sequester the peptides circulating in the serum thereby shifting the concentration gradient across the BBB and encouraging increased efflux from the brain parenchyma (Lemere et al. 2003). Indeed, anti-Aβ immunotherapy studies have reported increased serum concentrations of the peptide (DeMattos et al. 2001). Meanwhile the sequestering of monomers and oligomers in the CNS may both inhibit aggregation and neutralise the mechanisms required to induce synaptic dysfunction (Walsh et al. 2002). Reduction of the soluble Aβ concentration may promote the disaggregation of plaques, as the various species of Aβ are thought to exist in equilibrium (Benilova, Karran, and De Strooper 2012). However, despite a plethora of studies demonstrating the potential of anti-Aβ immunotherapies to reduce the amyloid burden and rescue the cognitive impairment of aged transgenic mouse models, as well as evidence of plaque clearance in human trials, no significant beneficial effect on human

cognitive decline has been reported. A thorough review of both active and passive immunotherapies in clinical trials of AD patients is presented in chapter 7.

Conclusion

The consistent failure of anti-amyloid therapies in clinical trials for AD patients has resulted in challenges to the amyloid hypothesis. As mentioned above, an updated hypothesis has modified the approach of clinicians, switching the focus from plaques to soluble oligomeric Aβ species while also recruiting patients at an earlier stage of the disease. The existence of mutations within the APP gene that significantly elevate or reduce the likelihood of developing AD is ostensibly impervious evidence supporting the protein's role in AD pathogenesis. However, explanations for the failure of anti-amyloid therapies must be promptly illuminated if the hypothesis is going to deliver a treatment. One explanation is that detectable pathology may already represent a stage beyond the remedial potential of anti-amyloid drugs; particularly considering current animal models (in which the treatments have shown success) reproduce preclinical stages of AD. However, in addition to medication being delayed beyond the therapeutic window, it is possible that premature administration of anti-amyloid treatment may also be detrimental. This major theme of this thesis is originally derived from the intriguing observation that BACE1 KO mice which exhibit deficits in spatial memory (Ohno et al. 2004, Laird et al. 2005, Kobayashi et al. 2008). Furthermore, pharmacological evidence also suggests that BACE1 inhibition is detrimental to cognition, as two separate inhibitors administered to WT mice both resulted in Y-maze alternation impairments (Filser et al. 2015). Therefore, while reducing the levels of Aβ has resulted in cognitive benefits in many studies involving transgenic APP models of AD, the detrimental effects in WT mice raises concerns for the welfare of patients; particularly with clinical trials aiming to recruit patients in earlier stages of the disease who may not have developed widespread amyloid deposition. In fact, during a clinical trial of 1454 patients diagnosed with prodromal AD, administration of the BACE inhibitor Verubecestat was actually associated with significantly worse cognitive decline and greater progression to AD than placebo treatment (Egan et al. 2019). This further suggests that amyloid inhibition as a therapeutic strategy may carry risks especially in those whose cognitive function is not severely compromised. One question that remains to be addressed is whether the detrimental effect of BACE inhibitors is caused by Aβ reduction or off-target effects. This issue will be addressed in Chapter 3 by using an antibody that binds to the β-cleavage site of APP and sterically inhibits cleavage, resulting in knock-down of Aβ (Thomas, Liddell, and Kidd 2011).

1.5 Modelling AD

One goal of this thesis was to investigate how the physiological role of Aβ changes during its accumulation leading to the toxic effect we see in AD. The failure of so many clinical trials of treatments had been supported by promising preclinical studies has led to the questioning the validity of genetically modified FAD models. In particular, the transgenic nature and hyperexpression of APP is under scrutiny. Recent evidence suggests that overexpression mechanisms may lead to artefactual effects in transgenic mice, particularly with APP as all of its fragments are overexpressed, meaning that the resulting phenotype is not specifically related to augmented Aβ levels. In order to elucidate these artefacts and assess the impact of increased Aβ expression in a more physiological manner, Takaomi Saido (RIKEN Centre for Brain Science, Japan) generated three novel "knock in" mouse models that express murine APP with a humanised Aβ sequence, under the control of the endogenous promoter. Therefore, APP is not overexpressed but Aβ production is upregulated due to the presence of FAD mutations, generating a more reliable *in vivo* system of the human disease. The focus of the current section will be to provide an overview of how first generation, transgenic models have shaped our understanding of amyloid pathology. It is important to comprehend the criticism concerning the relevance of these models, particularly within the context of the failure of promising therapies to deliver beneficial effects in clinical trials. Finally, this section will present current evidence of the phenotype observed in the second-generation, knock-in mice.

▪ 1.5.1 - First Generation Transgenic Models

Genetically modified mouse models have been used to model amyloid pathology and AD for two decades (Hsiao et al. 1996). These models exploit the discovery and sequencing of autosomal dominant, FAD-associated mutations in the genes encoding APP, PS1 and PS2 (Webster et al. 2014). Genetic constructs containing these human FAD mutations are expressed under the control of artificial promoters, which results in upregulated protein production above endogenous levels (Kobayashi and Chen 2005, Sasaguri et al. 2017). All the mutations linked to early onset AD result in increased amyloidogenic processing of APP, with the majority altering γ-secretase activity to increase the Aβ42/Aβ40 ratio, releasing peptides that are more likely to aggregate (Citron et al. 1997). Therefore, expression of these mutations in mice generates *in vivo* models of amyloidosis, which have permitted detailed investigations into the mechanisms of Aβ-

related pathogenesis. Common neuropathology observed in these mouse models are cerebral Aβ deposition and reactive gliosis: (activation and proliferation of microglia and astroglia that indicates neuroinflammation) (Irizarry et al. 1997, Games et al. 1995). Some models also exhibit the synaptic impairment, dendritic spine degeneration and hippocampal cell loss associated with high levels of Aβ (Redwine et al. 2003, Reilly et al. 2003, Jacobsen et al. 2006). Crucially, transgenic models also exhibit cognitive deficits, the major clinical hallmark of human AD. A comprehensive review of cognitive investigations using longitudinal designs in 10 distinct transgenic AD models revealed a common pattern: that spatial working memory (SWM) impairment manifests prior to the decline of other aspects of memory, such as object recognition (Webster et al. 2014). Inconsistencies in the reported age points of cognitive changes reflects the distinct background strains and specific nature of the transgene overexpression, as well as the precise protocols performed by each research group. As spatial memory tasks are well-characterised to depend on intact hippocampal activity, cross-sectional analysis suggests that this brain region is particularly susceptible to Aβ-related dysfunction (Morris, Hagan, and Rawlins 1986, Olton and Werz 1978, Aggleton, Hunt, and Rawlins 1986). Importantly, this recapitulates the clinical progression of cognitive impairment in human AD, of which a decline in MTL-dependent episodic memory is an early marker (Bateman et al. 2012).

The first mouse models of AD transgenically expressed mutant human APP (hAPP). The PDAPP mouse expressed hAPP containing the Indiana (V717F) mutation at over 10-fold higher levels than endogenous murine APP (Games et al. 1995). The PDAPP mouse exhibits plaques from 4-6 months of age, as well as dendritic spine loss and reactive gliosis. SWM deficits have been observed at 3 and 4 months of age in the radial arm maze (RAM) and Morris Water Maze (MWM), prior to plaque pathology (Dodart, Meziane, et al. 1999, Hartman et al. 2005). Another well-characterised human APP transgenic (hAPP-Tg) mouse model is the Tg2576, which expresses the Swedish (KM670/671NL) mutation located at the BACE1 cleavage site of APP (Hsiao et al. 1996). The Swedish mutation increases the affinity for the BACE1-APP interaction, resulting in a bias for β-, over α-secretase metabolism demonstrated by 5- and 14-fold increases in Aβ40 and Aβ42(43), respectively (Hsiao et al. 1996). The Tg2576 mouse develops plaques at 11-13 months of age, while synaptic impairment and degeneration was detected in the hippocampus at 4-5 months (Jacobsen et al. 2006). Deficits in spatial reference memory and alternation have been observed at 9-10 months of age, while visuospatial object recognition memory deficits

appeared in 14 month old mice (Hale and Good 2005). No single AD animal model recapitulates all the pathological features of the human disease, and multiple mutations are often combined to produce a more comprehensive set of pathological features. Overexpression or knock-in of pathogenic mutant PS1 alone induced neurodegeneration but not amyloid pathology, possibly due to insufficient quantity of Aβ (Chui et al. 1999). Alternatively, the three amino acid residues that distinguish murine Aβ from the human version may impede amyloidogenesis (Xu, Ran, et al. 2015). Crossing transgenic strains to combine human APP over-expression with mutations in PS1 accelerates AD-related neuropathology (Borchelt et al. 1997). These double transgenic mice include the APP/PS1 mouse, which develops plaques in the cortex from six weeks and in the hippocampus at 3-4 months of age (Radde et al. 2006). Cognitive deficits have been reported in spatial learning and memory at 7 months (Serneels et al. 2009) and reversal learning at 8 months (Radde et al. 2006). The APPswe/PS1dE9 strain developed detectable plaques at 4 months of age, while cognitive deficits were reported in the object-in-place task in 5 month old mice (Bonardi, Pardon, and Armstrong 2016, Garcia-Alloza et al. 2006).

The interest in mouse models expressing multiple mutations resulted in the generation of 5XFAD and 3xTg-AD mice, both of which develop cognitive deficits at a young age (<4 months). The 5XFAD model was developed by Oakley *et al* (2006) and combines three APP mutations with two in PS1, all driven by the Thy-1 promoter. The rapid onset of pathology includes Aβ accumulation and reactive gliosis from <2 months of age, while synaptic loss and cognitive deficits were detectable at 4-5 months of age (Oakley et al. 2006). However, rapid and severe amyloidosis in the 5XFAD model did not progress to NFT pathology in the absence of mutant tau. In order to concurrently observe both amyloid and tau pathogenesis, Oddo and colleagues (2003) developed the 3xTg-AD mouse, which overexpresses APP_{SWE} and Tau_{P301L} on a $PS1_{M146V}$ knock-in background. This model recapitulates both the Aβ plaque and NFT pathology exhibited by human AD patients, as well as the other hallmarks of synaptic and cognitive impairment (Billings et al. 2005, Oddo et al. 2003). However, it must be noted that the tau mutant expressed in these mice is not associated with AD but frontotemporal dementia with parkinsonism linked to chromosome 17 (Lewis et al. 2000). While some unknown aspect of murine physiology prevents NFT formation following expression of FAD mutations, KO of MAPT (microtubule associated protein tau: the gene encoding tau) conferred neuroprotection and ameliorated memory deficits in APP-Tg mice (Roberson et al. 2007, Ittner et al. 2010), suggesting that there is still some connection between amyloid and tau in these animals.

Class of Model	Strain(s)	Genetic Background	Promoter	Mutation(s)	General Phenotype	Specific Disadvantages
Single Transgenic APP-Tg	PDAPP J20 APP23 Tg2576	C57/Bl6 C57/Bl6 C57/Bl6 Bl6,SJL mixed	PDGF-β PDGF-β Mouse Thy1 Hamster prion protein	APP^{V717F} $APP^{KM670/671NL}$ APP^{V717F} $APP^{KM670/671NL}$ $APP^{KM670/671NL}$	Amyloid deposition from ~6 months Moderate behavioural phenotype Neuronal loss in some models	No NFTs Cognitive impairment preceding Aβ accumulation Mixed background in some cases
Double transgenic APP-Tg x PS1-Tg (or KI)	APP/PS1 5xFAD	C57/Bl6 (C57Bl/6 x SJL)F1 & C57Bl/6	Mouse Thy1 Mouse Thy1.2	$APP^{KM670/671NL}$ $PS1^{I166P}$ $APP^{KM670/671NL}$ APP^{I716V} APP^{V717I} $PS1^{I166P}$ $PS1^{I286V}$	Aβ accumulation from early age Moderate behavioural phenotype Neuronal loss in some models	Potential interaction of multiple mutations Mixed background in some cases Complicated cross-breeding
Triple Transgenic	3xTg-AD	C57Bl/6	Mouse Thy1.2 (APP, Tau) Endogenous (PS1)	$APP^{KM670/671NL}$ $MAPT^{P301L}$ $PS1^{M146V}$	Moderate to severe behavioural phenotype NFT formation Neuronal loss	Potential interaction of multiple mutations MAPT mutation not associated with AD Complicated cross-breeding Cognitive impairment preceding Aβ accumulation
Single APP Knock-In	*APP^{NLF}* *APP^{NLGF}*	C57Bl/6	Endogenous APP	$APP^{KM670/671NL}$ APP^{I716F} $APP^{KM670/671NL}$ APP^{I716F} APP^{E693G}	Minor behavioural phenotypes Aβ accumulation from an early age, alongside endogenous APP expression	Potential interaction of multiple mutations Lack of behavioural phenotypes Slow breeding due to genomic homozygosity

Table 1.1: Comparison of current APP mouse models of AD. This table summarises multiple classes of AD mouse model, to highlight their specific disadvantages, adapted from Sasaguri *et al* (2017). There are individual strains within each class that express various mutations. Transgenic mice have general disadvantages applicable to all of them, including random transgene insertion into the genome, APP overexpression-related artefacts, artificial promoters and the lack of relevant control mice.

Transgenic AD mouse models have been invaluable for researchers aiming to elucidate the mechanisms of amyloid accumulation and subsequent pathology. For example, while the lack of correlation between plaque burden and memory deficits initially confounded researchers, it triggered the shift in the understanding that soluble oligomeric species of Aβ delivered the neurotoxic activity of the peptide, rather than plaques (Chen et al. 2000). Evidence that gene knock-out of BACE1 abolishes cerebral amyloid deposition and the downstream neuropathology of synapse loss and cognitive impairment in all transgenic models tested to date strongly indicates a causative effect of Aβ accumulation' although the potential impact of βCTF overexpression cannot be excluded (Ohno et al. 2004, Ohno et al. 2007, Laird et al. 2005, McConlogue et al. 2007). The beneficial effect of genetic BACE1 depletion led to the screening of BACE1 inhibitors in these models. Small molecule inhibitors reduced cerebral amyloid load by up to 90% which accompanied rescue of learning and memory impairments (Kennedy et al. 2016, May et al. 2015, Neumann et al. 2015). Similarly, these models have been useful in the development of anti-amyloid immunotherapies such as aducanumab, which induced a dose-dependent reduction in the Aβ levels of Tg2576 mice (Sevigny et al. 2016). Furthermore, *in vivo* studies investigating the influence of γ-secretase on notch signalling predicted the adverse effects of inhibitor molecules observed in clinical trials (De Strooper et al. 1999).

▪ 1.5.2 – Criticism of 1st gen models

While the studies described in the previous section established the proof of principle of secretase inhibitors or anti-Aβ antibodies as putative therapeutic mechanisms to relieve amyloid pathology and memory deficits in first generation, transgenic mice; the failure of any treatments to show clinical benefit in trials of AD patients has brought their relevance as models into disrepute. The mutations expressed in these genetic models are associated with familial, not sporadic AD. Estimates for the proportion of AD cases that are familial range from 5% to less than 1% (Campion et al. 1999, Reitz and Mayeux 2014), and this percentage will decrease due to the aging global population. Almost all clinical trials for AD have recruited patients with sporadic AD, in which pathogenesis occurs by alternative genetic and environmental mechanisms to FAD (Bettens, Sleegers, and Van Broeckhoven 2013). Although amyloid deposition is hypothesised to be the central initiating factor in both forms of the disease, the use of FAD models to screen therapeutic treatments may not be relevant for the vast majority of patients (Hardy and Higgins 1992). For example, transgenic overexpression of mutated human APP may result in the generation of APP

fragments and Aβ species that are not present in the brains of sporadic AD patients (Sasaguri et al. 2017). Furthermore, many models involve multiple, combined mutations which do not naturally occur and are therefore not representative of any human form of the disease. Combinatorial models involve the cross-breeding of inbred mouse lines which further delineates the genetics and physiology from the original, natural state, generating artificial phenotypes (Saito et al. 2014). Meanwhile, although mice expressing mutant APP and/or PS1 exhibit the age-related plaque formation and cognitive deficits associated with AD, a complete recapitulation of the human condition seems impossible (LaFerla and Green 2012). Pathology does not always progress to the NFTs and neurodegeneration observed in late stage AD patients (Webster et al. 2014). In fact, mutations in the gene encoding the tau protein are required to produce tangle pathology (Lewis et al. 2001). These mutations are not associated with AD and therefore introduce further unphysiological alterations from both the human condition and wild-type mice (Lewis et al. 2000).

The most salient critique of transgenic models focuses on the overexpression mechanism utilised to generate the Aβ accumulation. Protein hyperexpression inherently confers potential cellular effects that are independent of the specific amplified protein. For example, insertion of the transgene into the mouse genome is random and potentially occurs within endogenous gene loci, impeding its expression (Kuro-o et al. 1997, Verret et al. 2012). Furthermore, the artificial promoter may result in non-specific tissue expression of the protein while also competing with endogenous promoters for transcription factors; both of which may influence cellular and network function in a spontaneous and uncontrolled manner (Matthaei 2007). Importantly, the inherent issues concerning the random insertion of transgenes, not least the variable copy number, diminish the reliability of wild-type negative control animals to reveal specific effects of the protein of interest (Sasaguri et al. 2017). Meanwhile, the variable combination of different artificial promoters, transgene constructs and background strains render comparisons between models difficult and restrict the presentation of a standardised phenotype (Webster et al. 2014). The nature of APP itself exacerbates the inherent variability of transgenic models, due to the multiple fragments produced from its metabolism, all of which are upregulated in mice overexpressing the protein. Therefore, the phenotypes observed in these hAPP transgenic mice may not be specifically caused by increased Aβ accumulation, but instead the unphysiological interaction of non-Aβ fragments with cellular proteins (Chang and Suh 2005, Mitani et al. 2012, Nhan, Chiang, and Koo 2015, Willem et al. 2015). For example, the Swedish mutation in the Tg2576 mouse confers increased production of βCTF, a

fragment that has demonstrated neurotoxic and memory impairing effects following its augmented expression (Nalbantoglu et al. 1997, Berger-Sweeney et al. 1999). Moreover, overexpression of full-length APP may disrupt its endogenous interactions with cellular proteins including JIP-1 (Chiba et al. 2014).

The confounds of APP overexpression manifest in artefactual, age-independent effects detectable prior to amyloid pathology. For example, the Dodart group observed anatomical and physiological abnormalities in PDAPP mice from as young as three months of age. They reported significant atrophy in the hippocampus, reduced cerebral glucose metabolism and mild learning deficits, suggesting that these results may represent effects of APP overexpression, rather than amyloid accumulation (Dodart, Meziane, et al. 1999, Dodart, Mathis, et al. 1999). Chen *et al* (2000) assessed SWM performance at both 6-9 and 13-15 months of age in the MWM. Transgenic mice performed significantly worse at the early time point, and this difference exacerbated with age. Further investigation elucidated an age-independent difference in training behaviour which was dissociated from age-dependent components that manifested at 13-15 months of age (Chen et al. 2000). In a separate study, Evans observed age-independent differences in navigational foraging strategy from 6-8 months old. The task involved searching for a liquid reward in a circular arrangement of six pots, and the experimenters reported that transgenic mice were more likely to visit neighbouring rather than distal pots and also to adopt a chaining response (consecutive visits to three or more adjacent pots) (Evans et al. 2018). They also revealed a separate age-dependent phenotype, as 14-16-month-old PDAPP mice performed significantly worse than WT littermates. However, it must be noted that in the absence of data from earlier time-points, one cannot exclude the possibility that 6 months simply represented the point at which the described phenotypes manifested in both studies. Another group observed age-independent deficits in spatial strategies used by PDAPP mice in the circular maze alongside significantly lower body temperature in both young (3-5) and aged (20-26 month old) transgenic mice compared to WT littermates (Huitron-Resendiz et al. 2002). Furthermore, young PDAPP mice exhibited significantly lower locomotor activity during the dark period and also a reduction in the generation of rapid-eye-movement sleep versus WT mice. This evidence of age-independent effects supports the notion that APP overexpression induces artefactual results which are particularly relevant in behavioural tests involving navigation. A more physiological method of generating AD models would generate more reliable data.

1.5.3 – Second Generation, Knock-In Models

In order to overcome the inherent unreliability of hAPP transgenic models and eliminate potential artefactual effects, a more elegant, "knock-in" method has been proposed. Specific gene-targeting techniques permit selective FAD mutations to be introduced within the endogenous murine APP gene. Therefore, mutant APP is expressed without transgene insertion, under the control of the endogenous promoter, avoiding potential artificial phenotypes. Reaume *et al* (1996) generated one such knock-in model which expressed APP at normal levels, however the incorporation of the Swedish double mutation resulted in 9-fold greater Aβ production. However, as further analysis revealed that these mice did not develop amyloid pathology up to 22 months of age, these mice were cross-bred with a mutant PS1 knock-in mouse to generate a double knock-in (Flood et al. 2002). These double gene targeted mice exhibited elevated Aβ42 resulting in amyloid deposition that began at 6 months of age and progressed linearly with age, in contrast to Tg2576 / PS1 knock-in mice in which deposition increased exponentially. The APP^{SWE}/PS1 double knock-in mice did not exhibit differences in locomotor or anxiety-related behaviours, however they did develop cognitive dysfunction with age observed in object recognition and the radial arm water maze from 11 and 15 months of age respectively (Webster, Bachstetter, and Van Eldik 2013). Despite the extensive amyloidosis, neuropathology did not progress to NFT formation and neurodegeneration, even in mice aged up to 27 months (Malthankar-Phatak et al. 2012). Another knock-in model was developed by Li *et al* (2014) which carried the Dutch (E693Q) and London (V717I) mutations alongside the Swedish. The Dutch mutation is not associated with FAD but causes cerebral amyloid angiopathy (CAA) leading to brain haemorrhage and early mortality in humans (Van Broeckhoven et al. 1990). Similar to the knock-in strain generated by Reaume, these mice did not develop significant Aβ deposits until they were crossed with PS1 mutants. The double knock-in mice exhibited deposition in both the cerebral parenchyma and vasculature, symptomatic of expression of the Dutch mutation. For that reason, this model has become more relevant for studying CAA. Meanwhile, the APP^{SWE}/PS1 double knock-in mice have also not been widely used by the AD community, potentially due to their double homozygous nature and late development of pathology, which leads to inefficient breeding and a long delay before observing significant effects.

Takaomi Saido and colleagues at the RIKEN Brain Science Institute, in Japan, have also generated knock-in models of FAD (Saito et al. 2014). They first humanised the Aβ

sequence by changing three residues within the endogenous mouse APP gene (G676R, F681Y and H684R), and subsequent expression of either one, two or three FAD mutations leads to three different knock-in strains. The *APP*$^{-NL}$ model includes the Swedish double mutation (KM670/671NL), which augments the interaction of BACE1 with APP, resulting in increased cleavage and Aβ generation (Citron et al. 1992). The *APP*$^{-NL-F}$ mouse also incorporates the I716F Iberian mutation, which promotes γ-secretase cleavage at the 42 site and augments the $A\beta_{42}$:$A\beta_{40}$ ratio by a factor of 30 *in vitro* (Lichtenthaler et al. 1999). Finally, the *APP*$^{-NL-G-F}$ mouse expresses both aforementioned alterations as well as the E694G Arctic mutation which occurs in the central domain of the Aβ sequence and renders the peptide more prone to oligomerisation (Cheng et al. 2004, Nilsberth et al. 2001). Importantly, homozygous models of these mice expressed APP and non-amyloidogenic fragments at the same level as WT littermates. βCTF increases with Aβ in all models in a gene dose-dependent manner, thereby promoting the *APP*$^{-NL}$ mouse as the ideal negative control for assessment of the specific effects of the augmented amyloid pathology exhibited in the *APP*$^{-NL-F}$ and *APP*$^{-NL-G-F}$ mice. However, it must be noted that using this strain in this way would introduce greater genetic heterogeneity between the test (*APP*$^{-NL-F}$) and negative control mice (*APP*$^{-NL}$), due to loss of littermate comparisons. Independent characterisation of 24-month-old *APP*$^{-NL}$ mice revealed no development of plaque pathology, reactive gliosis or cognitive deficits compared to age and sex matched C57Bl/6 mice (Salas et al. 2018). It is notable that this was consistent with the phenotype of the knock-in mice developed by Reaume *et al*, which shares the same mutant APP sequence (Reaume et al. 1996). This suggests that any pathogenic phenotypes demonstrated by *APP*$^{-NL-F}$ and *APP*$^{-NL-G-F}$ mice are linked to the effects of the further mutations incorporated into their genomes on Aβ pathology and are not associated with differences in βCTF levels.

Investigations using these knock-in models have already demonstrated results that suggest some pathological effects reported in transgenic animals were artefacts of APP overexpression. For example, the Saido group previously reported that Aβ plaque deposition was associated with a calcium-activated cysteine protease, calpain, in both APP23 transgenic mice and AD patients (Higuchi et al. 2012). Genetic deficiency of calpastatin, a protein that specifically inhibits calpain, exacerbated amyloidosis and downstream neuropathology pathology in the transgenic mice while the mortality rate increased to 50% by 10 weeks of age. However, when *APP*$^{-NL-F}$ mice were crossed with calpastatin KO animals, although amyloidosis was augmented, the early death phenotype

was eradicated (Saito et al. 2016). Furthermore, while studies in transgenic mice have proposed that one mechanism of amyloid pathogenesis involves Aβ initiating the conversion of p35 to p25 (a CDK5 activator), increased p25 generation was not observed in *APP*$^{-NL-F}$ mice, even following hyperactivation by calpastatin depletion (Seo et al. 2014, Saito et al. 2016). A third effect observed in multiple first generation models that has not been replicated in knock-in mice is the downregulation of $Na_v1.1$ sodium channels present in parvalbumin-positive interneurons (Verret et al. 2012, Saito et al. 2016, Kim et al. 2011). The decreased expression of this voltage gated sodium channel had been linked to the network hypersynchrony and memory deficits exhibited by hAPP transgenic mice after restoration of the channel to normal levels rescued deficits in the mice; however, the role of $Na_v1.1$ channels in AD pathogenesis will have to be re-examined. The Saido group have estimated that as many as 60% of the phenotypes reported in APP transgenic models may have been influenced by artefactual effects of overexpression.

While the second-generation AD models are theoretically more reliable systems in which to investigate mechanisms of amyloid pathology, they are not without limitations. They do not recapitulate progression to late-stage AD neuropathology, as NFT formation and neurodegeneration are absent. Therefore, these mice may be considered models of preclinical AD, a stage at which underlying amyloid pathology is developing and cognitive decline is beginning to manifest. It is possible that the progression of AD-associated pathology is limited by the life-span of the mice, as these processes occur over decades in humans while the mice do not outlive three years (Bateman et al. 2012). Furthermore, the combination of FAD mutations in the *APP*$^{-NL-F}$ and *APP*$^{-NL-F-G}$ mice does not represent a natural form of the disease, and multiple mutations may interact in an unforeseen manner, reducing the reliability of the *APP*$^{-NL-G-F}$ model to investigate mechanisms of Aβ oligomerisation and deposition (Sasaguri et al. 2017). Furthermore, although the chimeric APP expressed in these strains is a necessary confound to limit changes from control mice, it may result in different Aβ deposition mechanisms compared to the endogenous protein. These knock-in models represent more reliable models than first generation transgenic mice in which to screen potential AD treatments, however the Swedish mutation may result in the APP sequence exhibiting altered sensitivity to BACE1 inhibitors and this must be taken into account in preclinical studies. However, the fact that APP is expressed under the endogenous promoter in these mice constitutes a major advantage over first generation models because it ensures that results are not impacted by unphysiological APP expression.

1.5.4 – The *APP*$^{NL-F}$ Knock-In Model

The augmented production of $A\beta_4$ due to the presence of the Iberian mutation in *APP*$^{NL-F}$ mice results in progressive deposition of the peptide in the cerebral cortex and hippocampus detectable from 12 months of age (Saito et al. 2014). The plaques observed in these mice consisted mainly of a particular pathogenic species of Aβ ($A\beta_{1/3pE-42}$), thereby more faithfully resembling plaques found in human AD brains in comparison to those of other transgenic APP models, which are unphysiologically large and predominantly composed of $A\beta_{40}$ (Saido et al. 1995, Sasaguri et al. 2017). Heterozygous (*APP*$^{NL-F/WT}$) mice did not exhibit cortical amyloidosis until they were over 24 months of age. Amyloidosis in *APP*$^{NL-F}$ mice was accompanied by the neuropathology commonly associated with brains of AD patients or mouse models: activated microglia and astroglia cells were observed around Aβ plaques, while reduced immunoreactivity of synaptophysin and PSD95 suggested reduction of synaptic density. However, Sato and colleagues did not observe evidence of somatodendritic atrophy or hyperphosphorylated tau, indicating that these knock-in mice represent a model of preclinical AD. The Arctic mutation present in the Aβ sequence of *APP*$^{NL-G-F}$ mice resulted in earlier, more aggressive accumulation of pathology compared to the *APP*$^{NL-F}$ mice. Cortical amyloid deposition was detectable from 2 months and had approached saturation point by 7 months of age (Saito et al. 2014). These mice also exhibited significantly greater microglia and astrocyte activation in the cortex from 12 months of age (Masuda et al. 2016). Interestingly, there was a significant effect of sex in these mice, with 18-month-old females demonstrating more amyloid pathology and astrocytosis in both the cortex and hippocampus than their male counterparts. Meanwhile, the *APP*^{NL} mice did not develop detectable amyloid deposition when tested up to 18 months; although increased microgliosis was observed in the hippocampus when tested against WT mice at this age point.

The knock-in mice developed by the Saido group also develop cognitive impairment. The initial paper by Saito *et al* (2014) analysed spontaneous alternation behaviour in a Y-maze, reporting that while *APP*$^{NL-G-F}$ mice showed a deficit at 6 months of age, *APP*$^{NL-F}$ performance matched WT and NL levels. The *APP*$^{NL-F}$ animals were aged up to 18 months before they demonstrated decreased alternation behaviour and the *APP*^{NL} mice never developed this impairment. A follow up study aimed to characterise *APP*$^{NL-F}$ behaviour at 8-12 and 13-17 months of age using an IntelliCage system which permitted analysis of multiple cognitive domains (Masuda et al. 2016). Mice were housed in the

IntelliCages in mixed genotype groups of 10-12 and each received a subcutaneously implanted radio transponder in order to simultaneously assess individual behaviours. The four corners of the cage contained chambers from which water was available. Access to the chambers was controlled by doors, which could be opened by a nose poke at specific times during the training and testing schedules. The different paradigms available for assessment by the Intellicages revealed a deficit in place avoidance learning alongside, intriguingly, a significant enhancement of place preference reversal learning in the *APP*$^{-NL-F}$ mice at the younger age point. At the second age-point the *APP*$^{-NL-F}$ group had developed a deficit in place preference reversal learning; however, analysis of the place avoidance task was not accessible because none of the groups demonstrated retention. A compulsive phenotype was evident at both age points, measured via a delay discounting task in which the waiting time for a liquid reward to become available following a nose poke was increased each day. Premature licks during the delay period were counted as measures of compulsivity. Meanwhile, the *APP*$^{-NL-G-F}$ mice demonstrated similar behavioural phenotypes, exhibiting compulsivity and deficits in place avoidance and place preference avoidance learning at both age points (Masuda et al. 2016).

Since the initial publications by the Saido group, the cognitive impairment reported in *APP*$^{-NL-G-F}$ mice has not been consistently replicated and the number of studies that have aged the *APP*$^{-NL-F}$ mice up to 18 months is limited (figure 1.3). As these knock-in mice are a more accurate representation of age-related amyloid pathology than the first-generation transgenic mice, it is imperative that the effect of Aβ accumulation without APP overexpression on both cognitive and biochemical phenotypes is elucidated. This will also enable better understanding of the effects reported in transgenic mice that may be artefacts of the genetic mechanism of transgenic overexpression. The *APP*$^{-NL-F}$ model is the more appropriate to investigate these aims than the *APP*$^{-NL-G-F}$ because AD is associated with senescence, and so mice that develop pathology in early adulthood do not replicate this critical feature. Furthermore, the combination of three FAD mutations in the *APP*$^{-NL-G-F}$ mice may produce unphysiological interactions, thus rendering this model a less accurate representation of AD. Moreover, considering the *APP*$^{-NL-F}$ model exhibits a slow accumulation of Aβ, it presents the opportunity to investigate how the role of the peptide switches from performing the physiological functions described in section 1.2, to its putative involvement in the pathogenesis of AD detailed in section 1.3.

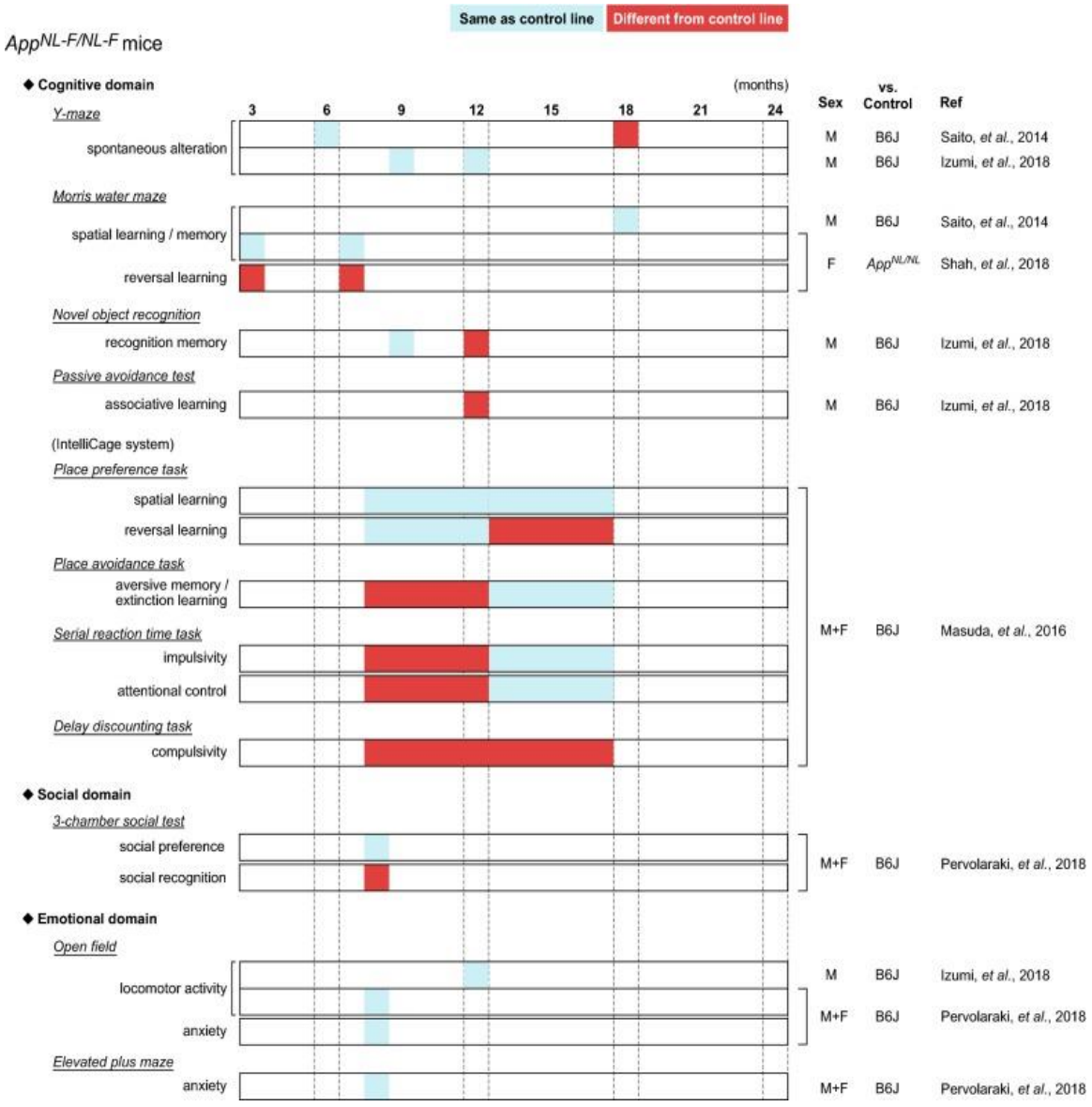


Figure 1.3: Summary of the current published data concerning the behavioural phenotype of *APP*$^{NL-F}$ mice. (Sakakibara *et al* 2019)
The type of behaviour tested and the specific task used are shown in the left column. Age in months is presented on the top line and increases horizontally. Blue and red colours demonstrate whether *APP*$^{NL-F}$ behaved the same (blue) or differently (red) than control mice. The "sex" column designates the sex of mice (M = male, F = female, M + F = both combined). The "vs control" column denotes the strain of mice used in comparison (B6J = C57Bl/6J).

1.6 Aims and Hypotheses

This chapter has described the physiological functions of APP and how increased concentration and aggregation of Aβ is involved in the pathogenesis of AD. However, despite this research into the mechanisms of amyloid pathology, no disease modifying therapies have been developed for the condition. Among the potential reasons for this lack of success is that the role of Aβ in normal neuronal function has been widely disregarded, resulting in the development of anti-amyloid therapies such as BACE1 inhibitors that have demonstrated detrimental effects in the absence of amyloid accumulation (Filser et al. 2015). However, it is unclear whether this effect manifested due to the impact on Aβ production or the reduced metabolism of alternative substrates. Therefore, the first aim of this thesis was to investigate whether specific inhibition of BACE1 dependent APP cleavage disrupted memory processes. Chapter 3 describes the administration of the anti-APP β-secretase cleavage site antibody 2B3 in young WT mice, with the hypothesis that it would disrupt object-place associative memory due to the reduced function of Aβ.

Another explanation for the failure of AD therapies is that model AD systems do not reliably recapitulate the mechanisms of the disease due to the presence of artefactual effects of APP overexpression. A more faithful genetic model, the *APP*$^{-NL-F}$, has been developed by using a knock-in strategy; although behavioural and biochemical phenotypes have yet to be elucidated. A major aim of this thesis was to breed a cohort of *APP*$^{-NL-F}$ mice and WT littermates and perform behavioural assessments at multiple age-points, as it was hypothesised that the mutations would manifest in an age-related accumulation of Aβ alongside a decline in visuo-spatial memory. In order to ensure any cognitive impairments were specifically within tasks involving spatial components, chapter 4 assesses the mice within object recognition paradigms while chapter 5 presents a foraging based working memory task. These tests were utilised as they provided the opportunity to dissociate assessment of spatial and non-spatial components. Chapter 6 involved *ex vivo* analysis of brain samples from aged *APP*$^{-NL-F}$ and WT mice in order to elucidate potential biochemical correlates underpinning the difference in cognitive performance.

Finally, chapter 7 sought to combine aspects of the two themes of this thesis and investigated whether selective inhibition of BACE1-dependent APP cleavage would result in opposite effects on cognition depending on the presence of amyloid pathology in aged *APP*$^{-NL-F}$ or WT mice.

Chapter 2 –

General Methods

2.1 Introduction

This chapter describes the methods that will be used through multiple experiments in this thesis. This includes the breeding, maintenance and cognitive testing of mouse colonies. The current chapter also describes the biochemical protocols used to quantify protein levels in brain tissue. Specific experimental designs, as well as any variations in techniques are detailed in the relevant chapters. All reagents were purchased from Sigma Aldrich (UK) or Fisher (UK) unless otherwise stated.

2.2 Breeding and Maintenance of NL-F Colony

▪ 2.2.1 – Housing Conditions and Breeding

Mice were housed in standard cages (48 x 15 x 13 cm) in holding rooms of stable temperature (21±2°C) and humidity (60±10%) on a 12-hour light/dark cycle, with *ad libitum* access to food and water. Animal were maintained according to the Animals in Scientific Procedures Act (1986), as well as UK Home Office and EU regulations.

APP^{NL-F} mice were obtained from the Wiseman group at UCL (3 males, 3 females, all heterozygous) and a colony was produced from pairings of these animals. All further breeding involved heterozygous pairs of *APP*$^{NL-F}$ mice. Pups were weaned at 21 days with males and females housed separately up to a maximum of 5 per cage. Ear-biopsies were taken from each mouse for identification and genotyping. Male *APP*$^{NL-F}$ and wildtype (WT) mice were age and/or littermate paired and grouped with another matched pair in cages of 4 wherever possible.

▪ 2.2.2 – Genotyping Using PCR and XbaI Digest

In order to identify homozygous, heterozygous and WT mice, a polymerase chain reaction (PCR) was used to amplify the mutated region within the amyloid precursor protein (APP) gene. The *APP*$^{NL-F}$ knock-in allele includes an XbaI restriction site inserted as part of the Swedish mutation, which was exploited in an XbaI restriction digest following PCR to distinguish genotypes, as the wildtype APP allele will not be cut. Therefore, a separate region containing an XbaI restriction site was also amplified in the PCR to act as a digest control.

A tissue sample (ear clip) was collected from each mouse at 3-7 weeks of age and frozen on dry ice. Tissue was digested and DNA extracted as follows: 75 µL NaOH extraction solution (25 mM NaOH, 0.2 mM EDTA) was added to the ear biopsy. This was incubated for 1 hour at 95°C before 100 µL neutralisation buffer (40 mM Tris-HCl, pH 5.5) was added. This was mixed and centrifuged at 17000 g for 5 minutes before either being used for PCR or stored at -20°C.

The PCR reaction mixture consisted of 5 µL Megamix Gold (Cat# 2MMG Microzone Ltd, West Sussex, UK), 3 µL nuclease free water, 1 µL of extracted DNA sample and 1 µL primer mix (2 µM concentration of each primer)}. Therefore, each reaction contained 10 µL and 0.2 µM of each primer. Two primer sets were used in the reaction: NLF-fw (5'-ACCAGTTTTTGATGGCGGAC-3') & NLF-rv (5'-TTTGGCCACACAGGCATTACA-3') amplified ~330 base pairs of the APP region containing the NL and F mutations, whilst primers Wdr26set1 (5'-TCCAGTTTGGCAAGGAAGGG-3') and Wdr26set2 (5'-CAGTGTGGCTATTGCTCTGG-3') amplified a ~700 base pair control region surrounding another XbaI restriction site.

Reaction mixtures were immediately transferred from ice to a thermocycler (MJ Research, Massachusetts, USA). The PCR program used the following conditions: 95 °C for 3 minutes, then 31 cycles of 3 minutes at 94°C (denature DNA), 30 seconds at 57.2°C (primer annealing) and 45 seconds at 72°C (strand extension), followed by a final extension period of 10 minutes at 72°C. Following the PCR reaction, the products were subjected to XbaI restriction digest. The reaction mixture consisted of the 10 µL PCR product, with the addition of 17 µL nuclease free H_2O, 3 µL 10x Fastdigest green loading buffer and 1 µL Fast digest XbaI enzyme (Cat# FD0684, ThermoFisher). The reaction tubes were replaced in the thermocycler at 37°C for 1 hour.

Products from the reactions were separated by gel electrophoresis, using a 1.5% agarose gel in TAE buffer on an ethidium bromide-free docking system (Bio-Rad, Hertfordshire, UK). DNA products were loaded with "Novel Juice" loading dye (GeneDirex, Newmarket, UK) at 1:5 Novel Juice:sample. Samples were run at 100 V for 65 minutes alongside a 100bp DNA ladder (GeneDirex), an H_2O (no DNA) control, a "no-cut" (no XbaI control) plus positive controls of known homozygous, heterozygous and wildtype *APP*$^{NL-F}$ mice. Gels were visualised using a Syngene GBOX Chemi-XX6 gel doc system with associated software (Syngene, Cambridge, UK).

As shown in figure 2.1, the XbaI control region DNA product appears at ~600bp (700bp in the no-cut control). The amplified APP region appears at ~340bp if wildtype (the same as in no-cut control samples), but ~280 if homozygous due to the XbaI digestion. Samples from heterozygous mice have multiple bands from ~360bp to ~280bp.

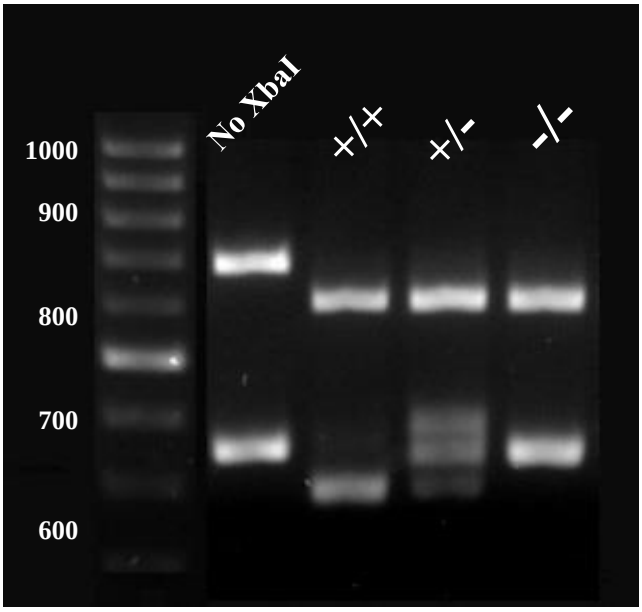


Figure 2.1: Representative image of DNA bands following PCR and electrophoresis of *APP*$^{NL-F}$ and WT mice. The XbaI restriction digest control region produces a band at 700 base pairs naturally, or 600 bp if cleaved. The diagnostic band for APP is at 340 bp if uncut, revealing a WT (-/-) genotype. Homozygous *APP*$^{NL-F}$ (+/+) mice show a cleaved band at 280 bp. Heterozygous (+/-) mice show both the 280 & 340 bp bands and an extra band at 400 bp.

2.3 Behaviour Testing

This section describes the apparatus and protocols used throughout this thesis to assess object-novelty (ON) and object-in-place (OiP) memory in mice. The method for measuring locomotive activity is described as part of the habituation protocol. Individual chapters will describe specific experimental designs.

▪ 2.3.1 – Apparatus

A square arena (60 x 60 cm wooden floor, with 40 cm high clear Perspex walls) was used throughout the experiments. The floor was painted white and the walls internally covered with an opaque, white film. The arena was positioned under the ceiling light to ensure even lighting in all corners. It was placed on a 50 cm tall table to elevate it off the floor of the quiet test room. The walls surrounding the arena were decorated with salient extra-maze cues to be visible from inside, including a striped curtain on one side and a

black curtain on another side that hid the experimenter. Another wall was covered in black spots ~15 cm in diameter. The position of these cues, as well as the arena and experimenter, were consistent throughout all testing, unless otherwise stated. Every test was recorded using a varifocal USB camera (ELP-USBFHD04H-BFV-U, Ailipu Technology Co Ltd, China) positioned above the centre of the arena and connected to a laptop, and saved onto a hard drive. Mice were tracked live using EthoVision XT 13 software (Noldus, UK) and interaction with objects was manually scored in addition to the EthoVision data.

▪ 2.3.2 – Open Field Test & Arena Habituation

Animals were always transported within the cardboard tubes from their home cage and placed in the centre of the arena facing the same wall throughout all procedures. Locomotive activity was assessed by Open Field test, whereby the animal was placed into the empty arena and allowed to freely explore for 10 minutes. Total distance moved was recorded by EthoVision tracking software. The central 30x30 cm square of the arena was described as the "Inner Zone" on the software, while the boundary between that and the walls of the arena was the "Outer Zone". The software recorded the amount of time spent within both zones by each mouse.

The Open Field test represented day 1 of habituation. On days 2 and 3, four different objects were placed in the arena and mice had 3 sessions of 5 minutes exploring the objects, with a 5-minute delay separating each session. Four new objects were used for day 3. Up to 20 mice were habituated together, before being split into 2 counterbalanced groups of 10 which began testing on either day 4 or 5. The order of different test and object sets were counterbalanced and no mouse saw the same object set more than once. Any differences in the testing protocol are specified in relevant chapters.

▪ 2.3.3 – Objects and Scoring

The objects used were made of a variety of materials, usually plastic and glass, and around 20 cm tall (figure 2.2). They were chosen with the aim of limiting mice climbing on them while being heavy enough to remain still while being investigated. They were grouped into object sets of various colours and shapes including shampoo bottles, etched champagne glass and Duplo towers. Objects were placed near the corners of the arena, 25-30 cm apart and ~15 cm from the walls. The arena and objects were cleaned with 70% alcohol prior to every session, in order to remove any odour cues which could affect discrimination.

Figure 2.2: Examples of objects used throughout object recognition tasks.

Object exploration was scored according to the protocol defined by Ennaceur & Delacour (1998): when the animal's head was within 2cm and interacting with the object. For test sessions, object exploration was translated into a "Discrimination Ratio" (DR), which was obtained with the calculation below. Random object exploration equates to a ratio of 0.5, i.e. equal exploration of both novel and familiar objects, therefore scores greater than this value indicates a preference to explore novelty.

$$\frac{\textbf{Total time spent exploring "Novel" object}}{\textbf{Total time spent exploring all (Novel + Familiar) objects}} = \textbf{DR}$$

EthoVision tracking software was utilised in order to automatically score object interaction and provide an objective comparison to manual scoring. A photo was taken of each object arrangement and a zone was drawn around each object (e.g. Object A), using the arena settings feature of the software. A second zone drawn by enlarging the original zone by 4 cm in both diameters, providing a 2 cm radius (Object A+2). Object interaction was scored when the animal's head was pointed towards Object A whilst also inside the "Object A+2" zone. Only experimenter scored data is presented due to the greater level of stringency it provides. For example: if the mouse was on top of the object, using it to explore around the room, or was sat within 2cm but facing away, it was not scored as exploration by the experimenter but may have been scored by the software. Statistical analysis of DRs calculated from the EthoVision-tracked object exploration resulted in identical conclusions to that of experimenter-tracked exploration in all experiments, and this tracking data is available on request

2.3.4 – Object Novelty

Object novelty recognition (ON) was tested with either a 2 or 4 object array in different experiments of this thesis and is therefore individually described. However, the protocol was consistent: the mouse was presented with an object array and allowed to explore for a 10-minute sample phase 1 (figure 2.3 A). The mouse was removed back into the home cage for a 5-minute rest period, during which the arena and objects were cleaned with 70% ethanol wipes. This exploration and rest cycle were repeated in sample phases 2 & 3 with the same objects. However, in the 5-minute delay following sample phase 3, either 1 or 2 objects were removed (depending on the 2 or 4 object array), and replaced with novel ones. The mouse was replaced in the arena to explore the objects for a 10 minute "test phase". Exploration of objects was scored as described above. The identity and location of the novel objects within each object set was counterbalanced.

2.3.5 – Object-in-Place

The Object-in-Place (OiP) protocol consistently used four objects placed 15 cm in from the corners and ~30 cm apart throughout this thesis (figure 2.3 B). The task used the same sample phase protocol of the ON task: three 10-minute sessions each separated by a 5-minute rest in the home cage. However, during the 5-minute delay prior to the test phase, the location of 2 diagonally opposite objects was switched. These were described as the novel objects for the analysis and exploration was scored as described above. The pair of objects switched and the novel location was counterbalanced within each object set, as well as the order of object sets if more than one was observed by each mouse.

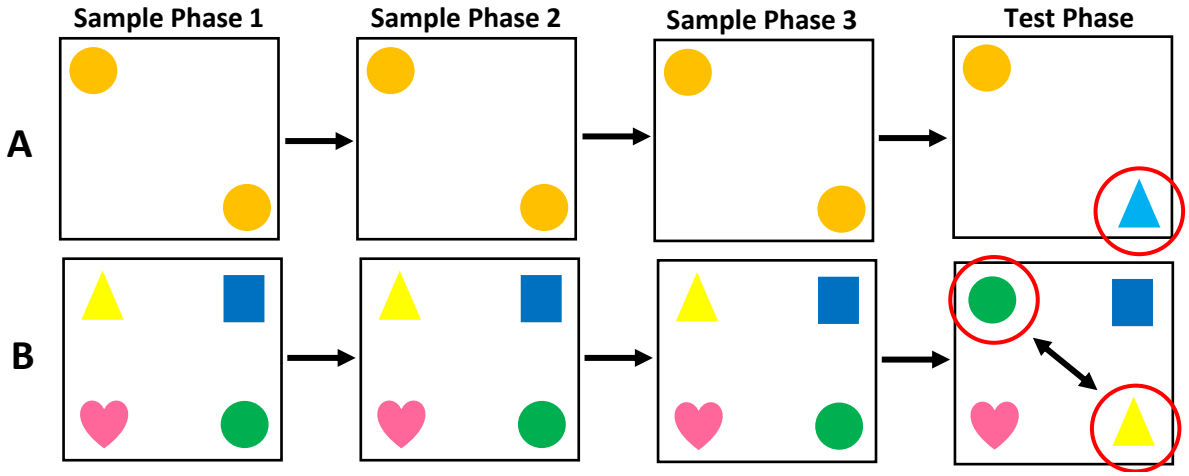


Figure 2.3: Diagrams of the object recognition protocols used in this thesis. All sample and test phases lasted 10 minutes and arrows represent a 5-minute delay. **(A)** Object novelty. **(B)** Object-in-Place. Red circles highlight the objects that are novel or have switched spatial locations.

2.3.6 – Data & Statistical Analysis

All object interaction data was collected with the EthoVision XT 13 software and exported to Excel for calculation of mean scores for each mouse and each group along with the associated standard error of the mean. All statistical analyses were performed using IBM SPSS statistics. Significance was determined using an α-level of 0.05 for all tests. Null hypothesis significance testing was performed throughout this thesis, using the tests detailed below. However, the limitations of such tests must be acknowledged, particularly concerning null results ($p>0.05$); as these tests lack the capacity to distinguish the true absence of an effect from a failure of an effect to reach significance.

The Shapiro-Wilke test was used to test for violations of normality in all data, while Levene's test checked for violations of equal variance. Where violation occurred, the data were transformed by the appropriate calculation in order to satisfy these assumptions, prior to analysis of variance (ANOVA), mixed measures ANOVAs and Student's t-test (independent samples, paired sampled or one sample were all employed where appropriate). Bonferroni adjustment was used on all significant interactions and main effects, adjusting for multiple post-hoc comparisons. Wherever transformation was not possible or did not eradicate violations of normal distribution, data were analysed by the equivalent non-parametric statistical test. For example, the Mann-Whitney U test was used as an equivalent of the independent samples t-test, Wilcoxon-Signed Rank for the Paired-Samples t-test, Kruskal-Wallis H Test for the One-Way ANOVA, Friedman Test for the Repeat Measures ANOVA.

2.4 *Intra-Cerebral Ventricular Infusion of 2B3*

The anti-APP antibody, 2B3, was developed by Thomas *et al* by methods previously described (Thomas et al. 2006). Their original hybridoma was sent to ProMab (California, USA), for larger scale production by ascites. Following purification and sterilisation, 2B3 was administered by intracerebroventricular (ICV) infusion via osmotic minipumps. The pumps released the antibody at a constant flow rate of 0.25 µL/hour over 14 days.

2.4.1 – Antibody Purification and Sterilisation

Concentrated ascites product from ProMab (California, USA), was thawed before being centrifuged at 1000 g and aliquoted after removing the top lipid layer. It was then

purified and concentrated with the MAb Trap™ affinity chromatography kit (GE Healthcare, Buckinghamshire, UK) according to the manufacturer's instructions. The concentrated 2B3 IgG solution was diluted 1:1 in the prepared binding buffer. The column was first washed through with dH_2O, then equilibrated with 3 mL binding buffer, at a consistent flow rate of 1 drop per second. The IgG solution was passed through the column, maximum 4.5 mL at a time, before 8 mL binding buffer was added to elute any non-2B3 material. The 2B3 antibody was released by passing 5 mL elution buffer through the column and collected in Eppendorf tubes with 60 µL neutralising buffer to maintain affinity. The column was then re-equilibrated with binding buffer for further purifications. The protein contents of the collection tubes were quantified by BCA assay (described in section 2.5.3) and the fractions containing 2B3 pooled.

The purified 2B3 antibody solution was then dialysed to replace the buffers from the MAb Trap kit with phosphate buffered saline (PBS; 137mM NaCl, 2.5mM KCl, 8mM Na_2HPO_4, 1.5mM KH_2PO_4, pH 7.2). The solution was injected into a Slide-A Lyzer Dialysis Casstte (3-12 mL volume, 10 kDa molecular weight cut off, Pierce), which was rotated in 2 L of PBS overnight at 4°C. Following dialysis, 2B3 was sterilised through a 0.22 µm filter, aliquoted and stored at -20°C. The final concentration was measured on a NanoVue spectrophotometer (GE Healthcare Ltd, Buckinghamshire, UK).

▪ 2.4.2 – Surgical Implantation of Minipump & Cannulae

Osmotic minipumps (model 1002, Alzet, UK), were filled with 200 µL of either purified 2B3 or control IgG1κ (mab201, Millipore, UK) and connected to a brain infusion kit (0004760, Alzet). This consisted of a 2 cm catheter, backfilled with the appropriate antibody and attached to a 28G cannula (0004760, Alzet). Mice were anaesthetised by isoflurane carried by O_2 and fitted into a stereotaxic frame. Metacam was injected subcutaneously at 30 mg/kg as analgesia prior to the surgery. Minipumps were subcutaneously implanted between the scapulae of the mice. The cannula was inserted at the following coordinates 3.0 mm ventral to the skull surface, 0.5 mm posterior and 1.2 mm lateral to bregma. Two screws were implanted alongside the cannula, enabling it to be fixed in place with dental cement. The skin was sutured around the cannula and the mouse was rehydrated with subcutaneous injection of mL gluco-saline into each flank. The mouse was allowed to recover in an incubator maintained at 30°C before being individually housed, (in order to limit damage to the sutures or the implants).

2.5 *Ex Vivo* Protocols

This section describes how mouse brains were prepared for biochemical analysis, and the methods used thereafter. Mice were culled by cervical dislocation and the brain was immediately removed. The hippocampus was dissected, and the cortex was divided into frontal and posterior regions, snap frozen in liquid nitrogen and stored at -80 °C. Proteins were extracted from the brain samples via two methods: the left hemisphere by homogenate extraction, while the right hemisphere underwent a protocol to isolate synaptosomes, allowing more efficient analysis of synaptic receptor expression. Extracted samples underwent protein analysis by either ELISA or Western Blot.

▪ 2.5.1 – Homogenate Extraction

Brain samples were homogenised in 2% sodium dodecyl sulphate at 75 mg/mL wet tissue/buffer. Protease inhibitor cocktail III (539134, Millipore) and phosphatase inhibitor cocktail V (524629, Millipore) were added to the homogenisation buffer at 1/100 and 1/50 dilution, respectively. Samples were homogenised in a Precellys 24 Dual (Bertin Technologies, Montigny le Bretonneux, France) at 5500 rpm for 2 x 30 seconds with a 30 second delay between before being rotated at 4°C overnight. The homogenate was centrifuged at 100,000 g (137,300 g) for 1 hour at 4°C before the supernatant was carefully removed, mixed and diluted 1/5 in EC Sodium Buffer (20mM Na_2HPO_4, 0.2mM EDTA, 0.4 M NaCl, 0.2% bovine serum albumin). All samples were stored at 20°C.

The insoluble pellet underwent further extraction by resuspension in 70% formic acid (Sigma Aldrich) at 150 mg/mL original tissue weight. The centrifugation was repeated at 100,000 g (137,300 g) for 1 hour at 4°C. The supernatant was diluted 1/20 in neutralising buffer (1M Tris, 0.5M Na_2HPO_4, pH 11) and stored at -20°C.

▪ 2.5.2 – Synaptosomal Extraction

Brain regions dissected from the right hemisphere were extracted using Syn-Per™ Synaptic Protein Extraction Reagent. Protease and phosphatase inhibitors were added to the Syn-Per just prior to the procedure in the same concentrations as above. The Syn-Per reagent was added at 10 mg/mL of the wet tissue weight, and homogenised manually with a microcentrifuge pestle. Samples were centrifuged at 1,200 g for 10 minutes at 4 °C. The supernatant was collected and 15 uL was taken as the homogenate fraction, while the pellet was discarded. The rest of the homogenate fraction was centrifuged at 13,000 g for 30

minutes (4 °C). The supernatant was taken as the cytosolic fraction and the synaptosomal pellet was resuspended in Syn-Per reagent at a final volume of 1.5 mg/mL of the initial tissue weight. All fractions were quantified by BCA assay and stored at -20°C.

▪ 2.5.3 – Bicinchoninic Acid Protein Assay

Protein concentrations of samples from both extraction protocols were quantified with a bicinchoninic acid (BCA) protein assay kit (ThermoScientific, UK). Bovine Serum Albumin (BSA) was serially diluted to prepare standards ranging from 2 – 0.003 mg/mL. 20 µL of these standards plus a blank (dH_2O) were analysed on a 96 well plate alongside 1 µL of each sample and 4 µL EC buffer. All standards and samples were analysed in duplicate. The assay was performed by first making up the BCA Working Reagent (50:1 Reagent A : Reagent B), 200 µL of which was added to each well. The plate was mixed for 30 seconds and incubated for 30 minutes at 37 °C. Absorbance at 540 nm was measured across the plate using a spectrophotometer. A standard curve was produced from the BSA standards, which was used to calculate the protein concentration of each sample.

▪ 2.5.4 – ELISA (Enzyme-Linked Immunosorbent Assay)

All ELISA kits quantifying human or murine Aβ were produced by Invitrogen (California, USA), unless otherwise stated, and the protocols were carried out according to the manufacturer's instructions. The provided Aβ standard was dissolved in filter sterilised reconstitution buffer (55 mM $NaHCO_3$, pH 9) for all kits, and this was serially diluted in the *Standard Diluent Buffer* to generate the range of standard concentrations as described by the instructions. Variations in protocol between the kits are detailed below. A mouse Aβ42 kit (#KMB3441) was trialled however the concentration in WT mice was below detectable levels and so no data is shown.

Mouse Amyloid Beta 40 (Aβ) (#KMB3481)

Following dilution of standards and samples, 100 µL was added to each well and left for 2 hours at room temperature (RT). The plate was washed 4 times with the diluted *Wash Buffer*, before 100 µL *detection antibody* was added to each well for 1 hour at RT. The horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody was diluted 1/1000 in the provided buffer and 100 µL was added to each well after 4 more washes and incubated for 30 minutes at RT. Finally, after another 4 washes, 100 µL *stabilised chromogen* was added to each well and left in the dark for 30 minutes at RT before 100 µL

stop solution was added, turning each well from blue to yellow. Absorbance at 450 nm was measured on a spectrophotometer and a standard curve was produced from the absorbance levels of the known standard concentrations. Concentrations of Aβ-40 were calculated from this standard curve, using Graph Pad Prism 4.0 and Microsoft Excel, normalised to the amount of protein in each sample.

Human Amyloid Beta (Aβ)

The protein standard for human Aβ40 (KHB3481) and Aβ42 (KHB3441) was reconstituted as above and the range of standard concentrations was produced according to the manufacturer's instructions. The protocol was identical to that of the mouse Aβ40 kit above, with one exception. Standards and samples were loaded onto the ELISA plate at a volume of 50 µL. 50 µL of the *detection antibody* was loaded on top of the samples and together this was left at RT for 3 hours, with shaking.

▪ 2.5.5 – Western Blot

Prior to Western blot (WB) analysis, extracted samples were diluted 2:1 in 3X sample buffer (6.3mM TrisBase, 0.8% SDS (w/v), 20% glycerol (v/v), 10% β-mercaptoethanol (v/v), 2% bromophenol blue (v/v)), and heated at 90°C for 45 minutes to reduce the proteins. Samples were stored at -20°C and, following the initial reduction, they were heated at 70°C for 5 minutes prior to loading in all other experiments. Cassettes (NC2010, LifeTechologies, USA) were filled with 10% polyacrylamide "separating" gels (3.3% (w/v) acrylamide, 375 mM TrisHCl, 0.1% SDS (w/v), 0.05% APS (w/v), 0.05% TEMED (v/v), all v/v) and topped with 5% polyacrylamide "stacking" gel. Samples were loaded onto these gels and separated by SDS-PAGE (Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis) in running buffer (25 mM Tris base, 190 mM glycine, 0.05% SDS, pH 8.3). Separated proteins were transferred from the gel onto 0.2µm nitrocellulose membrane (Amersham Biosciences, Little Chalfont, UK) in semi-dry blotting buffer (42.9 mM Tris base, 38.9 mM glycine, 0.038% SDS (w/v), 20% methanol (v/v).

The membrane containing the separated proteins was washed with Tris-buffered saline with Tween 20 (TBSt, 2 mM Tris, 15 mM NaCl, 0.1% Tween-20, pH 7.5) and then incubated at RT in 5% w/v BLOTTO (non-fat milk powder; Tesco, dissolved in TBSt) for 1 hour. Membranes were then incubated in primary antibody diluted in 1% BLOTTO overnight on a roller at 4°C (dilutions described in table 2.1). The next day, membranes

were washed in TBSt 3 times for 5 minutes each, before secondary antibody incubation in 1% BLOTTO at RT for 2 hours. The secondary antibodies were conjugated to horseradish peroxidase (HRP) and were selected depending on the host species of the primary antibodies (table 2.2). Membranes were washed as described above, before being incubated with Pierce™ Enhanced Chemiluminescence Western Blotting Substrate (ECL, Pierce Biotechnology, IL, USA) and visualised in a Syngene GBOX Chemi-XX6 gel doc system with associated software (Syngene, Cambridge, UK). Images were saved and the densities of the bands were quantified using ImageJ software.

Primary Antibody	Species	Dilution	Source, Catalogue #
APP (22C11)	Mouse	1:1000	Millipore, #mab348
BACE1	Rabbit	1:1000	Cell Signalling, #D10E5
PSD95	Rabbit	1:1000	AbCam, #ab18258
NMDA R1	Mouse	1:1000	BD Biosciences, #556308
NMDA NR2B NR2B p-Y1472	Rabbit	1:500 1:750	Millipore, #AB1557P Millipore, #AB5403
AMPA GluA1 GluA1-pS845	Rabbit	1:2500	Abcam, #ab31232 Abcam, #ab3901
α7-NAChR	Rabbit	1:500	Abcam, #ab10096
Tau p-Tau PHF-1 p-Tau CP13	Rabbit Mouse Mouse	1:500 1:250 1:250	Dako, A0024 Generous gift from P. Davies (Albert Einstein University, NY).
βActin (Pre-Conjugated to HRP)	Mouse	1:15,000	Sigma, A3854

Table 2.1: Primary antibodies used throughout this thesis in western blots.

Secondary Antibody	Species	Dilution	Source
Anti-Mouse	Horse	1:15,000	Vector, #PI-2000
Anti-Rabbit	Goat	1:15,000	Vector, #PI-1000

Table 2.2: Secondary antibodies used throughout this thesis in Western blots.

Chapter 3 –

Inhibiting Aβ in Young Wild-Type Mice

3.1 Chapter Overview

Chapter 3 describes experiments that investigated the normal physiological role of Aβ in cognition by selectively reducing its concentration in WT mice. The experiments utilised an antibody, 2B3, which binds to the β-secretase cleavage site of APP and sterically hinders cleavage. Experiment 1 involved infusing 2B3 directly into the hippocampus due to the region's association with Aβ activity and the ability to specifically assess function with spatial-based memory tests. Immunofluorescence and biochemical analysis revealed the presence and activity of the antibody did not persist beyond 5 days, which was not sufficient for cognitive testing.

Experiment 2 tested how chronic infusion of 2B3 affected Aβ production over a 14-day period. The 2B3 antibody was infused into the lateral ventricle via an osmotic minipump, resulting in a significant drop in hippocampal Aβ levels. The impact on novel object and object-in-place recognition memory in WT mice was also assessed. The results demonstrated that WT mice infused with 2B3 successfully performed an object recognition task at the same level as controls, but failed to detect novel object-place associations. To my knowledge, this is the first assessment of these cognitive tests following chronic anti-amyloid therapy in healthy WT mice.

3.2 Chapter Introduction

Following the identification of Aβ and the proposal of the amyloid cascade hypothesis (Hardy and Higgins 1992), the vast majority of research into AD has focused on the toxic effect of the peptide. It is now established that administration of high concentrations of Aβ to neuronal tissue culture induces apoptosis (Yankner, Duffy, and Kirschner 1990), while injection into the hippocampi of mice causes amnesia (Flood et al. 1994, Cleary et al. 2005). However, one aspect of Aβ that is not regularly discussed is that at low concentrations it can be neurotrophic (Whitson, Selkoe, and Cotman 1989, Yankner, Duffy, and Kirschner 1990). Moreover, application of anti-Aβ antibodies to neuronal cultures reduced cell viability, further suggesting that the peptide plays an important normal physiological role (Plant et al. 2003). Due to the amyloid cascade hypothesis driving the field for nearly three decades, the majority of therapies in clinical development for Alzheimer's disease patients are aimed at reducing Aβ levels. Given that amyloid

production has been the focus of therapies, it is critical to understand its normal function and role in memory processes. Knowledge of its normal physiological functions will further aid understanding of the potential effects of its reduction in pre-symptomatic individuals.

Previous research has suggested that APP, and particularly the Aβ peptide, may be required for normal function. Mice with genetic knock-out of the APP gene develop age-dependent deficits in neuronal morphology, synaptic plasticity, passive avoidance memory and spatial learning in the Morris water maze (Dawson et al. 1999, Ring et al. 2007, Seabrook et al. 1999, Phinney et al. 1999, Senechal et al. 2007). However, due to the multiple cleavage products produced by APP metabolism, results using genetic knockout cannot specify the consequences of aberrant Aβ production. For example, multiple studies have now shown that the phenotype of APP-KO mice can be rescued by the expression of sAPPα (Ring et al. 2007, Tyan et al. 2012). There is also evidence that manipulating the cleavage of Aβ from APP also exerts detrimental effects on neuronal function. BACE1 knock-out mice show changes in axon guidance, myelination, exploration and anxiety in the open field and elevated plus maze, as well as impaired pre-pulse inhibition of startle and hypersensitivity to seizures (Hitt et al. 2010, Hu et al. 2006, Willem et al. 2006, Dominguez et al. 2005, Harrison et al. 2003, Savonenko et al. 2008). Importantly within the context of the current study, BACE1-KO mutants also exhibit impairment in synaptic plasticity and deficits in spatial learning and fear conditioning (Laird et al. 2005, Ohno et al. 2004, Ohno et al. 2007, Kobayashi et al. 2008). It is also worth noting that reduction of β-cleavage upregulates sAPPα expression up to 250% in BACE-null mutants. Therefore, unlike APP-KO mice, the cognitive deficit cannot be attributed to a loss of this domain (Luo et al. 2001). However, similarly to APP manipulation, effects of BACE1 inhibition cannot be exclusively Aβ-dependent.

Many of the effects measured in BACE1-null mice may actually be due to the loss of cleavage of one of over 40 other substrates of the enzyme, including neureglin-1 and β-subunits of voltage-gated sodium channels (Zhu et al. 2018, Hitt et al. 2012, Hu et al. 2013, Savonenko et al. 2008, Kim et al. 2011, Wong et al. 2005). Another drawback of knock-out models is that, as the genetic manipulation is present from conception, any phenotype may manifest either due to loss of the function of the protein during development or compensatory mechanisms. In fact, conditional knock-out of BACE1 in adult mice resulted in a less severe phenotype resulting in no myelination, epileptiform and cognitive effects

(Ou-Yang et al. 2018). The mice did still demonstrate alterations in axonal organisation, indicating that BACE1-mediated cleavage of the cell adhesion protein close homologue of L1 (CHL1) remains a critical process in axonal guidance through to adulthood.

A more elegant method to measure the effect of reducing Aβ may be to use small molecule β-secretase inhibitors, particularly because of their clinical relevance. While multiple groups have published the effects of BACE inhibitors in preclinical models of Alzheimer's disease (Kennedy et al. 2016, May et al. 2015), data concerning their impact in WT mice are limited. A study by Filser *et al* (2012) reported that chronic (15 day) administration of a BACE inhibitor in 2-month-old WT mice induced a 75% drop in Aβ alongside a loss of dendritic spine density, synaptic plasticity impairments and deficits in spontaneous alternation and novel object recognition. A follow up study with a different inhibitor confirmed the effect on dendritic spines, however, it concluded that this effect was transient, contingent upon withdrawal of the treatment (Blume et al. 2018). Experiments aimed at further increasing the specificity of Aβ inhibition have utilised antibodies binding to regions of the APP protein. The first example of such an approach involved intracerebroventricular (icv) infusion of anti-APP antibodies into rats which significantly reduced memory performance in a passive avoidance test (Huber et al. 1993). More recent experiments with antibodies binding specifically to the Aβ peptide itself resulted in memory deficits in the water maze, contextual fear conditioning and a T-maze passive avoidance test (Garcia-Osta and Alberini 2009, Morley and Farr 2012, Puzzo et al. 2011). Moreover, these studies also demonstrated that these antibodies caused dysfunction of synaptic plasticity mechanisms in hippocampal slices, indicating that the peptide may be important in induction of LTP. All of the impairments described following antibody treatment were rescued by concurrent administration of Aβ. Intriguingly, these groups also demonstrated that picomolar concentrations of Aβ alone actually enhanced memory and facilitated synaptic plasticity in WT mice (Puzzo et al. 2008, Morley et al. 2010).

The experiments in this chapter aimed to investigate the impact of specific inhibition of APP cleavage by BACE. To overcome the challenges associated with the multiple substrates of BACE, an antibody directed towards the β-cleavage site on APP was infused into the CNS (Thomas et al. 2006). Thomas *et al* designed 2B12, a mAb raised against a 15-residue peptide sequence representing the β-cleavage site of APP (Thomas et al. 2006). Thomas et al (ibid.) showed that 2B12 inhibited BACE cleavage of APP and reduce the concentration of Aβ40 in human astrocytoma and neuroblastoma cell lines

expressing endogenous APP. Thomas and colleagues later showed that another antibody, 2B3, exhibited greater affinity for APP and a more potent effect on Aβ40, Aβ42 and βCTF compared to 2B12, while levels of sAPPα remained unchanged (Thomas, Liddell, and Kidd 2011). 2B3 remained bound to its antigen even following an hour-long incubation at pH4, equivalent to the acidic environments present in the endocytic compartments, where BACE1 interacts with APP (Vassar et al. 1999). The group later replicated the effect of 2B3 on Aβ40 in primary cortical neurons derived from a transgenic AD mouse model expressing the *hAPP* London mutation (Thomas et al. 2013). Most recently, Evans *et al* (2019) administered 2B3 to aged PDAPP transgenic mice, expressing the V717F Indiana mutation, over 14 days, via intracerebroventricular (icv) infusion. The group reported a significant reduction of soluble Aβ40 and βCTF in the hippocampus and rescue of a visuospatial associative recognition memory deficit.

The mechanism of 2B3 avoids the issues associated with manipulation of alternative BACE substrates or APP metabolites described above. However, one drawback of immunotherapies is their availability in the brain. Penetration of any peripherally administered drugs into the brain, particularly macromolecules such as antibodies, is severely limited by the blood brain barrier (BBB), a layer of endothelial cells that selectively blocks molecules entering the brain parenchyma from capillaries. In fact, the BBB may block up to 98% of small molecules while quantification of antibody penetration is estimated at 0.1% (Pardridge 2005, Banks et al. 2002). Therefore direct, intracerebral injection ensured the maximum concentration entered the desired area, especially when localised to a specific brain region. The hippocampus is one of the most vulnerable brain regions to amyloid pathology, and dysfunction within it leads to the memory loss observed in early stage Alzheimer disease patients (Braak and Braak 1991). The substantial evidence concerning the pathological effect of Aβ in this region, as well as the numerous cognitive tests developed to specifically measure its function, highlight the hippocampus as a critical region to analyse the physiological role of the peptide. Therefore, the experiments presented in this Chapter involved infusion of 2B3 into the hippocampus of WT mice in order to investigate the biochemical and cognitive effects.

3.3 Experiment 1: Acute Inhibition of hippocampal Aβ

3.3.1 – Introduction

The aim of this experiment was to investigate whether a measurable reduction of Aβ concentration could be induced following direct infusion of an anti-APP antibody, 2B3, throughout the hippocampus. It further aimed to ascertain the duration of this effect, in order to confirm the possibility of investigating the impact of Aβ inhibition on cognitive performance.

3.3.2 – Experiment 1 Methods

Subjects, Design

Young C57Bl/6 mice (3-4 months old) underwent bilateral hippocampus injections. All mice had 2B3 infused into one hemisphere and a control antibody into the other. The mice were culled after either 1- or 5-days following surgery (n=8 for both timepoints), and hippocampi were immediately dissected and flash frozen on liquid nitrogen. One mouse had to be removed from the within-subjects comparison after 1 day of treatment due to an error in the extraction of the 2B3-treated sample. This meant that for the comparison of treatment versus duration, there was n=8 in the 5-day group and n=7 in the 1-day group. A further group of 8 mice did not undergo surgery and were compared alongside the others.

The control antibody was the same isotype as 2B3 (IgG1κ) and was raised against rabbit IgG light chain (1.3 mg/mL, mab201, Millipore, UK). The origin and purification of the 2B3 antibody was described in section 2.4 and the concentration was 2.31 mg/mL. The extraction protocol for the soluble fraction is described in section 2.5, and the concentration of Aβ was quantified using a mouse Aβ40 ELISA kit (KMB3481, Invitrogen, California, USA). Aβ42, measured by ELISA, was not detectable in WT mice (data not shown). This was consistent with a previous study measuring Aβ42 changes in WT mice (Iaccarino et al. 2016)

Surgery

Mice were anaesthetised with Isoflurane [2-chloro-2-(difluoromethoxy)-1,1,1-triflouro-(ethane)] carried by oxygen throughout the surgery. They were fixed into a stereotaxic frame and the scalp incised and retracted to expose the skull. A bone flap was removed over both hippocampi spanning the range of the injection coordinates in table 3.1.

Mab201 control IgG or 2B3 was delivered through a 25G microinjection 2 µL Hamilton Syringe (#20751, Hamilton Company, Reno, USA) at 0.3 µL per minute into each coordinate up to the volume described. Volumes were based on previous studies using intrahippocampal infusions. The needle remained in place for 2 minutes following the end of each infusion, before being slowly withdrawn. Following all infusions, the scalp was sutured and the mouse was rehydrated with 1 mL gluco-saline injected subcutaneously into each flank. Mice were placed in a recovery chamber with temperature maintained at ~30 °C until they were fully alert and responsive, before being placed in a clean home cage with tissue paper on the floor. They were provided with sweetened porridge (ReadyBrek®) to encourage eating, alongside the usual *ad libitum* access to chow and water. Mice were treated with 30 mg/kg Metacam analgesia immediately before, and each day following the surgery until they were sacrificed.

Site	Stereotaxic Coordinates			
	Anterior / Posterior	**Lateral (+/-)**	**Ventral**	**Volume (µL)**
1	-1.7	1.3	-2.5	0.75
2	-2.3	2.5	-2.5	1.0
3	-2.9	3.1	-2.8, -3.6	0.8
4	-3.4	3.1	-3.2, -4.2	0.7

Table 3.1: Stereotaxic co-ordinates used for direct infusions of 2B3 or control IgG into the hippocampus. Co-ordinates are labelled as distance (mm) posterior and lateral from bregma, and ventral from the dura.

Fluorescent Labelling of 2B3

Purification of 2B3 was performed as described in section 2.4. A fluorescent tag was conjugated to the antibody using the Alexa Fluor™ 647 Protein Labelling Kit (A10239, Invitrogen, California, USA) according to the protocol outlined in the manufacturer's guidelines. The labelling reaction was performed by adding 50 µL of 1 M sodium bicarbonate to 0.5 mL of the 2B3 solution. This was mixed with the provided vial of reactive dye and stirred for 1 hour at room temperature. Purification resin was pipetted into the column and the dye/2B3 reaction product was loaded on top. The elution buffer was diluted and slowly added on top of the column and as it ran, the first coloured band was

collected because it contained the labelled antibody. The concentration of labelled antibody was calculated using the equation in the protocol and a NanoVue spectrophotometer (GE Healthcare Ltd, Buckinghamshire, UK).

Immunofluorescence

Fluorescently labelled 2B3 (2.15 mg/mL) was injected into the hippocampus of one hemisphere according to the surgery protocol above. Mice were euthanised after 1 or 5 days and brains were immersed in Optical Cutting Temperature compound and flash-frozen in liquid nitrogen. The brains were sectioned at 20 μm on a cryostat. As the brain was sectioned, photographs of coronal sections were taken throughout, recording the extent of the coloured dye. Sections were mounted onto slides and coverslips were fixed on top with Vectashield mounting medium with DAPI stain (H-1500, Vector Laboratories, Peterborough, UK). Fluorescence imaging was performed using a Leica DM5000B fluorescence microscope.

▪ 3.3.3 – Experiment 1 Results

Imaging Fluorescently Labelled 2B3

Fluorophore tagged 2B3 solution was injected into the hippocampus of one hemisphere of a 5-month old C57Bl/6 mouse. Figure 3.1 presents photographs depicting the frozen brain 1-day post-injection as it was being sectioned. The blue dye of the 2B3 solution can be observed throughout one hippocampus around the injection sites (panel A). Panel B demonstrates the immunofluorescence images taken of sections of the same brain. The fluorescently labelled 2B3 can be seen in red throughout the hippocampus, in which the cell nuclei are identified by the blue DAPI counterstain. The fluorescent signal was not observable in the opposite hemisphere. In the brain collected 5 days post injection, neither blue dye nor immunofluorescence were detectable in the hippocampus (Figure 3.1C).

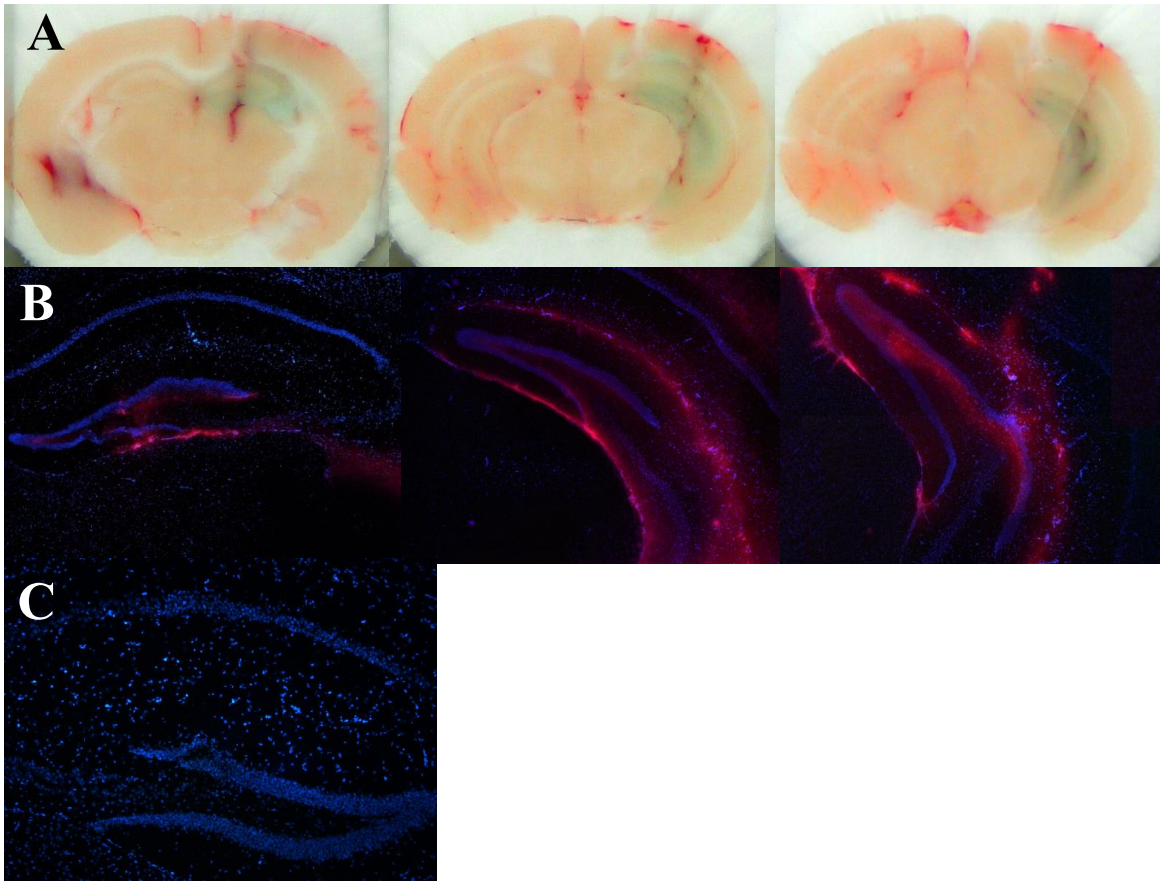


Figure 3.1: Detection of fluorescently labelled 2B3 in the hippocampus, 24 hours and 5 days post-injection. (A) Photographs of injection sites in a frozen mouse brain. fluorescently tagged Bb3 is visible due to the blue dye. **(B)** Immunofluorescence microscope images of the hippocampus 24 hours following injection of fluorescent tagged 2B3 (red) and DAPI counterstain (blue). **(C)** Representative image of the hippocampus 5 days following injection.

2B3 Reduction of β-Amyloid

In order to measure the effect of 2B3 on amyloid concentrations in the hippocampus, mice underwent contralateral infusions of 2B3 and IgG into either hemisphere, permitting a within-subjects comparison of Aβ40 concentrations between each hippocampus. Mice were culled after either 1- or 5-days post-surgery, in order to compare the duration of any effect. ELISA results for the four groups were checked for normality of distribution via the Shapiro-Wilke test, and all showed $p>0.5$. The results were then analysed by a mixed measures ANOVA using treatment (2B3 or IgG) as a within-subjects' factor, and duration (1 or 5 days) as the between-subjects factor. Levene's test and Box's test revealed no violations of homogeneity of variance or covariance.

The ANOVA revealed a significant treatment*duration interaction $F(1,13) = 6.4$, $p=0.025$ (Figure 3.2). There was no significant main effect of treatment $F(1,13) = 3.3$, $p=0.095$; or duration $F(1,13)=0.98$, $p=0.34$. Simple main effects analysis on the interaction term revealed that 2B3 significantly reduced Aβ40 concentration compared to the IgG-injected hippocampi when measured 1-day post-surgery ($p=0.011$) but not at 5-days ($p=0.6$). Furthermore, there was no difference in Aβ40 concentration between day 1 and 5 for the IgG-treated hippocampi ($p=0.444$), but Aβ40 levels significantly increased in the 2B3-treated hippocampi over the 5 days ($p=0.027$).

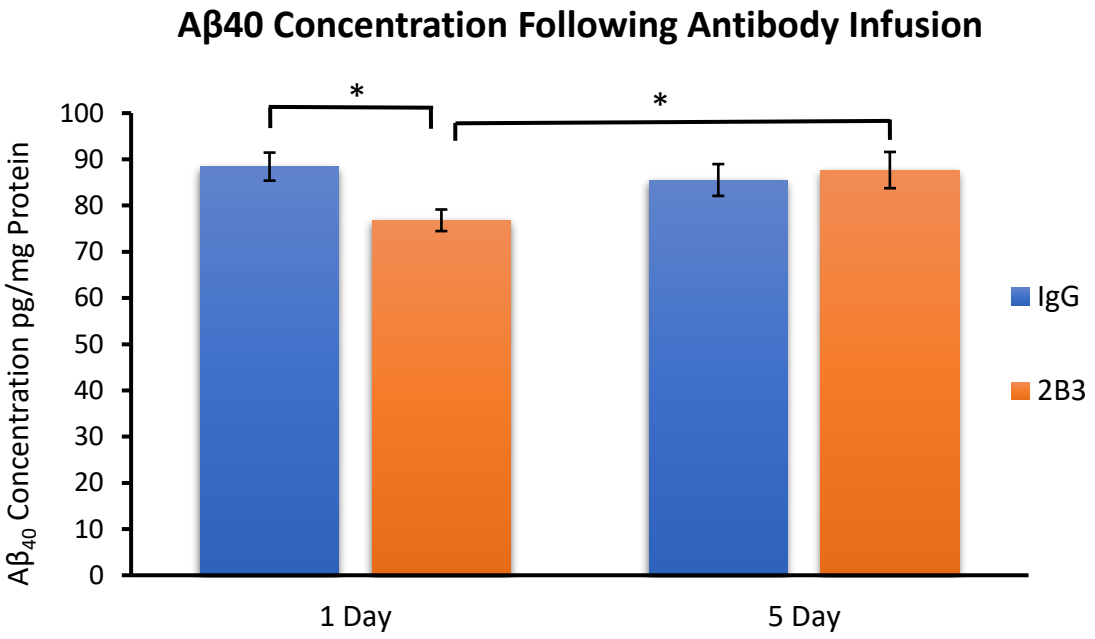


Figure 3.2: Aβ concentration following direct infusion of 2B3 in the hippocampus, measured at 1- or 5-days following surgery and compared to the contralateral hippocampus which had been infused with control IgG. n=7 at 1 day and 8 at 5 days. Error bars represent S.E.M. *=$p<0.05$, 2x2 mixed measures ANOVA.

Effect of Surgery on beta-Amyloid

A group of untreated 8 mice were culled alongside the mice which had undergone surgery. The Aβ40 concentration in their hippocampi were compared to the control IgG-treated mice in order to assess any effect of surgery. There were three groups: no surgery, 1-day post-surgery and 5 days post-surgery. None of the datasets violated the assumption of normality (Shapiro-Wilke $p>0.05$), and they were analysed by a one-way ANOVA. The ANOVA reported a significant difference between groups $F(2,23) = 3.87$, $p=0.037$ (Figure 3.3). *Post hoc* analysis with Bonferroni correction revealed a significant increase in hippocampal Aβ40 1-day following surgery ($p=0.044$). However, this did not reach

significance when measured in animals culled 5-days following surgery (p=0.161). There was no difference between the two surgery groups (p=1.0).

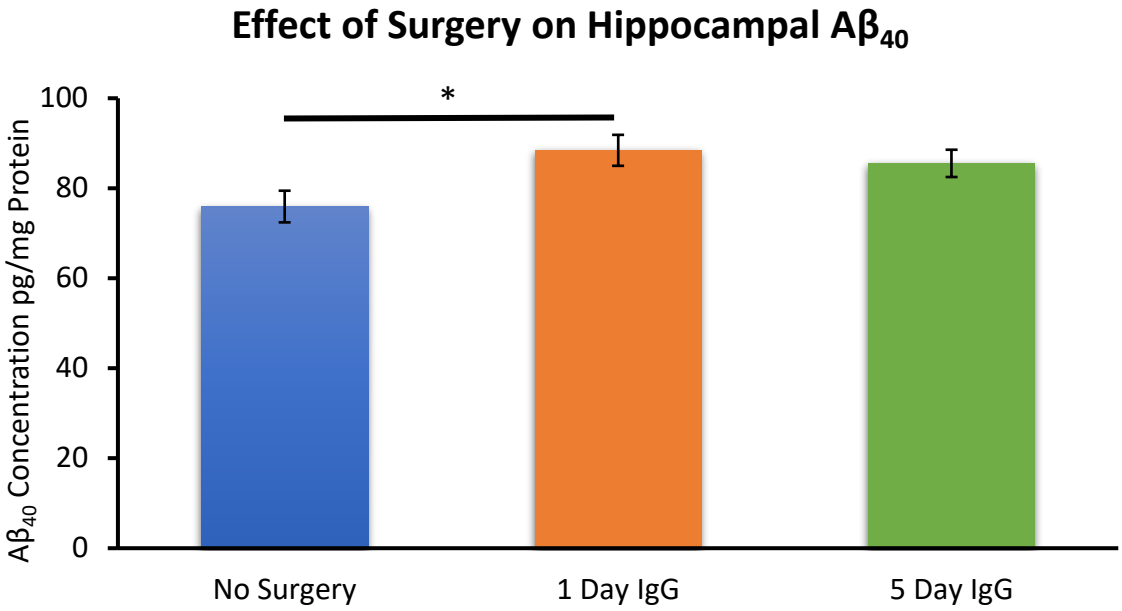


Figure 3.3: Mean Aβ40 concentration in the hippocampus of mice following hippocampal infusions of a control IgG after 1 or 5 days compared to a group that had no surgery. Error bars represent standard error of the mean. N=8 for all groups, *p<0.05.

▪ 3.3.4 – Experiment 1 Discussion

The aim of this experiment was to assess whether direct injection of 2B3 into the hippocampus could induce a measurable reduction in Aβ compared to the contralateral hippocampus, which was treated with a control antibody that does not bind mouse protein. The results of the ELISA revealed a significant reduction in Aβ40 concentration in the hippocampi treated with 2B3 at 1-day post infusion. This effect was absent at 5 days, presumably due to diffusion of the antibody out of the hippocampus and recovery of peptide concentrations to normal levels. This theory is supported by the fluorescently labelled 2B3 being visible in the brain 1 day after infusion, but not detectable 4 days later. Measurement of Aβ42 was attempted, however, the concentration was below detectable levels in all groups (data not shown). The 2B3 antibody has previously demonstrated the capability to inhibit β-secretase cleavage of APP and thus reduce the concentration of Aβ40 in mouse primary cortical neuronal cultures derived from a transgenic mouse model expressing the London mutation in APP (Thomas et al. 2013). Endogenous Aβ concentrations in WT mice are estimated to be in the picomolar range (Puzzo et al. 2011,

Cirrito et al. 2005). The low concentration makes it difficult to detect significant changes during manipulation of the peptide, particularly considering the high level of background noise identified in commercial ELISA kits (Teich, Patel, and Arancio 2013). 2B3 was compared to a control IgG with each infused into the contralateral hippocampus of the same mouse. This within-subjects design was hypothesised to reduce variation. This is the first time an antibody has been used as the control condition when testing the effect of 2B3 *in vivo*. The results confirm that the reduction of Aβ is linked to the specific activity of 2B3 to inhibit β-secretase cleavage rather than simply the presence of an IgG.

There are two potential explanations for why the effect of 2B3 on Aβ40 did not persist over 5 days: there could be an antagonistic increase in β-secretase activity or APP expression, to counteract the inhibition of β-cleavage and return the Aβ level to normal. Alternatively, the 2B3 IgG may have been cleared from the hippocampal parenchyma to an extent that its activity is negligible after 5 days. The absence of the fluorescently labelled 2B3 after 5 days indicates that the second possibility is more likely. As macromolecules, antibodies do not readily cross the BBB, however, they may be actively effluxed by the neonatal Fc receptor in rodents (Cooper et al. 2013, Deane et al. 2005). One group calculated the half-life of an IgG antibody following intracerebral injection as 48 minutes (Zhang and Pardridge 2001). Despite this experiment involving a different isotype (IgG2a) and species (rat), it is still relevant to support the theory that the infused 2B3 may have been cleared by the 5-day timepoint. One conclusion from this study is that the reduction in Aβ40 did not persist for long enough to permit an in-depth assessment of cognitive function without implantation of a cannula to permit acute infusions.

The level of Aβ40 in was found to be significantly higher in hippocampi harvested 1-day following control IgG infusion compared to a non-surgery control group. Elevated Aβ concentrations have frequently been linked to isoflurane anaesthesia, both in humans and mice (Xie and Xu 2013). Furthermore, general anaesthesia has also been linked to an exacerbation of Alzheimer-like pathology in both species (Tang et al. 2011, Zhang et al. 2013, Bianchi et al. 2008). One group has analysed the acute effect of isoflurane anaesthesia on APP metabolism over 24 hours in 5 month old WT mice (Xie et al. 2008). Mice, placed in an anaesthetising chamber, received 2 hours of 1.4% isoflurane in 100% oxygen. Control mice underwent the same procedure without the anaesthetic. They reported robust increases in BACE expression in the prefrontal cortex at 12- and 24-hours post-surgery and consequently, the concentration of Aβ was also increased at 24 hours.

Together, these results indicate the possibility that 2B3 may have blocked the isoflurane-induced increase in Aβ production.

In conclusion, Experiment 1 revealed that 2B3 reduced Aβ40 levels in the hippocampus of WT mice 24 hours after direct injection. However, this effect did not persist over 5 days, likely due to clearance of the antibody. Therefore, a chronic delivery method was subsequently adopted in order to assess the impact of decreased Aβ on object recognition.

3.4 Experiment 2: Chronic Inhibition of Aβ in WT Mice

▪ 3.4.1 – Introduction

The aim of this experiment was to measure the biochemical and cognitive impact of chronic 2B3 infusion into the lateral ventricle of WT mice over 14 days. Specifically, this experiment quantified the expression of proteins involved in APP metabolism. The hypothesis was that infusion of 2B3 would inhibit Aβ production in the hippocampus by steric hindrance of β-secretase cleavage of APP. The chronic (14-day) nature of the icv infusions permitted a detailed cognitive assessment of Aβ reduction. Two object recognition tasks were used to assess the nature of networks influenced by 2B3. Object novelty and object-in-place (OiP) tests were performed in this experiment in a counterbalanced order. The former task depends on function of the perirhinal cortex during short-term memory tests, while the latter task also requires intact hippocampal function (Barker and Warburton 2011). The combined use of both tasks permits analysis of any non-specific effect of 2B3 on performance (for further detail see chapter 4). The short timeframe in which cognitive testing was possible mandated a test with minimal training requirements and object recognition tests rely on the spontaneous exploratory behaviour of mice and their natural reaction to novel stimuli.

In summary, the aim of this experiment was to assess how manipulation of Aβ levels would impact cognitive performance during chronic infusion of 2B3. It was hypothesised that reduction of the Aβ peptide in the hippocampus would lead to a deficit in memory processes.

3.4.2 – Experiment 2 Methods

Subjects, Design

Five-month-old male C57Bl/6 mice underwent chronic intracerebroventricular (icv) administration of either 2B3 or a control antibody of the same isotype raised against rabbit IgG light chain (IgG1κ, mab201, Millipore, UK). There were 8 mice in each group, however, one of the mice in the 2B3 group was removed from the analysis because the catheter came loose from the brain infusion cannula prior to the commencement of behaviour testing, leaving group numbers as following: 2B3 infusion n=7; control IgG infusion n=8. The implantation of osmotic minipumps and infusion cannulas in the lateral ventricle was performed as described in section 2.4. ELISA and Western blot protocols are described in section 2.5.

The within-subjects design of this experiment is depicted in Figure 3.4. Mice were assessed on both object novelty and object-in-place recognition before and during chronic 2B3 infusion. The task order was counterbalanced, as was the use of object sets between both timepoints. Each mouse started the 6-day protocol on the 9th day after having the surgery. Mice were sacrificed 14 days after surgery and hippocampus and cortex were dissected, flash frozen in liquid nitrogen and protein was extracted following the protocols on page 52.

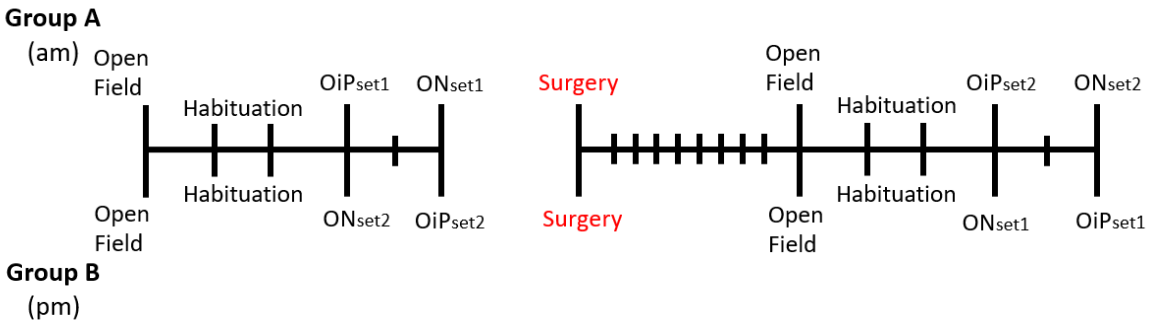


Figure 3.4: Experimental design assessing Object Novelty (ON) and Object-in-Place (OiP) memory before and following icv infusion of 2B3. The cohort of mice was split into groups A and B in order to counterbalance the order of tests. Each vertical line represents a new day. Truncated lines are rest days.

Object Recognition Tests

The ON and OiP protocols are described in section 2.3 of this thesis. This experiment utilised a 2-object array for the ON task. Two identical objects were placed in opposite corners of the arena for the 3 sample phases (each 10 minutes long). During the 5-minute delay, prior to the test phase, one of the objects was replaced with a novel item. The identity of the novel object within each set (two pairs of identical objects) was counterbalanced. The OiP protocol was run as previously described, with 4 different objects. During the test phase two of the objects had switched positions. Calculation of discrimination ratios and statistical analysis were performed as previously described in 2.3.

▪ 3.4.3 – Experiment 2 Results

APP Processing

Following the chronic icv infusion of 2B3 or a control IgG over 14 days, the hippocampi and cortices were collected for the assessment of Aβ40 levels by ELISA. The concentration in each 2B3-treated sample was normalised to the average of the IgG-treated samples in order to express percentage reductions which are presented in figure 3.5. The two datasets for the normalised hippocampal concentration were normally distributed as assessed by the Shaprio-Wilke test ($p>0.05$) and they were analysed by an independent samples t-test. The test revealed a significant difference between the groups $t(1,14)=2.046$, $p=0.029$ (Figure 3.5A).

The Aβ40 concentration in the cortices was also analysed in order to assess the penetration of 2B3 to the cortex and any potential effect in that region. After normalisation to the control concentration, Shapiro-Wilke test confirmed the results of both groups had normal distributions. They were analysed by an independent samples t-test, which revealed no difference between the groups $t(1,13)=0.366$, $p=0.722$ (Figure 3.5B).

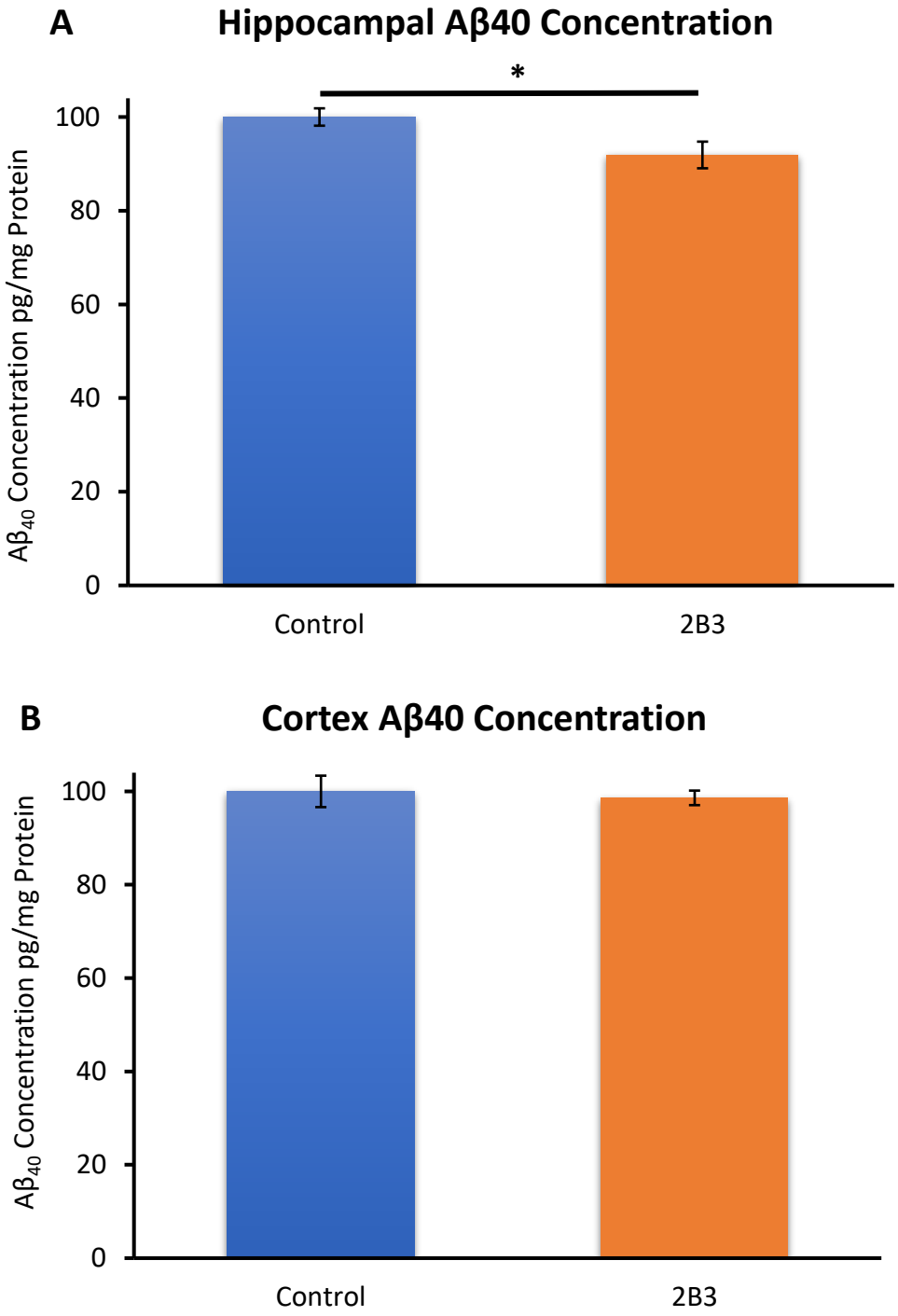


Figure 3.5: Icv infusion of 2B3 reduced concentration of Aβ40 in the hippocampus but not the cortex. The concentration for each 2B3 treated mouse was normalised to the average result of mice infused with the control antibody to produce a percentage reduction in the hippocampus (A) and cortex (B). N=8 for the control group, n=7 for 2B3. Error bars represent SEM. * $p<0.05$, independent t-test.

APP and BACE1 Expression

Western blot analysis was performed to detect whether any changes in Aβ concentration were mediated by alterations of endogenous APP or BACE1 expression. Figure 3.6 depicts representative blots (A) and the quantitative comparisons (B). The quantified expression densities were compared by independent Student's t-tests. There was no difference in the levels of APP t(1,13) = -0.77, p=0.458; or BACE1 t(1,13) = -0.26, p=0.797.

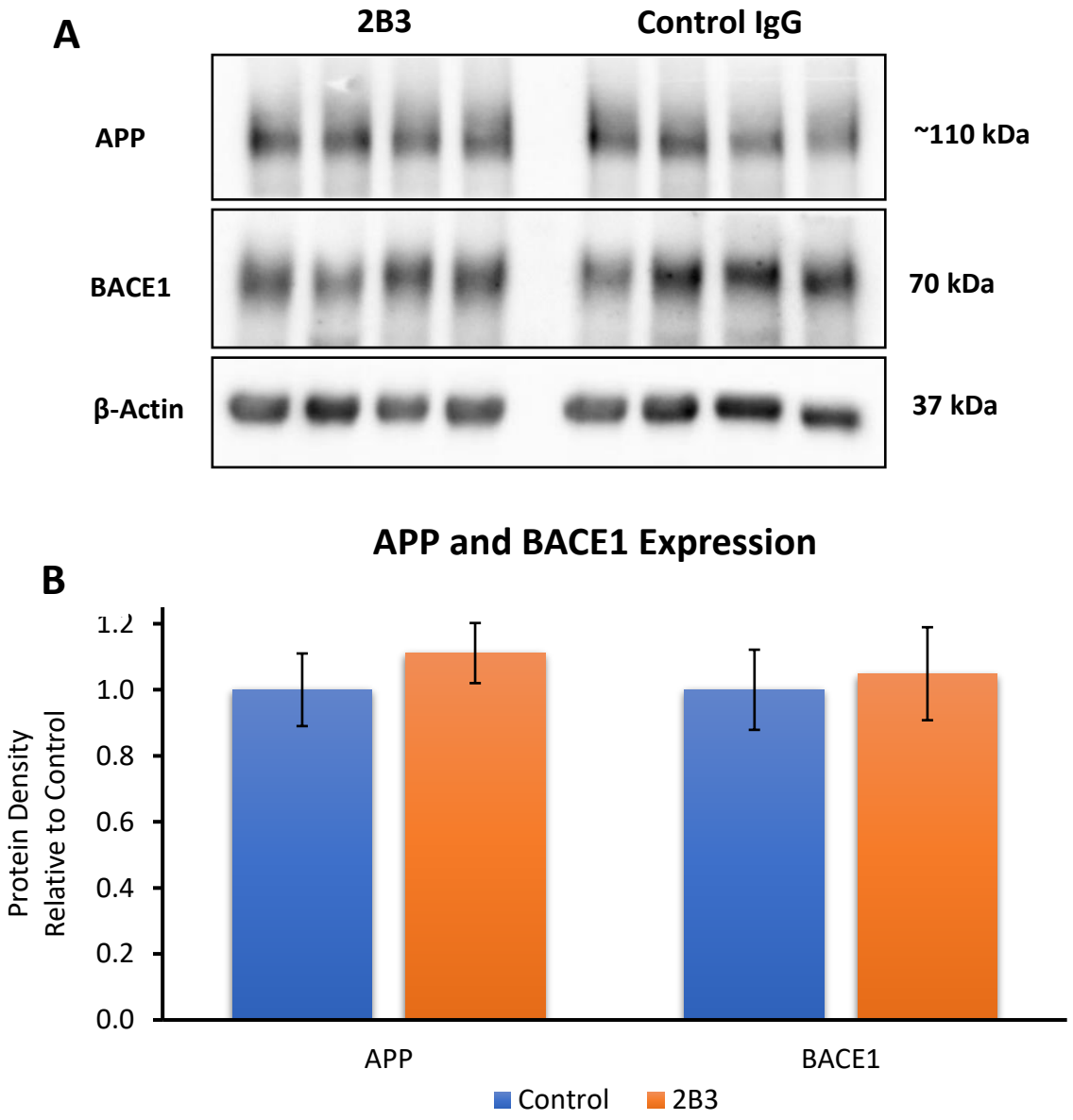


Figure 3.6: 2B3 infusion did not affect expression levels of APP and BACE1. **(A)** Representative Western blot images of APP and BACE1 expression in hippocampal homogenates following icv infusion of control (n=8) or 2B3 (n=7) antibodies. **(B)** No significant differences were detected after quantification of densities normalised to the control level (p>0.05, independent t-tests). Error bars show SEM.

Open Field

The protocol for recognition memory assessment involved free exploration of the empty arena as the first point of habituation. This provided the opportunity to investigate non-cognitive motor effects of both general surgery and 2B3 administration. The total distance travelled by both groups before and during antibody administration was recorded and is presented in Figure 3.7. Visual inspection suggested a decrease in general locomotive activity after surgery for both groups. The datasets were normally distributed as revealed by the Shapiro-Wilke test ($p>0.05$). They were analysed by a mixed measures ANOVA with surgery time point as the within subjects' factor and treatment type as the between subjects' factor. The results of the ANOVA indicated no significant main effect of surgery time point $F(1,13) = 2.4$, $p=0.148$; no main effect of antibody treatment $F(1,13) = 0.23$, $p=0.639$; and no surgery*treatment interaction, $F(1,13) = 0.029$, $p=0.868$. In summary, neither surgery nor 2B3 treatment exerted any effects on the general locomotive behaviour of the mice.

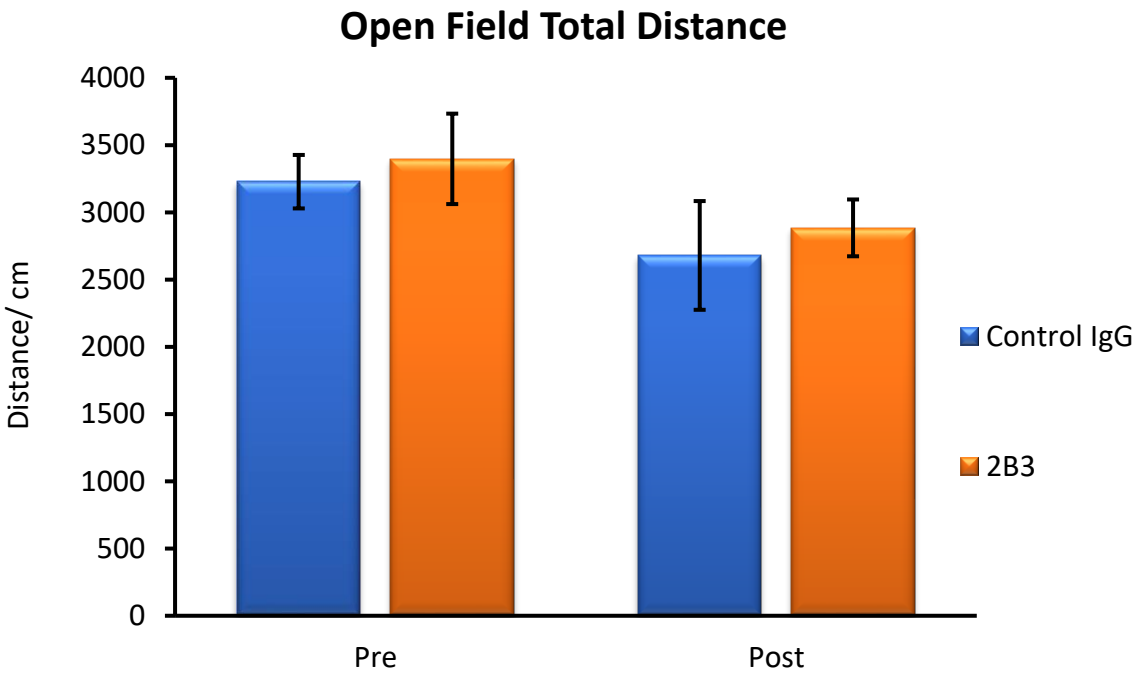


Figure 3.7: Total distance moved in the open field test pre and post-infusion of 2B3 or the control antibody. Control IgG n= 8; 2B3 n=7. Error bars represent SEM.

Open Field Inner Zone Exploration

The Open Field test permits assessment of anxiety-related exploratory behaviour. Figure 3.8 depicts the ratio of time spent in the exposed inner zone (central 30 x 30 cm square) of the 60 x 60 cm arena compared to the total duration of the trial. Results of each group both before and during treatment are shown. All four groups demonstrated normal distributions and they were compared by a mixed measured ANOVA using treatment as the between subjects' factor and pre- vs post- surgery timepoint as the within subjects' factor. The ANOVA reported a significant main effect of surgery timepoint $F(1,13) = 9.6$, $p=0.008$, as mice entered the zone less post-implantation of the minipump and cannula. There was no main effect of treatment $F(1,13) = 0.49$, $p=0.498$ and no surgery*treatment interaction $F(1,13) = 0.72$, $p=0.413$.

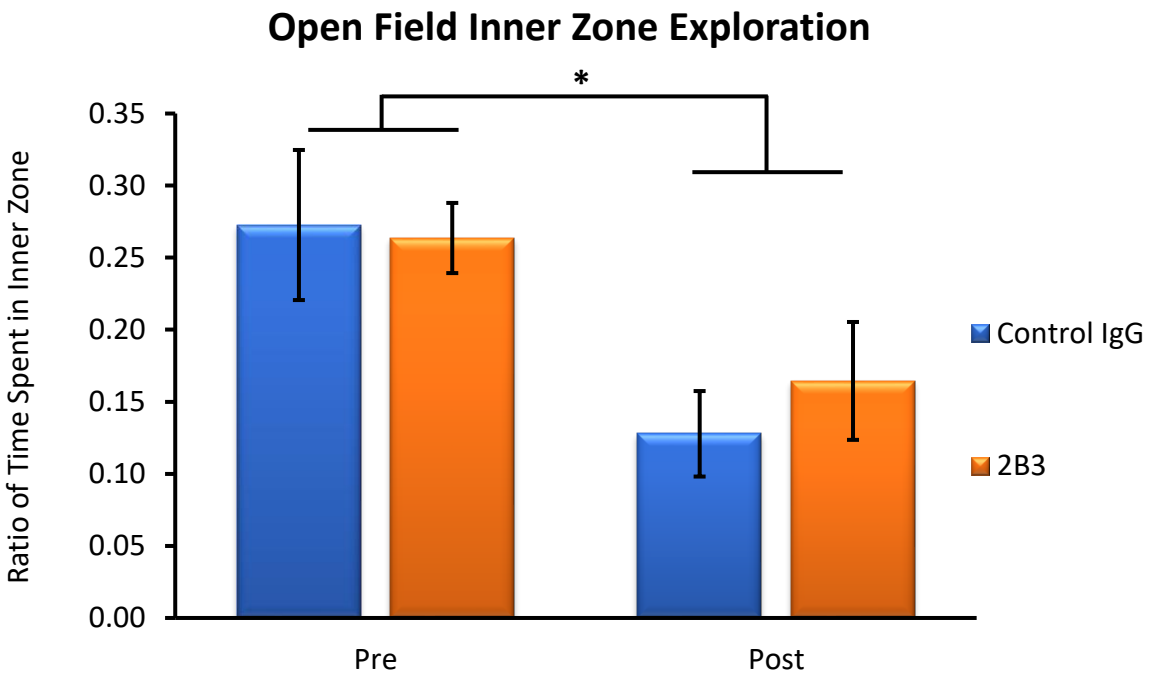


Figure 3.8: Exploration of the "inner zone" of the open field arena pre- and post-infusion of 2B3 or IgG antibody. Control IgG n= 8; 2B3 n=7. Error bars represent SEM, * $p<0.05$, 2x2 mixed measures ANOVA.

Object Novelty

Sample Phase Contact Times

During the object novelty tasks before and during chronic infusion of 2B3 or control IgG, total contact with both objects was scored and the results are summarised in table 3.1. The data suggest a slight decrease in object contact following surgery. There was also a general decrease in exploration as the sample phases progressed. The datasets were normally distributed as assessed by the Shapiro-Wilke test ($p>0.05$). Levene's & Box's tests revealed there was equality of error variances and covariance matrices ($p>0.05$). The data were analysed by a 3-way mixed measures ANOVA, with pre- vs post- surgery timepoint and sample phase as within subjects' factors and treatment as the between subjects' factor.

The ANOVA demonstrated significant main effects of surgery timepoint (pre- vs post-) $F(1,13) = 6.25$, $p=0.019$ and sample phase $F(2,26) = 4.6$, $p=0.004$. There was no main effect of treatment $F(1,13) = 0.01$, $p=0.983$; no surgery timepoint*treatment interaction $F(1,13) = 3.9$, $p=0.071$; no sample phase*treatment interaction $F(2,26) = 0.71$, $p=0.389$; no sample phase*surgery interaction $F(2,26) = 0.53$, $p=0.532$ and no sample phase*surgery*treatment interaction $F(2,26) = 1.33$, $p=0.242$. *Post hoc* analysis of the main effect of sample phase showed that although mice exhibited lower object exploration between phases 1 & 3, this was not significant ($p=0.066$). The main effect of surgery demonstrated that in general, mice explored objects less after having surgery.

ON Sample Phase Contact					
		Control IgG		2B3	
		Pre	Post	Pre	Post
Sample Phase 1	**Mean**	**43**	**51**	**63**	**44**
	SD	18	26	29	18
Sample Phase 2	**Mean**	**43**	**42**	**45**	**34**
	SD	22	30	14	15
Sample Phase 3	**Mean**	**44**	**31**	**43**	**29**
	SD	18	13	18	12

Table 3.2: Mean contact times across the object novelty (ON) contact phases pre- and post-infusion of 2B3 or control antibody. Contact time was measured in seconds. Data represent both mean scores and standard deviation (SD).

Test Phase Contact Times

Figure 3.9 presents the average contact times for exploration of either the familiar or novel object in the test phase for both groups before and during antibody infusion. Inspection of the data suggests all groups preferentially explored the novel object. All datasets were normally distributed (Shapiro-Wilke test $p>0.05$). the data were analysed by a three-way mixed measures ANOVA with object type and pre-vs post- surgery timepoint as within subjects' factors, and antibody treatment as the between subjects' factor. The test revealed a significant main effect of object type $F(1,13) = 52.6$, $p<0.0005$. There were no significant effects of pre- vs post-surgery timepoint $F(1,13) = 0.03$, $p=0.865$ or treatment $F(1,13) = 0.29$, $p=0.600$. There were no significant interactions: surgery*object*treatment interaction $F(1,13) = 0.72$, $p=0.412$; surgery*treatment $F(1,13) = 0.25$, $p=0.628$; object*treatment $F(1,13) = 0.029$, $p=0.867$; surgery*object $F(1,13) = 0.001$, $p=0.982$.

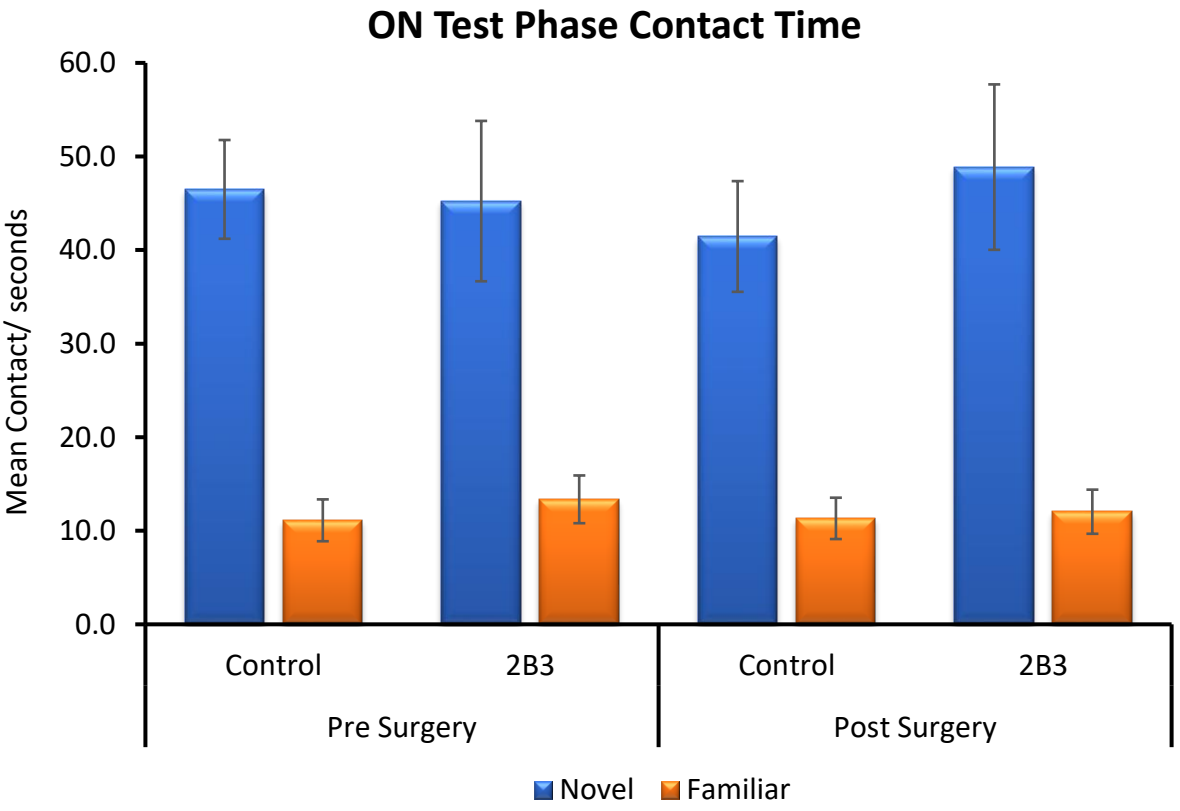


Figure 3.9: Contact times for novel and familiar objects in the test phase of the object novelty (ON) task pre- and post-infusion of 2B3 or control antibody. Control IgG n= 8; 2B3 n=7. Error bars represent SEM.

Discrimination Ratio

The contact time for the familiar and novel object in the ON task test phase were converted into discrimination ratios. These are presented in Figure 3.10 for both treatment groups. The Shapiro-Wilke test disclosed that not all groups exhibited normal distributions ($p>0.05$), so they were all transformed by the reflect and square root calculation. The transformed data were compared by a mixed measures ANOVA with surgery state as the within subjects' factor and treatment antibody as the between subjects' factor. The ANOVA revealed no significant main effect of treatment $F(1,13) = 0.07$, $p=0.796$ or surgery $F(1,13) = 0.009$, $p=0.925$. There was no significant surgery*treatment interaction $F(1,13) = 0.79$, $p=0.389$.

The DRs of the four groups were compared to the predicted score of chance (0.5, highlighted in the graph) by a one-sample t-test. The equivalent test statistic was calculated by performing the same transformation on an imaginary score of 0.5. The one-sample t-tests revealed that all groups performed significantly better than chance: Control pre surgery $t(7) = -6.3$, $p<0.0005$; 2B3 pre surgery $t(6) = -7.2$, $p<0.0005$; Control post-surgery $t(7) = -5.8$, $p=0.001$; 2B3 post-surgery $t(6) = -5.3$, $p=0.002$.

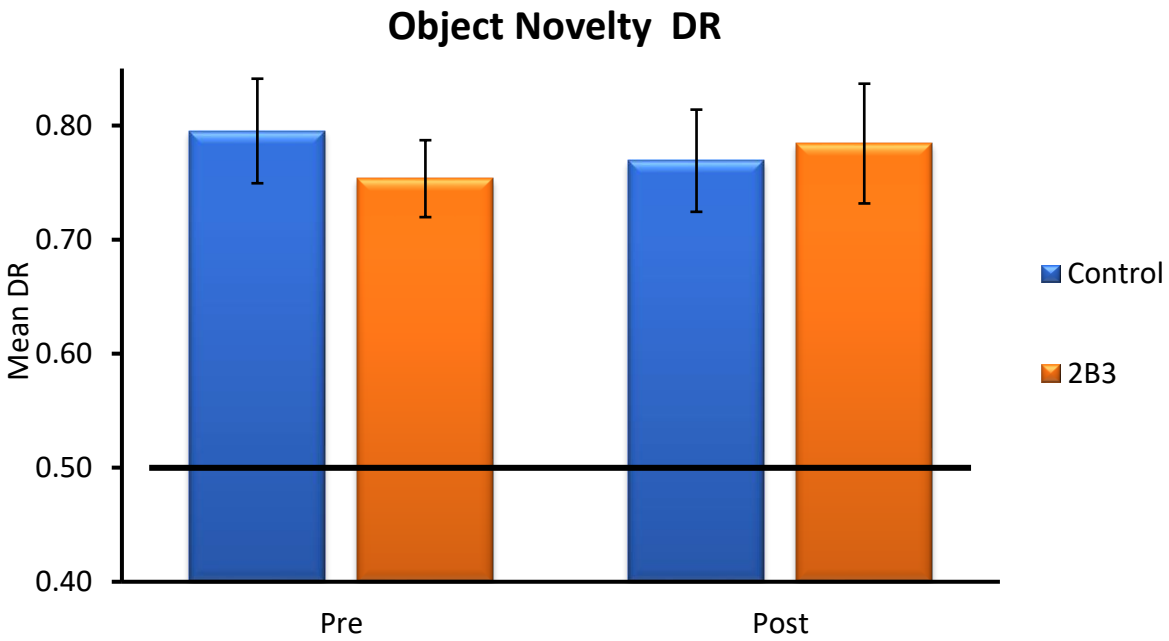


Figure 3.10: 2B3-treated WT mice display intact object novelty recognition. Mean discrimination ratios (DR) pre- and post-infusion of 2B3 or control antibody. The predicted random chance score of 0.5 is highlighted. Control IgG n= 8; 2B3 n=7. Error bars represent SEM.

Object-in-Place

Sample Phase Contact Times

Table 3.2 shows the mean total contact time throughout the sample phases of the OiP task. Inspection of the data suggests a decrease in total contact after having surgery. The 2B3 group seemed to exhibit greater contact both pre- and post-surgery. Furthermore, sample phase 3 appeared to involve less exploration of objects than the other phases. Specific datasets were not normally distributed (Shapiro-Wilke test $p>0.05$), so were all transformed by square root. The sample phase contact times were analysed by a three-way mixed measures analysis of variance test (ANOVA) with sample phase and surgery timepoint (pre- vs post-) as within-subject factors and treatment group as the between subjects' factor.

The ANOVA revealed a significant main effect of sample phase $F(2,26) = 8.8$, $p<0.001$. There was no main effect of surgery timepoint (pre- vs post-) $F(1,13) = 3.22$, $p=0.106$ or treatment $F(1,13) = 2.6$, $p=0.134$. There were no significant interactions: phase*surgery*treatment $F(2,26) = 0.32$, $p=0.639$; phase*surgery timepoint $F(2,26) = 0.94$, $p=0.444$; surgery*treatment $F(1,13) = 0.02$, $p=0.878$; phase*treatment $F(2,26) = 2.2$, $p=0.229$. Pairwise comparisons on the main effect of sample phase showed that while there was no difference in contact time between sample phases 1 and 2 ($p=1.0$), there was significantly reduced contact with objects in sample phase 3 compared to both phase 1 ($p=0.033$) and phase 2 ($p=0.007$). These data illustrate that, across all groups, mice showed habituation of exploratory behaviour across the sample phases.

OiP Sample Phase Contact					
		Control IgG		2B3	
		Pre	Post	Pre	Post
Sample Phase 1	**Mean**	**73**	**69**	**105**	**91**
	SD	29	23	35	34
Sample Phase 2	**Mean**	**94**	**72**	**104**	**80**
	SD	34	22	33	19
Sample Phase 3	**Mean**	**71**	**56**	**71**	**65**
	SD	35	17	26	22

Table 3.3: Mean contact times across the object-in-place (OiP) sample phases pre- and post-infusion of 2B3 or control antibody. Contact time was measured in seconds. Data are mean and standard deviation (SD).

Test Phase Contact Times

The contact times for objects in familiar or novel locations in the OiP test phase are presented in Figure 3.11. Visual inspection of the data suggests the control mice explored the objects in the novel location more, in contrast to the behaviour shown by mice infused with 2B3. The datasets were analysed by a three-way mixed measures ANOVA with Object location and Surgery state as within subjects' factors, and Treatment group as the between subjects' factor.

The ANOVA revealed a significant main effect of object location $F(1,13) = 44$, $p<0.0005$; showing that overall, mice spent more time exploring the objects in novel locations. There were no significant effects of surgery $F(1,13) = 3.3$, $p=0.093$ or treatment $F(1,13) = 0.14$, $p=0.714$. There were no significant interactions. Three-way object*surgery*treatment interaction $F(1,13) = 2.8$, $p=0.119$, object*surgery interaction $F(1,13) = 4.4$, $p=0.056$, surgery*treatment interaction, $F(1,13) = 3.2$, $p=0.098$ and object*treatment $F(1,13) = 2.7$, $p=0.122$.

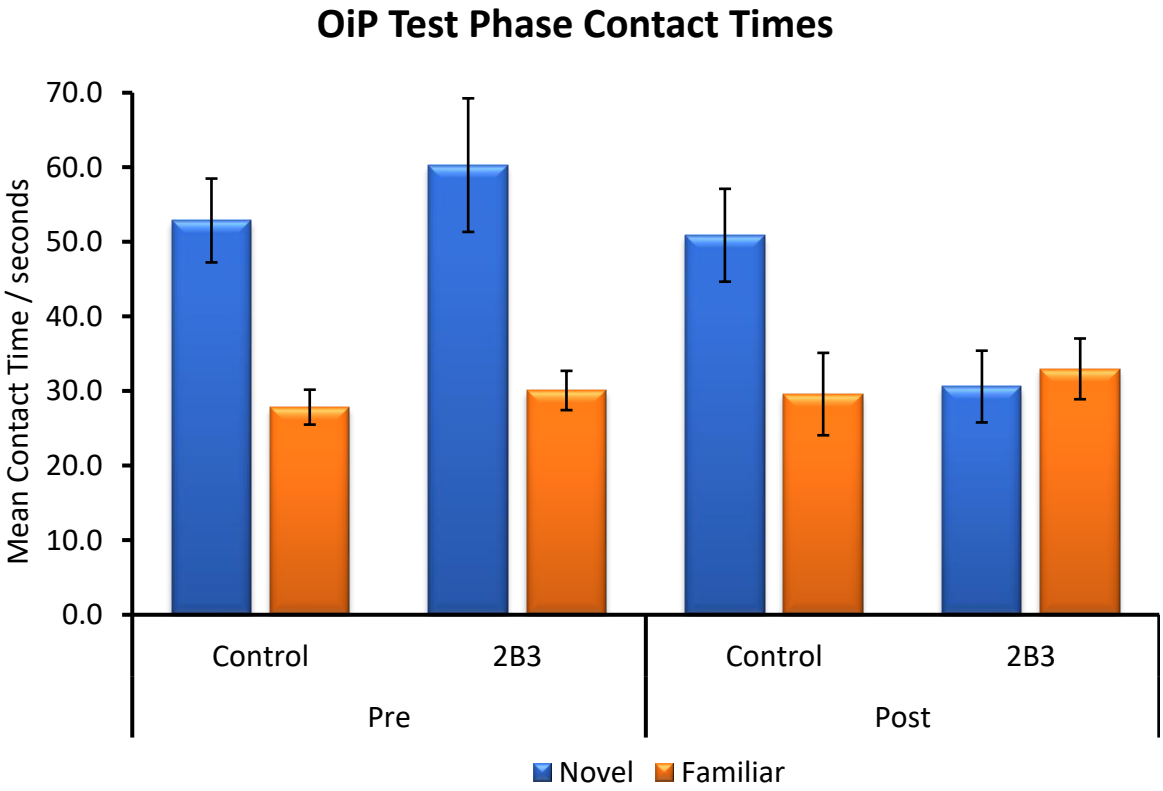


Figure 3.11: Mean contact times of WT mice with objects in either familiar or novel locations in the test phase of the OiP task pre- and post-infusion of 2B3. Error bars represent standard error of the mean (SEM). Control IgG n= 8; 2B3 n=7.

Test Phase Discrimination Ratio

The test phase contact times were converted into discrimination ratios and these are presented in Figure 3.12. Visual inspection indicates the group treated with 2B3 had a lower DR that was not above chance level. The datasets were all normally distributed and were analysed by a 2x2 mixed measures ANOVA. Surgery state (timepoint: pre or post) was the within subjects' factor, and treatment group was the between subjects' factor. The ANOVA revealed no significant surgery*treatment interaction $F(1,13) = 2.9$, $p=0.114$. There was no main effect of surgery $F(1,13) = 3.0$, $p=0.109$ but a significant main effect of treatment $F(1,13) = 8.8$, $p=0.011$. Pairwise comparisons on this main effect demonstrated that overall, mice in the 2B3 group exhibited a lower DR compared to the control IgG-treated group.

The group DR scores were compared to the predicted random chance score of 0.5 by one-sample t-tests. Both groups significantly discriminated at greater than chance level before surgery (IgG $t(1,7) = 4.4$, $p=0.003$; 2b3 $t(1,6) = 3.8$, $p=0.009$). However, when tested during chronic administration, only the performance of the control group was above chance $t(1,7) = 3.6$, $p=0.009$; while the 2B3-treated group failed to discriminate between objects $t(1,6) = -0.49$, $p=0.640$. Collectively these data demonstrate that, while the ability to discriminate objects in different locations was numerically reduced to chance in mice treated with 2B3, the lack of a surgery timepoint*treatment interaction limits the strength of this conclusion.

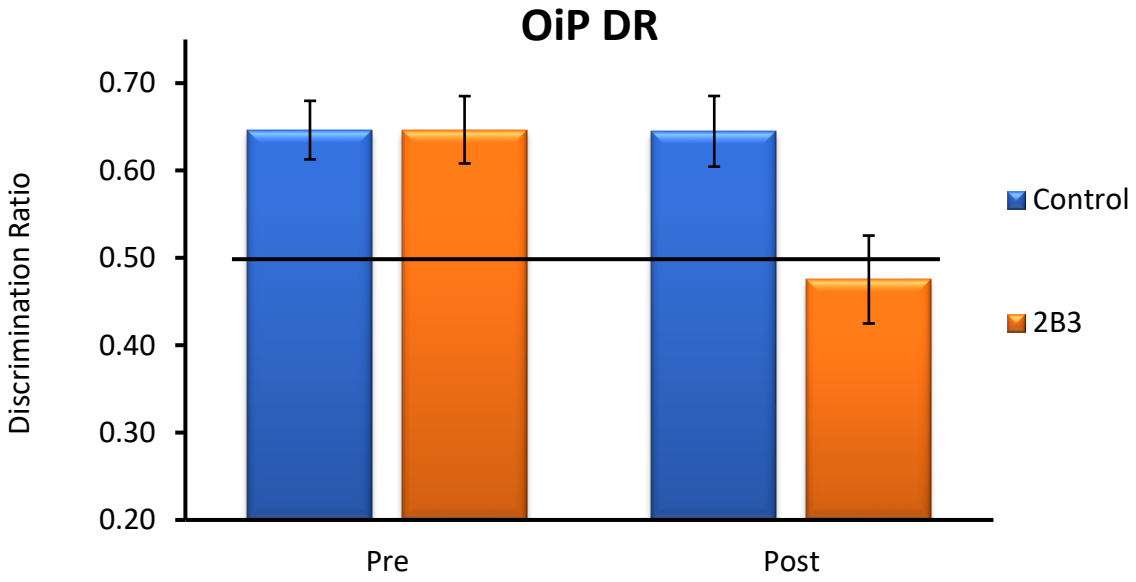


Figure 3.12: 2B3-treated WT mice fail to discriminate novel object-place associations above chance. Mean discrimination ratios in the object-in-place task pre- and post-infusion of 2B3**.** the score predicted by random performance, 0.5, is highlighted. Error bars represent SEM. Control IgG n= 8; 2B3 n=7.

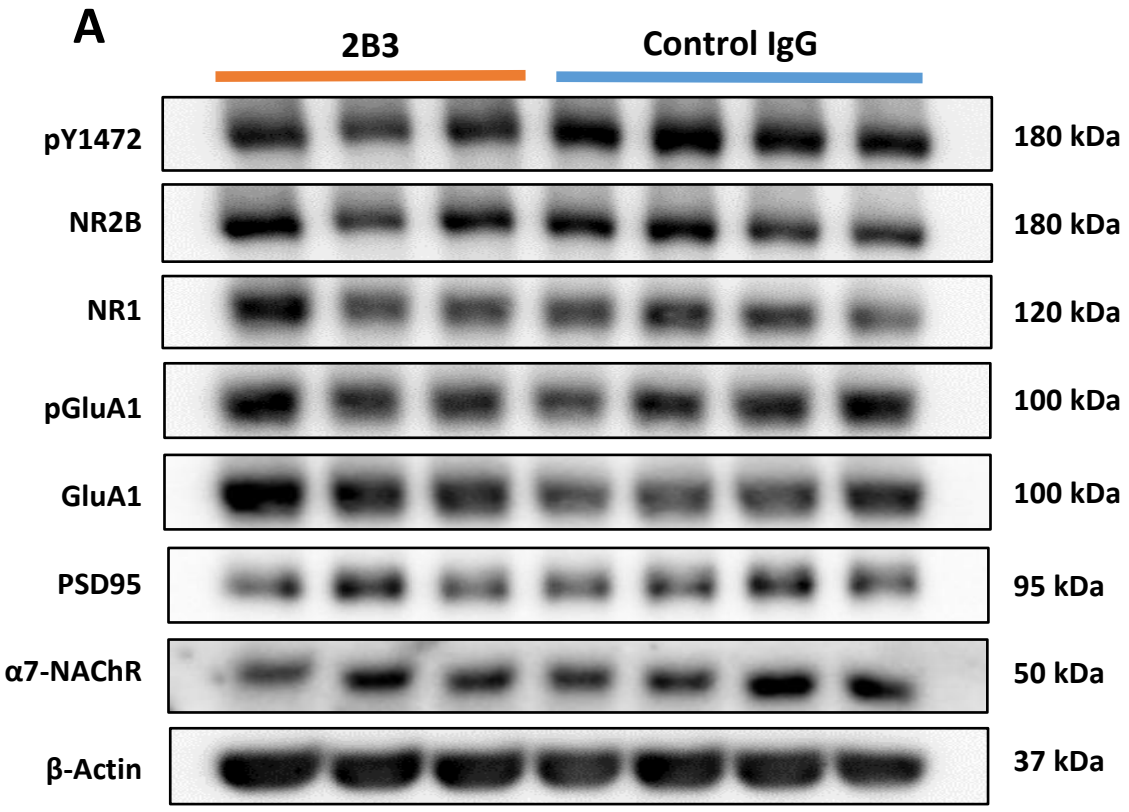
Synaptic Receptor Expression

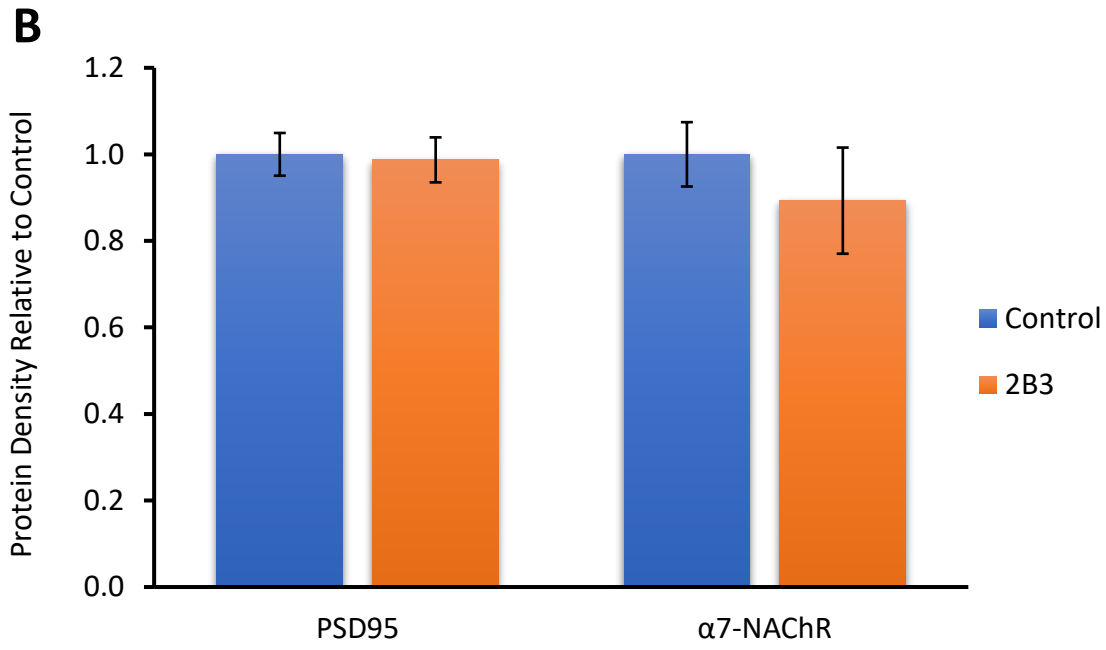
` Following the results of the OiP test, synaptosome preparations were extracted from the right hippocampi of the mice in order to analyse synaptic markers and receptor dynamics by Western blot (figure 3.13). Glutamate receptor expression in the hippocampus was investigated because these have been shown to be required for associative recognition memory (Barker and Warburton 2015). The data were normalised to the control treated mice for all proteins and statistically compared by independent samples t-tests.

There were no significant differences in the total expression of post synaptic density scaffolding protein (PSD95) $t(13) = 0.059$, $p=0.602$, indicating that any changes in receptor expression cannot be attributed to differences in total synaptic density. There was also no effect of 2B3 on α7-NAChR expression $t(13) = 0.457$, $p=0.384$ (figure 3.13 A).

Figure 3.13 C shows that total NMDA receptor (NR1) expression was unchanged by 2B3 infusion $t(13) = 1.35$, $p=0.326$. An NMDA receptor subtype that has been shown to be critical to OiP performance, NR2B showed no significant difference in its total expression $t(13) = -0.469$, $p=0.647$. Expression of the phosphorylated receptor at the regulatory tyrosine residue Y1472 (pY1472) was not significantly affected by 2B3 infusion $t(13) = 1.79$, $p=0.096$. However, calculation of the ratio of pY1472 to total NR2B (pY1472 : NR2B) showed a significant reduction in the amount of pY1472 relative to the total level of NR2B $t(13) = 2.8$, $p=0.011$.

AMPA receptor dynamics were analysed by examining the total expression and phosphorylation of the GluA1 subunit. There was no effect of 2B3 infusion in the total level of GluA1 $t(13) = 0.94$, $p=0.365$ or pGluA1 $t(13) = 0.072$, $p=0.944$. However, there was a significant decrease in the relative phosphorylation (pGluA1 : GluA1), $t(13) = 2.3$, $p=0.037$.


A
2B3
Control IgG
pY1472
NR2B
NR1
pGluA1
GluA1
PSD95
α7-NAChR
β-Actin
180 kDa
180 kDa
120 kDa
100 kDa
100 kDa
95 kDa
50 kDa
37 kDa


B
Protein Density Relative to Control
1.2
1.0
0.8
0.6
0.4
0.2
0.0
PSD95
α7-NAChR
Control
2B3

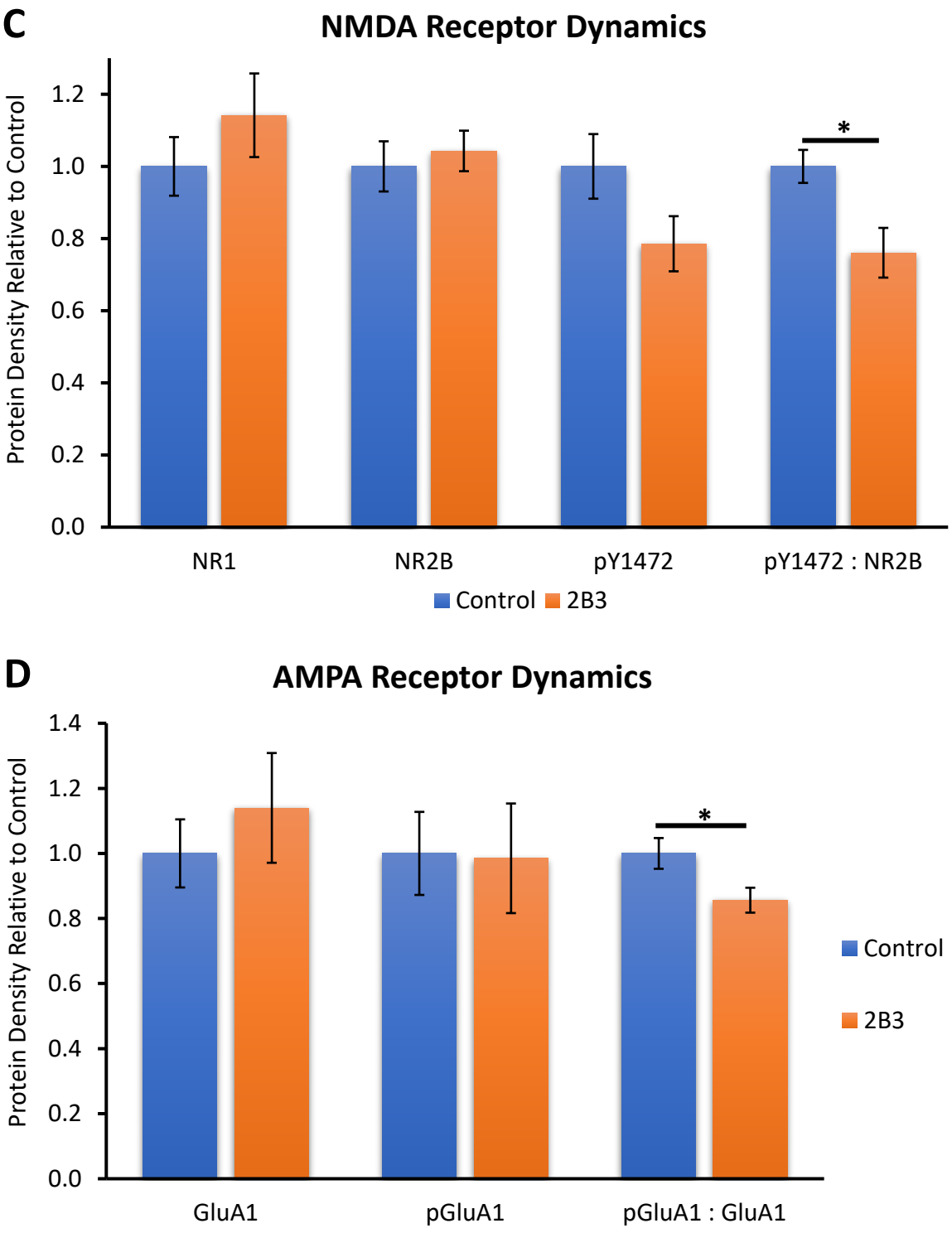


Figure 3.13: Western blot analysis of synaptic receptor expression in WT mice following 2B3 infusion. Hippocampal synaptosomes were prepared from mice that had been infused with either 2B3 or a control IgG. **(A)** western blot images were used to analyse expression of synaptic markers and receptors. **(B)** Quantification of PSD95 (postsynaptic density scaffolding protein), total nicotinic acetylcholine α7 receptor (α7-NAChR). C: Quantification of total NMDA receptor expression (NR1), total NR2B and both the total pY1472 expression and after normalisation to NR2B (p1472 : NR2B). **(C)** quantification of total GluA1 subunit expression of AMPA receptors, along with total pGluA1 and the relative phosphorylation. Error bars represent SEM. Control IgG n= 8; 2B3 n=7. *p<0.05, independent samples t-test.

3.4.4 – Experiment 2 Discussion

This experiment revealed that chronic icv infusion of 2B3 over 14 days induced a significant reduction in the concentration of Aβ40 in the hippocampus, but not the cortex. The effect on hippocampal Aβ is consistent with Experiment 1 and the absence of measurable effect in the cortex may simply reflect the lack of penetration by 2B3 to that region over the period of infusion. Analysis of endogenous APP and BACE1 expression levels in the hippocampus revealed no changes between the 2B3 and IgG control-treated samples, further validating the conclusion that changes in Aβ were mediated by specific inhibition of APP cleavage by BACE rather than changes in expression.

Cognitive tests demonstrated that, while infusion of 2B3 had no effect on object novelty recognition, mice receiving the antibody failed to discriminate novel object-place associations above chance. The lack of significant interactions in the test phase behavioural data does not strictly support the hypothesis that a reduction of Aβ by administration of 2B3 disrupted associative recognition memory. The experiment appeared to be underpowered for the conservative nature of the ANOVA test. A *post hoc* power analysis performed on the pre- vs post-surgery timepoint*treatment interaction for the OiP DR scores using SPSS calculated the observed power as 0.349. While this does suggest a lack of statistical power, it is important to note that increasing sample size may not necessarily produce a significant result. Averaging over multiple trials would reduce the variation in the DR scores for the 2B3-treated mice which performed at the level of random chance. However, the fact these mice were not able to discriminate between the objects above chance indicates that there was an impact on their processing of object-place associations. This ability is well evidenced to depend on hippocampal function (Barker and Warburton 2011, Barker et al. 2007).

During the object novelty task, the mice demonstrated lower total object contact in the sample phases after having the surgery. However, there was no interaction with treatment group, meaning that this was probably a result of undergoing surgery or simply an artefact of repeated testing. This effect of pre- vs post- surgery timepoint was not present in the OiP data, meaning that it was not robust enough to be maintained across tasks. 2B3 did not alter total contact times, therefore did not impact general exploration or motivation to contact objects. In fact, results from the Open Field test showed no effect on general locomotor behaviour, however, entry into the central zone was decreased in all mice after

surgery. While the significant decrease in central zone exploration (Figure 3.7) could be caused by surgery, it must be acknowledged that it is the second time mice experienced the apparatus after habituation and further object memory tests.

Western blot analysis of synaptosomes revealed no effect of 2B3 on synaptic density or total NMDA receptor expression. However, the relative phosphorylation of the NMDA subunit NR2B was significantly reduced in the 2B3-treated animals. Previous studies by the Warburton group have demonstrated that NMDA and AMPA receptors in the hippocampus are involved in associative recognition memory (Barker and Warburton 2015). In particular, the NMDA subunit NR2B is required for OiP memory, as shown by Evans *et al* (2019) who reported that administration of the selective NR2B inhibitor, RO25-6981, resulted in a deficit in OiP discrimination performance. Activation of NMDA receptors leads to the induction of synaptic plasticity due to expression or internalisation of AMPA receptors from the synapse, which is regulated by phosphorylation of the GluA1 subunit at serine residue 845 (Lu et al. 2001, Lee et al. 2003). Therefore, the decrease in relative GluA1 phosphorylation observed in the current experiment may represent a downstream effect of the reduced NR2B activation and propose a mechanism for the failure of the 2B3-treated mice to discriminate object-place associations above chance level. Further discussion of the putative role of Aβ is provided in sections 7.5 and 8.3.

3.5 Chapter Discussion

This Chapter presents the first experiments investigating the effect of specific inhibition of β-cleavage of APP on WT mice using antibody-mediated steric hindrance. Experiment 2 revealed that chronic icv infusion of 2B3 significantly reduced Aβ40 in the hippocampus. Mice administered 2B3 showed control levels of object novelty detection that were reliably above chance but failed to show the same level of performance when tested on the OiP associative recognition memory task. The latter observation is consistent with the view that Aβ is involved in normal learning and memory processes (Puzzo et al. 2008, Puzzo et al. 2011, Morley et al. 2010)

Although the two tasks differed in the complexity of the sample arrays (two objects versus four), the absence of a deficit in the object novelty is consistent with evidence that the effect of the 2B3 antibody did not extend to the cortex. There is a plethora of data

showing that the perirhinal cortex is required for visual object novelty detection (Barker and Warburton 2011, Good et al. 2007, Forwood, Winters, and Bussey 2005, Warburton and Brown 2015). Of course, the perirhinal cortex represents a small part of the tissue analysed in experiment 2 and so the specific effect of 2B3 within this region requires further investigation. Lesion studies by Barker and Warburton (2011) demonstrated that disrupted performance on short-term OiP associative recognition tasks alongside intact ON memory may be caused by disruption of the medial prefrontal cortex (mPFC)-hippocampus-perirhinal cortex network, suggesting that communication between regions in the network is critical for associative memory.

While this is the first example of specific inhibition of APP cleavage by BACE impacting on cognitive processes in WT mice, it is consistent with previous studies. Genetic studies have shown that knock-out of the APP or BACE genes leads to impairment of cognitive function, among other phenotypes (Zhu et al. 2018, Müller, Deller, and Korte 2017), while chronic administration of a small molecule BACE inhibitor disrupted both synaptic plasticity and memory (Filser et al. 2015). However, these approaches were not specific to modulation of Aβ, due to the various other metabolites of APP and substrates of BACE1 as well as the influence of both proteins during development (Vassar et al. 1999, Willem et al. 2006). In more specific manipulations of Aβ, antibodies with high affinity for the peptide have been shown to impair memory in three separate studies. These groups validated their conclusion that the loss of Aβ activity was the causative factor in their reported memory deficits by showing that concurrent application of exogenous Aβ42 rescued performance. Both icv and intra-hippocampal injection of such antibodies prior to training induced a deficit in a T-maze passive avoidance task, as mice required a significantly greater number of training trials to reach the criterion for learning (Morley et al. 2010, Garcia-Osta and Alberini 2009). Puzzo *et al* (2011) reported that injection of anti-Aβ antibodies 15 minutes prior to training impaired spatial reference learning and memory in the MWM, as well as contextual (but not cue-related) fear conditioning. No effect on memory performance was observed if infusion occurred post-training, suggesting that the peptide may be involved in encoding processes. The chronic infusion of 2B3 ensured that the antibody was present from the beginning of the encoding phase. Therefore, the inability to discriminate novel object-place associations may have manifested due to a failure to encode the original spatial arrangement of objects. However, habituation of exploratory activity during the sample phase indicated that encoding of the objects themselves proceeded normally in mice administered 2B3 (as reflected by a decline in contact times).

Despite the considerable number of anti-amyloid drugs entering clinical trials as treatments for Alzheimer's disease (39 were counted as of January 2018), none have been approved since 2003 (Cummings et al. 2018). Clinical trials for AD therapies involve cognitive assessment to determine to what extent the treatment has reduced the decline of patients (Vassar 2014). For that reason, identifying any physiological functions of Aβ in cognition is even more pertinent. If healthy levels of the peptide play an important role in neuronal function, then anti-amyloid mechanisms may exacerbate rather than improve whatever symptoms the patient is experiencing. In fact, multiple cognitive trials have reported that some potential treatments actually worsened the cognitive decline in patients compared to placebo (Coric et al. 2012, Doody et al. 2013, Schneeberger et al. 2009). It is possible that this was due to the loss of physiological Aβ function and that this is also responsible for the inability of anti-Aβ therapies to produce efficacious phase III results. Recent trials have attempted to recruit patients at earlier stages of the disease to try and match their pathology with that exhibited by the preclinical AD models in which the treatments have shown beneficial effects. However, the data in the chapter indicate that administering anti-amyloid therapies to patients without amyloid burden may be detrimental to cognition.

In conclusion, the experiments in this chapter revealed that icv infusion of 2B3 over 14 days significantly reduced Aβ40 in the hippocampus but not the cortex. In addition, 2B3 disrupted OiP memory while leaving object novelty detection intact – this pattern is consistent with disruption of hippocampal function. These results indicate that Aβ is necessary for a physiological role in hippocampal neuronal function and this must be taken into account during the design of anti-amyloid therapeutics for AD patients.

Chapter 4 –

Characterising Object Recognition Memory in *APP*$^{-NL-F}$ Mice

4.1 Chapter Overview

Genetic mutations found in Familiar Alzheimer Disease (FAD) patients, who develop an early onset form of AD, have permitted the expression of these mutations to investigate how AD pathology influences rodent behaviour. Numerous transgenic models, which express the human gene at artificially high levels, have been developed over the last decade with many of them showing age-dependent and independent cognitive (e.g., memory) and non-cognitive (motoric, emotion-related) deficits (Lalonde, Fukuchi, and Strazielle 2012).

This chapter describes the longitudinal assessment of emotional and cognitive domains of the APP^{-NL-F} mouse model, which uses a knock-in method and expresses mutated human APP at endogenous levels. The experiments were designed with the aim of assessing whether the knock-in mice show age-dependent changes in behaviour compared to wild-type cage mates. Open Field and Elevated Plus Maze tests were used to assess locomotor and anxiety related phenotypes. Cognitive deficits were investigated using a battery of object recognition tasks. Manipulating the parameters of each of these tasks dissociated aspects of memory and permitted mapping of the pattern of performance in separate tasks to the functional efficiency of different brain regions and networks (Barker et al. 2007, Barker and Warburton 2011).

The data presented in this chapter describe deficits in associative recognition memory performance in APP^{-NL-F} mice that starts at 16-17 months of age, while memory for object novelty remains intact. These results are discussed in the context of previous (transgenic) mouse models and our current understanding of the neural basis of associative recognition memory.

4.2 Chapter Introduction

Object recognition paradigms were first described by Ennaceur and Delacour in 1988 as a spontaneous test of memory in rodents that utilised their innate tendency to explore objects with a bias towards those that were unfamiliar (Ennaceur and Delacour 1988). The report highlighted the benefit of not requiring specific training and positive/negative reinforcement used in contemporary memory tests to encourage engagement (Barnes 1979, Aggleton 1985). Furthermore, the increased stress associated

with these tests can impact memory performance, particularly in the Morris water maze, in which mice are forced to swim in cold water (Kim and Diamond 2002, Aguilar-Valles et al. 2005). The protocol described by Ennaceur and Delacour involved a rat exploring two identical objects in an arena for a 3 or 5 minute "sample trial". After a "delay" interval up to 24 hours, the animal returned to the arena to find one object remaining from the sample trial alongside a "novel" object that it had never seen before. The authors measured exploration of each object by recording the time elapsed while rat's nose was directed towards the object within a radius of 2 cm. They reported that rats exhibited an inherent proclivity to explore the novel object, and that this ability to discriminate was sensitive to the amount of exploration time allowed in the sample phase, and the length of the delay interval prior to the test phase. This basic observation has been replicated substantially in the literature in both rats and mice.

The versatility of the object recognition paradigm manifests in its ability to test multiple aspects of memory including encoding, retrieval and retention, by manipulating the parameters of the sample phase as well as different properties of objects. It has been employed in various applications of psychological, pharmacological and anatomical research (Lueptow 2017). Lesion studies have identified critical roles played by specific neural systems in memory for distinct spatial and temporal properties of objects (Warburton and Brown 2010). Indeed, recent research has informed a previously controversial literature regarding the role of the hippocampus (Barker and Warburton 2011). While some studies reported significant deficits in object recognition following hippocampal lesions; (Clark, Zola, and Squire 2000, Broadbent et al. 2010) there is greater evidence that performance is not affected (Good et al. 2007, Winters et al. 2004, Warburton and Brown 2015, Mumby et al. 2002, Forwood, Winters, and Bussey 2005, Langston and Wood 2010). In contrast, the discrimination of novel and familiar objects is severely impaired after lesions to the perirhinal cortex (Mumby and Pinel 1994, Bussey, Muir, and Aggleton 1999, Ennaceur, Neave, and Aggleton 1996). A 2013 review by Warburton *et al* indicated unpublished results revealing that involvement of the hippocampus in object novelty recognition depends on whether the objects in the acquisition phase are identical or distinct (Warburton, Barker, and Brown 2013). Furthermore, there is evidence that NMDA receptors in the hippocampus contribute to task performance when delays of >3 hours are used: ie for long term memory retrieval (Baker and Kim 2002).

In contrast to novelty/familiarity discrimination, lesions of the hippocampus disrupt memory for spatial or location properties of objects. The object location (OL) task includes the same sample phase protocol as the object novelty (ON), however instead of swapping one object for a new object, one of the objects is moved to a novel spatial location (figure 4.1). During the test phase, the rat is presented with the same two objects as the sample phase, but one is now in a location that was previously unoccupied. Control rodents will preferentially explore the object in a novel location but rats with hippocampal lesions fail to show this pattern of exploration of the hippocampus (HPC). In contrast rats with perirhinal cortex (PRC) show a normal pattern of preferentially exploring the object in a new location (Barker and Warburton 2011). There are also recognition tasks that require intact function of both the PRC & HPC. The object-in-place task (OiP) requires the ability to form an association of a specific object with a spatial location. As both object and spatial information is encoded, it is not surprising that the PRC & HPC and their interaction is vital (as illustrated by cross hemisphere lesions technique) (Barker and Warburton 2011, Hannesson et al. 2004). Another neuroanatomical region that has a role in memory of object-place associations is the medial prefrontal cortex (mPFC), lesions of which abolish object-in-place and temporal order memory performance (Barker et al. 2007, Barker et al. 2017, Bachevalier and Nemanic 2008).

The final variation of object recognition described here is the temporal order task (TO). Episodic memory involves representations of temporal information, and Alzheimer patients have a decreased ability to remember the sequential order of words, objects and places (Bellassen et al. 2012). Temporal order memory has also been shown to depend on hippocampal function in both rodents and humans (DeCoteau and Kesner 2000, Barker and Warburton 2011, Kumaran and Maguire 2006). During the task described in the current chapter, mice were exposed to two different pairs of identical objects, with a 1-hour separation. In order to ensure that the task was not confounded by the relative strengths of memory for different objects, a long (3-hour) delay was used between the second exposure (sample phase B) and the test phase, in which the animal was presented with one object from each sample phase. Healthy control animals preferentially explore the object encountered in the first sample phase (i.e. the object least recently presented). This task also requires intact function of the PRC, HPC & mPFC, as well as the network through which the regions communicate (Mitchell and Laiacona 1998, Chiba, Kesner, and Reynolds 1994, Fortin, Agster, and Eichenbaum 2002).

Disconnection studies by Barker and colleagues (2007 & 2011) demonstrated that interaction between the mPFR, PRC and HPC is essential for the associative recognition tasks (OiP & TO). While single bilateral lesions of either the PRC or HPC disrupted ON and OL tasks respectively, recognition remained intact after ipsilateral lesions of both regions in one hemisphere, showing that subjects were capable of performing the tasks with one intact hemisphere. Performance in both ON & OL tasks was also unimpaired after bilateral mPFC lesions. However, bilateral lesions of any of the 3 brain regions disrupted discrimination in the OiP and TO tasks. Intriguingly, while ipsilateral lesions of any combination of two out of the 3 regions did not impair associative recognition, any unilateral lesions of 2 different regions in contralateral hemispheres disrupted them. For example, lesions of the PRC in one hemisphere and the HPC in the other. The effect of different lesions and their combinations is summarised in table 4.1. The data from the two studies demonstrates that associative recognition memory is underpinned by a PRC-HPC-mPFC circuit, so that intact connections between each region are vital for encoding of a multi-dimensional stimulus of what-where or what-when. This information is particularly relevant to mouse models of Alzheimer's Disease, which often don't exhibit neurodegeneration, because any pattern of deficits observed in object recognition paradigms may be due to a subtle disruption of the neural networks.

Left Hemisphere Lesions	**Right Hemisphere Lesions**	**Intact Recognition**	**Disrupted Recognition**
PRC	PRC	OL	ON, OiP, TO
HPC	HPC	ON	OL, OiP, TO
mPFC	mPFC	ON, OL	OiP, TO
PRC & HPC PRC & mPFC HPC & mPFC	None	ON, OL OiP, TO	None
PRC PRC HPC	HPC mPFC mPFC	ON OL	OiP TO

Table 4.1: The effects of distinct combinations of lesions on performance in multiple object recognition tasks. Summary of the studies by Barker and colleagues (2008, 2011). PRC (perirhinal cortex); HPC (hippocampus); mPFC (medial prefrontal cortex); OL (object location); ON (object novelty); TO (temporal order); OiP (object-in-place).

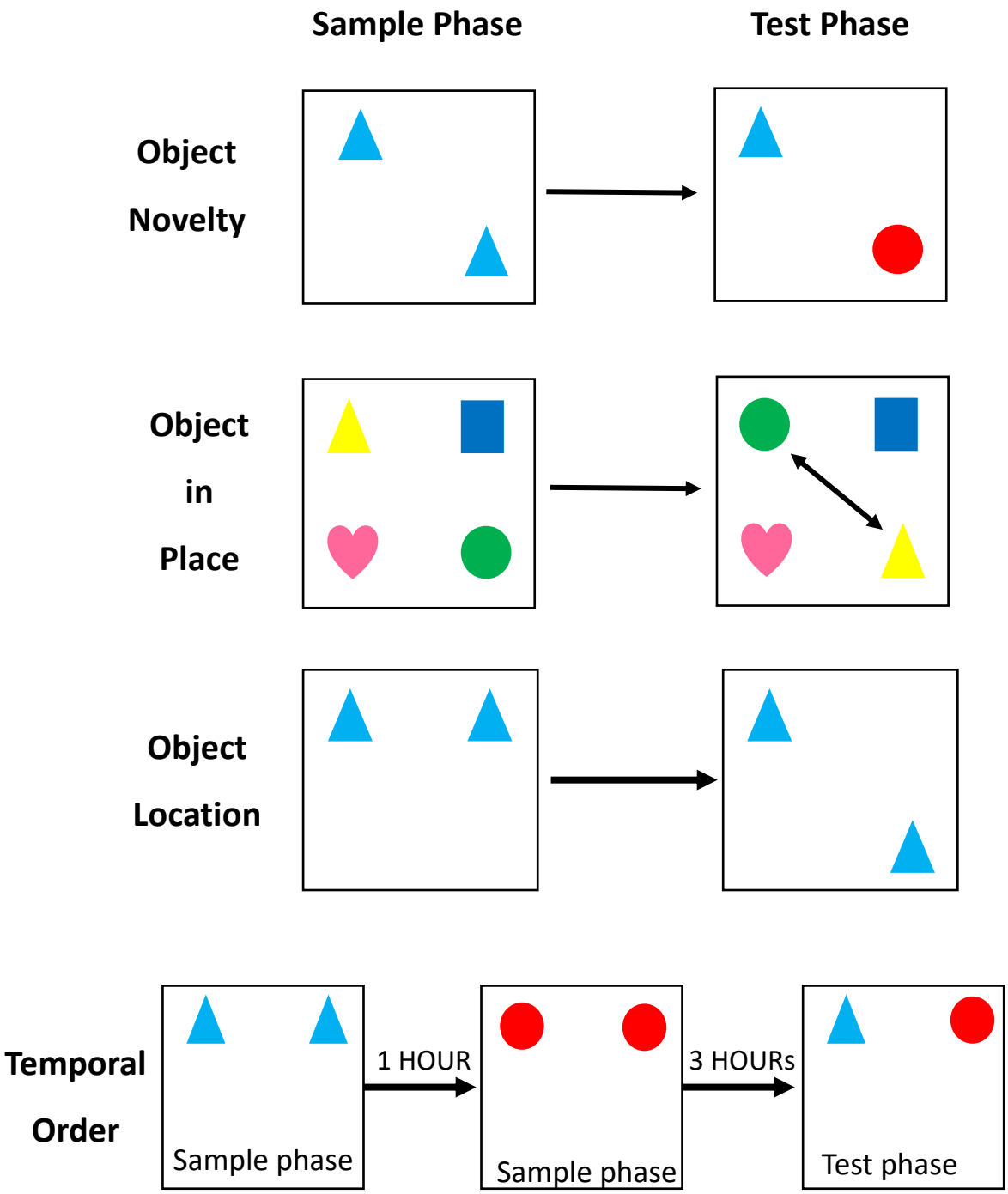


Figure 4.1: Diagrams depicting the object recognition protocols used in this thesis to test different aspects of memory.

Object Memory in Rodent Models of Amyloid Pathology

Transgenic mouse models that overexpress human amyloid precursor protein containing mutations found in familial AD have been used to study the cognitive effects of amyloid accumulation for over 20 years (Hsiao et al. 1996, Games et al. 1995). These models, such as the Tg2576 and PDAPP have consistently exhibited a phenotype involving age-dependent spatial memory deficits that coincide with amyloid deposition, often discovered in the Morris water maze (Chen et al. 2000, Westerman et al. 2002). Deficits in processing of spatial information have also been reported in object recognition protocols, with transgenic mice failing to discriminate novel object-place associations in the OiP task, while simultaneously demonstrating intact object novelty memory (Hsiao et al. 1996, Hale and Good 2005, Good and Hale 2007, Evans et al. 2018). The studies by Hale & Good also reported that their aged Tg2576 colony successfully identified when objects were presented in previously unoccupied locations (OL task). However, the mice failed to recognise when an object had moved to a position previously occupied by a different object (OiP task). This is strong evidence that the mice are able to encode a representation of both object and spatial information, but not combine the two dimensions into unified (or configural) association of a specific object in a specific location (Good and Hale 2007). Analysing this pattern of deficit within the context of the previously discussed studies by Barker *et al* would suggest that in aged Tg2576 mice the perirhinal cortex and hippocampus are able to carry out some functions associated with object processing, but the accumulation of amyloid disrupts the ability of the perirhinal-hippocampal-medial prefrontal cortex circuit to integrate information about object spatio-temporal attributes. There is evidence of Aβ42 accumulation in the mPFC in 6-month-old Tg2576, correlating to an age dependent deficit in reversal learning (Zhuo et al. 2008). Pathology in this brain region may contribute to impaired performance on the OiP task.

Pathology within the entorhinal cortex (EC) may also be relevant, as firstly this is one of the earliest affected brain regions in AD patients (Harris et al. 2010). Secondly, Aβ accumulation has been reported in the EC of both Tg2576 and PDAPP models (Xu, Fitzgerald, et al. 2015, Reilly et al. 2003). Thirdly, selective overexpression of APP in the EC was shown to induce spatial learning and memory deficits in mice (Harris et al. 2010). Fourthly, there is evidence from lesion studies that this structure is required for integration of associative recognition memory (object-place, object-context, object-place-context), but not non-associative tasks (ON, OL) (Wilson et al. 2013, Bannerman et al. 2001). Thus, the

evidence from the transgenic rodent models of amyloid pathology support the suggestion that the functional integrity of medial temporal structures and frontal lobe structures are sensitive to amyloid accumulation.

There is currently no substantial literature concerning the *APP*$^{-NL-F}$ knock-in mouse model performing object recognition tasks. A review of the current evidence of a cognitive phenotype in this and the *APP*$^{-NL-G-F}$ knock in models is covered in section 1.5.4 of this thesis, but studies relevant to object recognition tests will be highlighted here. It is worth noting that these studies often report inconsistent habituation and test protocols, which is important because sampling time and delay duration are known to affect performance in object recognition tasks (Ennaceur and Delacour 1988, Antunes and Biala 2012). To date, there is only one published study in which object recognition is examined in the *APP*$^{-NL-F}$ mouse: Izumi and colleagues reported that while female *APP*$^{-NL-F}$ mice explored novel objects significantly more than familiar at 9 months of age but not at 12 (Izumi et al. 2018). Their protocol involved a 24-hour delay between sampling and testing, which suggests that long-term memory (LTM) for object familiarity/novelty discriminations is impaired in 12-month old *APP*$^{-NL-F}$ mice. The neural systems that support long- (as opposed to short) -term object novelty memory remains in dispute with some evidence suggesting LTM may require hippocampal NMDA receptors (Baker and Kim 2002). The question as to whether *APP*$^{-NL-F}$ mice display changes in memory for the spatial properties of objects at the same or earlier timepoint remains unclear.

There are two published studies testing object recognition in the *APP*$^{-NL-G-F}$ mouse, in which the knocked-in Aβ sequence also contains the arctic mutation causing increased aggregation of peptides and cognitive deficits at an earlier timepoint. Whyte and colleagues (2018) reported a non-significant reduction in the discrimination by *APP*$^{-NL-G-F}$ mice compared to controls when tested at 6 months of age. Mehla *et al* (2019) also described no statistical difference at 6 months, however when tested at 9 and 12 months of age, the *APP*$^{-NL-G-F}$ mice had developed a deficit in their ability to discriminate novel from familiar objects (Mehla et al. 2019).

Non-Cognitive Phenotypes of Amyloid models

Differences in non-cognitive behaviours such as anxiety or locomotive activity may confound analysis of performance if they alter the engagement of one group with the task. While the cognitive symptoms of Alzheimer Disease are well known, the majority of patients also suffer from emotional changes that include depression, aggression and anxiety

(Craig et al. 2005, Shin et al. 2005). Patients can often exhibit seemingly opposite symptoms, for example either apathy or agitation, and either increased anxiety or disinhibition (Chung and Cummings 2000). This makes predictions of murine phenotypes difficult: individual mice may mirror the variability in human patients and become either hypo- or hyperactive due to apathy or agitation, even showing opposite behaviour in different arenas, with similar potential in anxiety tests. Indeed, tests for locomotor activity and anxiety behaviour are highly variable even between studies using the same mouse model, making it impossible to draw overarching conclusions concerning the general phenotype of AD mice (Lalonde, Fukuchi, and Strazielle 2012). It was important to establish any differences in anxiety or locomotive behaviours in this specific cohort of mice because they may confound results of cognitive tasks by impacting on the animal's propensity to interact with objects (Middei et al. 2006). If one genotype exhibits increased or decreased contact with objects in the sample phase, it may introduce a sampling bias whereby one group has had greater opportunity to encode an object.

Investigation of anxiety and locomotor behaviours are frequently assessed using the Elevated Plus Maze and Open Field tests, which are two pieces of apparatus that allow rodents to express their natural exploratory activity. The Open Field is a simple square arena, while the EPM is a raised platform with four arms arranged in a cross. Two arms have high black walls and two are open to their surroundings. Both arenas include darker regions (the perimeter of the open field and the "closed" arms of the EPM, and normal animals will exhibit a preference to remain here rather than venture out into the open regions (Rodgers and Cole 1993). Measuring the amount of time each animal spends in these open regions allows a comparison of anxiety-like behaviour between groups. More anxious animals will spend less time in the open areas and vice versa for less anxious animals. Measurement of the total distance travelled by each animal allows comparison of locomotive activity.

The literature surrounding non-cognitive phenotypes in Alzheimer mouse models is controversial. The most regularly tested transgenic model for locomotive activity is the Tg2576 mouse, which expresses the Swedish mutation. While there are some studies showing no effect, the prevailing result describes a hyperactive phenotype, potentially due to increased neuroinflammation evidenced by the increased activity being rescued by Ibuprofen treatment (Deacon et al. 2009, Lim et al. 2001). Anxiety results are similarly controversial: a review by Lalonde *et al* counted reports of reduced anxiety in 8 AD mouse

models out of the 12 that displayed sensitivity to the test.(Lalonde, Fukuchi, and Strazielle 2012) The age at testing as well as various other environmental and methodological confounds offer potential reasons why comparisons of non-cognitive domains are so variable.

While it is necessary to acknowledge the high level of variability between studies of anxiety phenotypes in AD models, it would be remiss not to briefly describe the current literature concerning the *APP*$^{-NL-F}$ and *APP*$^{-NL-G-F}$ knock-in models. Three studies have reported increased exploration of the EPM open arms by the *APP*$^{-NL-G-F}$ knock-in mouse, detected from as young as three months of age (Latif-Hernandez et al. 2019, Sakakibara et al. 2018, Pervolaraki et al. 2019). This indicates that reduced anxiety phenotypes reported in transgenic mice were not artefacts of APP overexpression. While the phenotype present in the *APP*$^{-NL-G-F}$ mice was not shown to be age dependent, this may have been due to the speed at which amyloid accumulates in this model and the lack of testing at younger age points. Sakakibara *et al* reported increased effect sizes when *APP*$^{-NL-G-F}$ mice were assessed at 15-18 months of age, but performance at the two age points were not statistically compared. The paradoxical, opposing, arena-dependent effects on anxiety were evident in the study by Pervolaraki and colleagues, which reported reduced anxiety of the *APP*$^{-NL-G-F}$ mice in the EPM, but increased anxiety when examined in the Open Field. Currently, there are no reports of altered anxiety or locomotor phenotypes in the *APP*$^{-NL-F}$ mouse, but this is potentially due to mice not being tested beyond 12 months of age (Izumi et al. 2018). Tests of locomotive activity in the *APP*$^{-NL-G-F}$ is again varied, with one study showing hypoactivity at 6 months; (Whyte et al. 2018) while two others found no difference compared to control mice up to 12 months of age (Latif-Hernandez et al. 2019, Mehla et al. 2019).

The aim of this chapter is to first characterise any non-cognitive phenotypes in the *APP*$^{-NL-F}$ mouse model compared to WT littermates and then memory performance through a battery of object recognition-based tasks. Individual aims and hypotheses are presented during introductions to the relevant experiments.

4.3 Experiment 3: Anxiety & Locomotive Assessment

▪ 4.3.1 – Introduction

Assessments of anxiety and locomotor behaviour were undertaken at two age points with the aim of characterising any phenotype of the *APP*$^{-NL-F}$ knock-in mutations as well as any age dependent effects. Identifying any differences in performance would become important during analysis of cognitive test results. The hypothesis for this experiment was that the knock-in mice may develop anxiolytic behaviour when aged, spending more time exploring the open arms of the EPM. This prediction was derived from evidence that the more aggressive pathology in the *APP*$^{-NL-G-F}$ model generated reduced anxiety as young as three months of age. The lack of phenotype currently identified in *APP*$^{-NL-F}$ literature may be due to the fact they have not been tested at >12 months of age. Therefore, we hypothesised that at 16 months old, amyloid pathology would have developed to a level similar to that found in young *APP*$^{-NL-G-F}$ mice, and we would observe a decrease in anxiety behaviours. We did not expect to see differences in locomotor behaviour because no robust phenotype has been described in either the *APP*$^{-NL-F}$ or *APP*$^{-NL-G-F}$.

▪ 4.3.2 – Experiment 3 Methods

Subjects

This experiment involved male *APP*$^{-NL-F}$ and WT mice who were tested twice: at both 6-8 and 16-17 months old. The total number of mice in each group was 32 (WT) and 38 (*APP*$^{-NL-F}$). These mice underwent extensive handling and testing in other behavioural paradigms following the first time-point measured in these experiments.

Apparatus

The elevated plus maze consisted of a white floored platform 75 cm above the ground with four arms arranged in a cross (figure 4.2). Arms were 40 cm long and 7 cm wide, and were symmetrical in that those opposite one another were of the same design. Two arms were “open” meaning they had 1cm high walls allowing mice to see over them to the room and floor. The other arms were “closed”, they had 15 cm high black walls restricting any view. The open field arena and procedure was described in Chapter 2 as it is relevant to multiple chapters in this thesis.

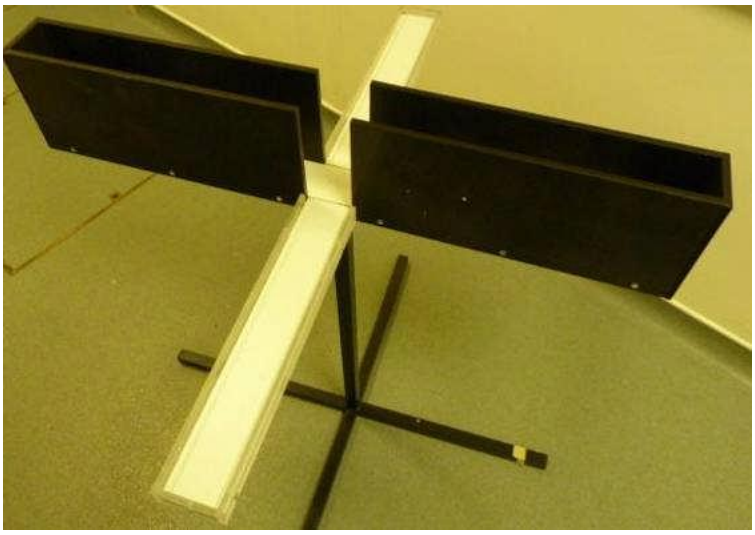


Figure 4.2: Photo of the elevated plus maze apparatus. The EPM was a raised platform with two open arms and 2 closed arms. Normal mice will spend a greater amount of time in the closed arms.

Procedure

The maze was thoroughly cleaned with 70% ethanol wipes before testing each mouse, to remove any odour cues. Mice were picked up in a cardboard tube from their home cage and placed in the centre of the maze, facing the same open arm each time. They were allowed to freely explore for 5 minutes, recorded by a camera overhead connected to a laptop. Recordings of each mouse's activity were taken and tracked by EthoVision XT 13 software. An "arena" was drawn around the maze in the videos, identifying the open & closed arms as distinct zones. Entry into the open arms was defined as all three body points of nose, centre and tail base being in the open arm zone. An exploration ratio was calculated for each mouse as the amount of time spent in the open arms divided by the total time in the maze (300s).

4.3.3 – Experiment 3 Results

Elevated Plus Maze

Performance of *APP*$^{NL-F}$ & WT mice in the elevated plus maze (EPM) was analysed by calculation of the time spent in the open arms as a ratio of total time in the maze. The data is displayed in figure 4.3 A. Visual inspection suggested that there was a small increase in open arm exploration by the *APP*$^{NL-F}$ mice compared to the WT mice. Furthermore, exploration appeared to decrease with age in both groups. However, these impressions were not confirmed by statistical analysis. A 2-way mixed ANOVA was performed with age as a within subjects' factor and genotype as the between-subject factor. There was no significant main effect of age $F(1,68) = 3.65$, $p=0.06$; no main effect of genotype $F(1,68) = 0.959$, $p=331$ and no age*genotype interaction $F(1,68) = 0.002$, $p=0.965$.

The total distance moved by each mouse was calculated as a measure of general locomotive activity in the apparatus, Figure 4.3 B. Data were analysed by a 2-way mixed measures ANOVA, which did not reveal significant main effects of age $F(1,68) = 0.099$, $p=0.754$; or genotype $F(1,68) = 0.452$, $p=0.504$; and there was no age*genotype interaction $F(1,68) = 0.055$, $p=0.816$.

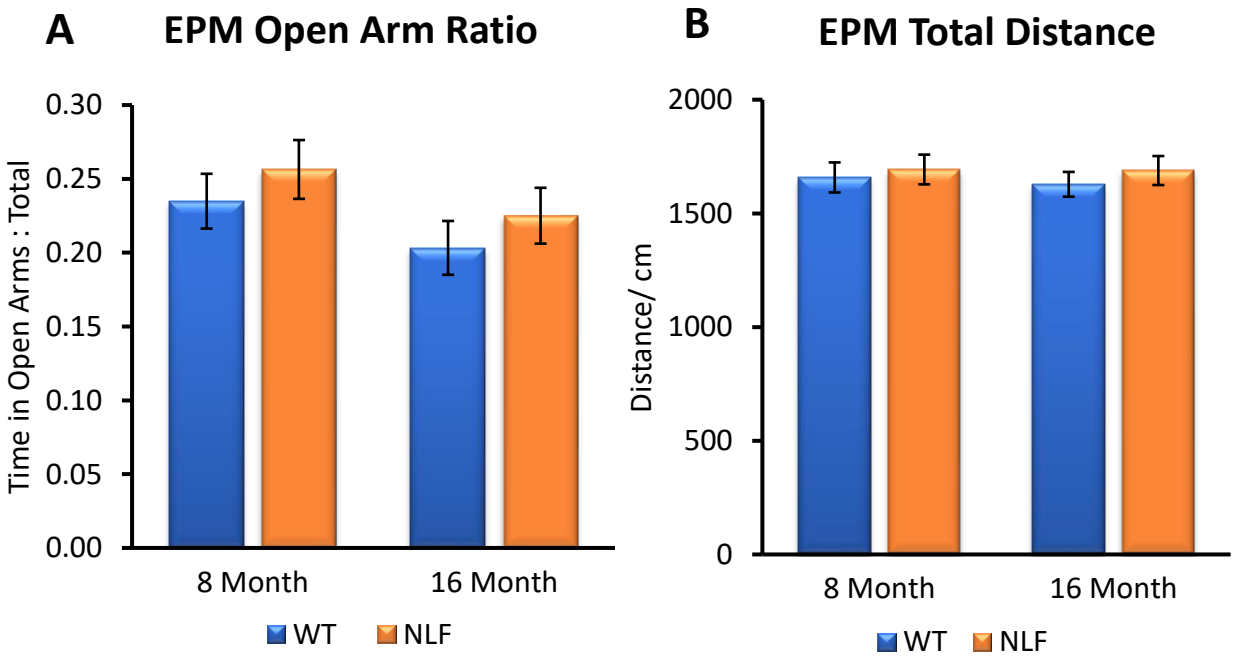


Figure 4.3: Elevated plus maze results for *APP*$^{NL-F}$ and WT mice.
(A) The ratio of time spent in the open arms as a ratio of the total time spent in the maze. (B) Mean distance travelled by the mice throughout exploration of the EPM. Error bars show the standard error of the mean (SEM). WT n=32, *APP*$^{NL-F}$ n=38

Open Field

The Open Field test was used to compare general locomotive activity in the WT and *APP*$^{NL-F}$ mice, as well as for a secondary assessment of anxiety related behaviour. The mean total distance travelled by the mice in each group is presented in figure 4.4 A. The Shapiro-Wilke test identified violations of distributional normality. The data was therefore transformed by taking the inverse of each score, to remedy the violations. It was then analysed by a 2-way mixed measures ANOVA with age and genotype as factors. The ANOVA revealed a significant main effect of age $F(1,68) = 29.1$, $p<0.0005$, as both WT & *APP*$^{NL-F}$ mice showed lower locomotive activity at the older time point. There was no main effect of genotype $F(1,68) = 0.236$, $p=0.629$; and no age*genotype interaction $F(1,68) = 0.886$, $p<0.350$.

The central 30 x 30 cm square of the arena was described as the "inner zone". The amount of time each mouse spent within this zone was recorded and expressed as a ratio of total exploration time. The results are displayed in figure 4.4 B. Some of the datasets were not normally distributed (S-W $p<0.05$), so were transformed by square root transformation. The data was analysed by a mixed measures ANOVA with age as a within subjects' factor and genotype as the between subjects' factor. There was a significant main effect of age $F(1,68) = 4.4$, $p=0.039$, but no main effect of genotype $F(1,68) = 0.061$, $p=0.806$; or age*genotype interaction $F(1,68) = 0.961$, $p<0.330$.

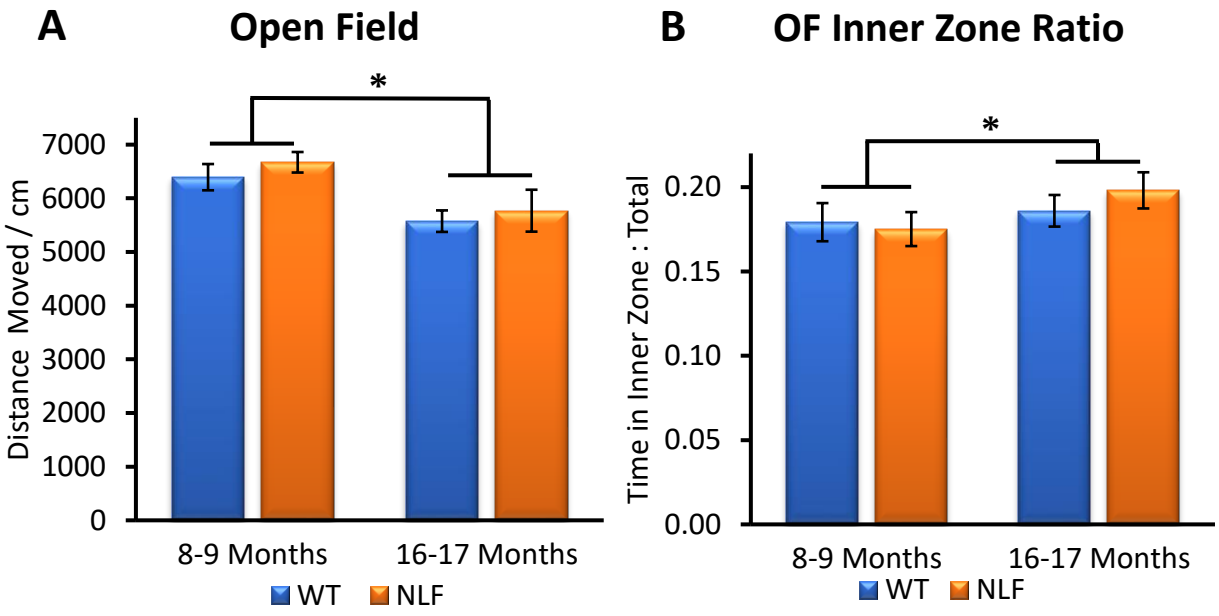


Figure 4.4: Open field (OF) results for *APP*$^{NL-F}$ and WT mice. (A) Total distance moved by each genotype at two age-points. (C) Ratio of time spent in the inner zone out of total time in the arena. Error bars show the standard error of the mean. *p<0.05, mixed measures ANOVA. WT n=32, *APP*$^{NL-F}$ n=38.

4.3.4 – Experiment 3 Discussion

The aim of this experiment was to determine if *APP*$^{-NL-F}$ mice show anxiety/locomotor changes that may interact with age and thus amyloid pathology. The knock-in mice were tested in the Elevated Plus Maze and Open Field and compared to Wild-Type littermates at two age points. This was the first study to assess these behaviours in the *APP*$^{-NL-F}$ model at >12 months of age. The results revealed no effect of genotype at either 7-8 or 16-17 months on preference for the closed vs open arms of the plus maze or locomotive activity. Comparisons with other *APP*$^{-NL-F}$ studies are limited, because they did not test the mice any older than 12 months. However, it is worth noting that Pervolaraki *et al* (2019) observed no effects in the EPM or Open Field at 8 months, and that their results correspond with those reported here. Izumi *et al* (2018) similarly reported no locomotor phenotype in the Open Field. The hypothesis that anxiolytic behaviour may develop with age did not come to fruition indicating that even at 17 months old, the amyloid pathology in the *APP*$^{-NL-F}$ model was not great enough to induce changes in this behaviour. This presents further evidence that this model has a milder phenotype than the triple knock-in *APP*$^{-NL-G-F}$ mouse, which has had anxiolytic behaviour reported from as young as 3 months of age.(Latif-Hernandez et al. 2017, Sakakibara et al. 2018, Pervolaraki et al. 2019) As this mouse has a more aggressive pathology and phenotype, it remains to be seen whether the *APP*$^{-NL-F}$ mice would ever go on to develop a measurably reduced anxiety with more severe amyloidosis, or whether the reported phenotype is specific to the *APP*$^{-NL-G-F}$ mice.

Effects of age were more apparent in these experiments, and highlighted an interesting dichotomy for the effect of age on exploration of the two anxiogenic zones in the separate apparatus. Aged mice in the EPM showed a trend for lower exploration of open arms, but were significantly more likely to enter the inner zone of the open field. This phenomenon of seemingly opposite effects is not uncommon, in fact has recently been described by one study of the *APP*$^{-NL-G-F}$ mice.(Pervolaraki et al. 2019) There are several potential explanations for the difference in results in this study, particularly the fact that the mice receive greater exposure to the open field arena. Following the open field test at 8 months, the mice undertook extensive cognitive tests in the same arena. However, the EPM is only encountered in a single trial. The extra habituation to the open field arena while at 8-10 months could explain the lowered anxiety exhibited by the mice independent of genotype when tested at 16 months. Furthermore, exploration in the EPM has been shown to fall during repeated exposure.(Bertoglio and Carobrez 2000, Carobrez and

Bertoglio 2005) Similarly, there was a significant drop in locomotor activity in the Open Field due to age, across both genotypes. This was to be expected, as C57Bl/6 mice are known to exhibit reduced activity over the course of their lifespan (Dean et al. 1981).

In summary, these experiments did not reveal any differences between the genotypes in terms of their anxiety or locomotive behaviours and there also were no changes in their responses to age. There was a general decrease in activity when the mice were tested at the aged timepoint which is common, especially after factoring in the effect of repeated testing. The lack of genotype effects will ensure that any differences in cognitive performance cannot be attributed to variations in the behaviours tested in this experiment.

4.4 Experiment 4: Object Novelty Memory in *APP*$^{-NL-F}$ Mice

▪ 4.4.1 – Introduction

The dearth of studies of object recognition in the *APP*$^{-NL-F}$ knock-in mouse highlights the need for a further systematic investigation. The current study used different object memory tasks to assess the age-of-onset and nature of cognitive changes in *APP*$^{-NL-F}$ mice. The aim of experiments 5 to 9 was to test the *APP*$^{-NL-F}$ mice on a battery of object memory tasks designed to interrogate different attributes of object memory, in order to identify which phenotypes and thus putative neural systems were sensitive to beta-amyloid aggregation in *APP*$^{-NL-F}$ mice. Four object arrays were used for both the ON and OiP tasks, to ensure that they were of comparable complexity. This information will provide assessment of changes in medial temporal lobe-frontal networks. Due to the admittedly limited evidence, the hypothesis was that the *APP*$^{-NL-F}$ mice would exhibit an age-related decline in memory for object identity. The aim of these experiments was to broaden the understanding of recognition processes in this model by assessing whether hippocampal dependent associative memory shows a similar pattern of decline.

▪ 4.4.2 – Methods

Subjects & Design

Male *APP*$^{-NL-F}$ (N=16) mice were tested and compared to male WT (N=16) cage mates at 8-10 and 16-17 months of age in experiments 5 and 6. The apparatus, habituation protocol and scoring method for the object recognition tasks is described in Chapter 2.

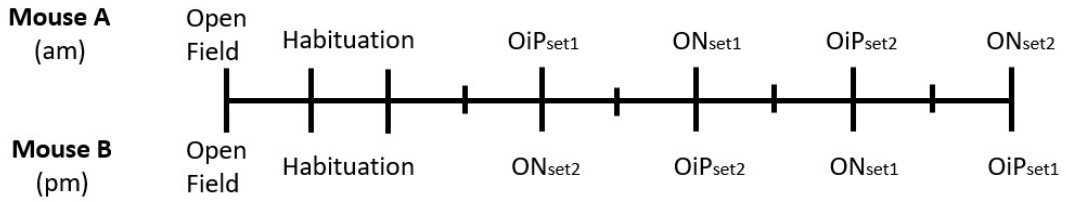


Figure 4.5: Experimental design of the counterbalanced object novelty (ON) and object-in-place (OiP) tests. Each vertical line represents a new day. The truncated lines are rest days. Each mouse performed this protocol at 8-9 and 16-17 months-of-age.

The experiments were designed to reduce impact of variables such as task order, time of day, object set and the identity/location of the novel object, by counterbalancing between and within the two periods of testing. For example, the mice were split into two groups which received object sets 1 & 2 at 8-10 months and sets 3 & 4 at 16-17 months, or vice versa. The order of ON and OiP tests performed, as well as the object sets, were counterbalanced within both groups. Figure 4.5 is a schematic depicting a representative protocol for two non-paired mice within the same group.

Procedure

The ON and OiP protocols were described in Chapter 2 of this thesis, with a note that the object array varies between experiments. Both tasks used a four-object array here. Briefly, the protocols involved 3 sample phases of 10-minutes duration each with a 5-minute delay interval. Another 5-minute delay preceded the test phase, in which two objects either had their location switched (OiP) or were replaced with two novel objects (ON). A cartoon depicting the OiP and 2-object ON protocols is shown in figure 4.1. The object novelty test in this experiment uses 4 different objects in the acquisition phase and two new different objects are added for the test phase (figure 4.6).

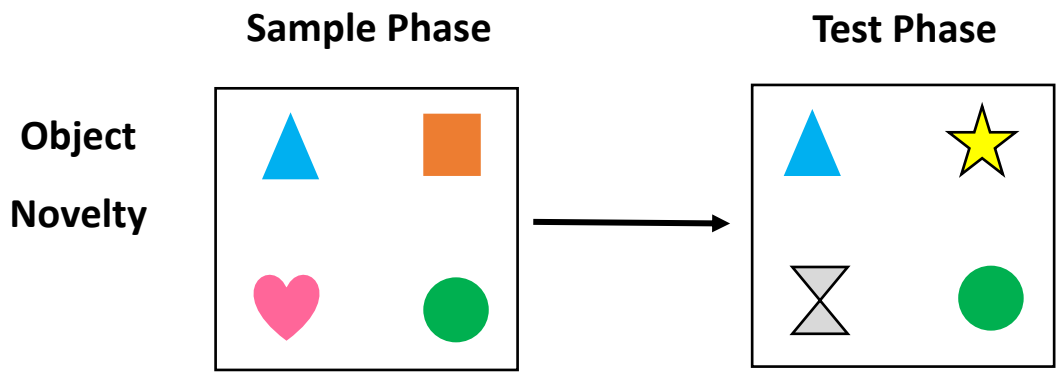


Figure 4.6: Cartoon depicting the object-novelty protocol using four-object array.

Statistical Analysis

Discrimination Ratios (DRs) were calculated via the equation below. All statistical testing was performed with SPSS and is described in Chapter 2.

$$\frac{\textbf{Total time spent exploring "Novel" object}}{\textbf{Total time spent exploring all (Novel + Familiar) objects}} = \textbf{DR}$$

▪ 4.4.3 – Experiment 4 Results

Sample Phase Contact Times

The total interaction time with all four objects throughout the sample phases at 8-9 and 16-17 months is shown in table 4.2. The Shapiro-Wilke test revealed that all datasets were normally distributed, while Levene's test confirmed that there was homogeneity of variances ($p>0.05$). Mauchley's test of sphericity reported that the assumption of sphericity was met for the sample phase factor ($p>0.05$). The data were analysed by three-way repeated measures ANOVA, with age, sample phase and genotype as factors.

The ANOVA revealed a significant main effect of age $F(1,30) = 8.9$, $p=0.006$, and a main effect of phase $F(2,60) = 18.1$, $p<0.0005$. There was no main effect of genotype $F(1,30) = 0.092$, $p=0.763$. There was a significant age*phase interaction $F(2,60) = 4.927$, $p=0.01$. No other interactions or main effects were significant: age*genotype $F(1,30) = 0.24$, $p=0.629$; phase*genotype $F(2,60) = 0.522$, $p=0.596$; age*phase*genotype $F(2,60) = 0.34$, $p=0.710$.

Tests for simple main effects within the significant age*phase interaction revealed that there was increased contact time for all mice in sample phases 1 & 2 at the older timepoint (phase 1 $p<0.0005$; phase 2 $p=0.016$), but not sample phase 3 ($p=0.548$). There was also a significant simple main effect of phase on contact time at 8-10 months between phases 1 & 3, but not between 1 & 2 or 2 & 3 (1 vs 2 $p=1$; 1 vs 3 $p=0.049$; 2 vs 3 $p=0.07$). At 16-17 months, while there was no difference in contact time between phases 1 & 2, there was a significant drop in exploration between phases 2 & 3 and 1 & 3 (1 vs 2 $p=0.493$, 1 vs 3 $p<0.0005$; 1 vs 2 $p<0.0005$).

Collectively, analysis of this data revealed that all mice spent more time interacting with objects at the older time point during sample phases 1 & 2, when collapsed over

genotype. There was also a general reduction in exploration over the course of the three sample phases, as mice habituated to the objects in the arena and provides evidence consistent with the view that WT and *APP*$^{NL-F}$ mice across all ages were able to process information about the objects.

	ON Sample Phase Contact Times				
		WT		*APP*$^{NL-F}$	
	Age/ Months	8 - 10	16 - 17	8 - 10	16 - 17
Sample Phase 1	**Mean**	**58.7**	**78.6**	**60.1**	**72.9**
	SD	19.7	20.5	15.0	18.8
Sample Phase 2	**Mean**	**55.1**	**69.2**	**58.5**	**68.9**
	SD	22.6	25.8	21.0	25.3
Sample Phase 3	**Mean**	**52.6**	**54.9**	**48.5**	**51.3**
	SD	18.1	19.0	19.6	15.6

Table 4.2: Mean contact times (seconds) for WT and *APP*$^{NL-F}$ mice in the object novelty (ON) task across two age-points. SD (standard deviation of the mean). n=16 for both groups.

Test Phase Contact Times

Figure 4.7 shows contact times with novel or familiar objects in the test phase for WT and *APP*$^{NL-F}$ mice at 8-10 and 16-17 months old. The figure shows that, at both ages, both the WT and *APP*$^{NL-F}$ mice spent more time interacting with the novel object compared to the familiar. High variability of contact times resulted in the Shapiro-Wilke test revealing violations of normality of the distributions ($p<0.05$). A Square Root transformation of the data removed the violations ($p>0.05$) and it was analysed by a repeat measures ANOVA with age, object type and genotype as factors.

The ANOVA revealed a significant main effect of object type $F(1,30) = 408$, $p<0.0005$. There was no main effect of age $F(1,30) = 0.26$, $p=0.616$; or genotype $F(1,30) = 0.414$, $p=0.525$. There was no significant interactions: age*genotype*object, $F(1,30) = 0.19$, $p=0.666$; age*genotype $F(1,30) = 2.54$, $p=0.121$; age*object $F(1,30) = 0.45$, $p=0.507$; object*genotype $F(1,30) = 0.55$, $p=0.463$.

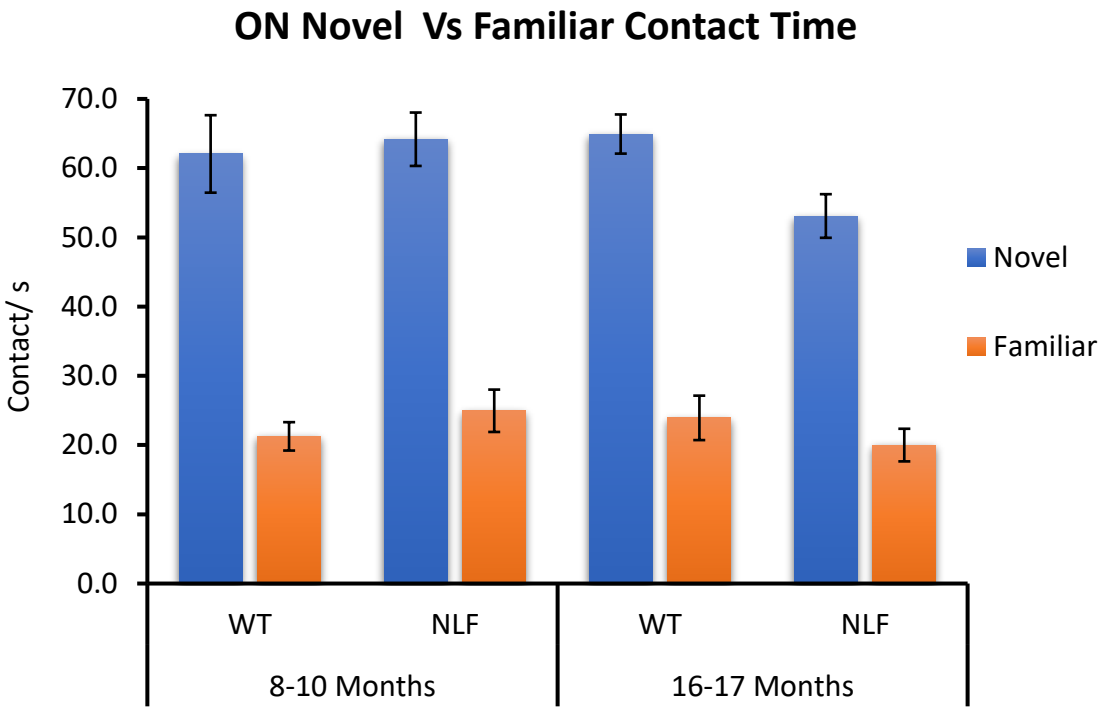


Figure 4.7: Mean contact times of WT and *APP*$^{NL-F}$ mice for familiar or novel objects in the test phase of the object novelty task. WT and *APP*$^{NL-F}$ contact time at two age points with error bars showing SEM. N=16 for all groups.

Discrimination Ratios

The interaction with the familiar or novel objects was converted into a discrimination ratio to take into account the individual variation in contact times between mice. These ratios are shown in figure 4.8. None of the datasets violated the assumptions of normal distributions (Shapiro-Wilke test $p>0.05$), or homogeneity of variances (Levene's test $p>0.05$). A mixed measures ANOVA was performed on the data with age and genotype as factors. There was no interaction between age*genotype $F(1,30) = 0.018$, $p=0.895$. There was no main effect of genotype $F(1,30) = 0.155$, $p=.697$; and no main effect of age $F(1,30) = 0.218$, $p=0.895$.

One sample t-tests performed on all four groups showed that they were all significantly above 0.5. WT at 9 months and 17 months $t(15)=12.3$ and 13.9, $p<.0005$ and *APP*$^{NL-F}$ at 9 and 17 months $t(15)=11.8$ and 17.1, $p<0.0005$, respectively. These data show that the WT and *APP*$^{NL-F}$ mice both maintain intact ability to recognise novel objects at a comparable efficiency up to 17 months of age.

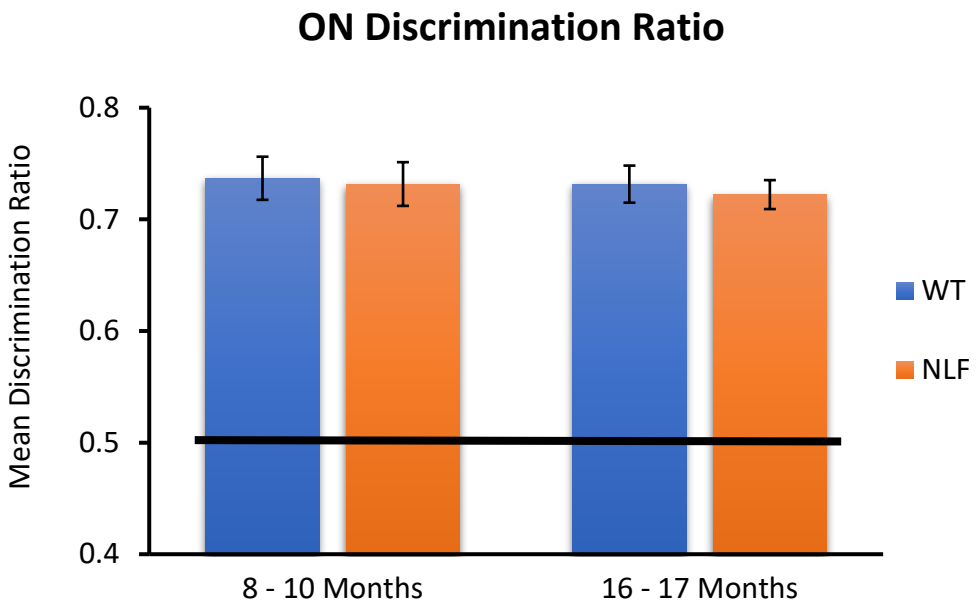


Figure 4.8: *APP*$^{NL-F}$ mice display intact object novelty recognition up to 17 months of age. Discrimination ratios (DR) for WT and *APP*$^{NL-F}$ mice in the object novelty (ON) task at two age-points. The black bar represents 0.5 or no object preference. Error bars show SEM. N=16 for all groups.

4.5 Experiment 5: Object-in-Place Memory in *APP*$^{NL-F}$ Mice

▪ 4.5.1 – Experiment 5 Results

Sample Phase Contact Times

Table 4.3 shows the average contact time with all objects through the OiP sample phases for both WT and *APP*$^{NL-F}$ mice at 8-10 & 16-17 months of age. Inspection of the data suggests there was increased interaction with objects at the older timepoint, while in all groups, contact times decreased through the sample phases. All datasets were normally distributed (S-W test $p>0.05$), so the data were analysed by a three-way repeated measures ANOVA with age, sample phase and genotype as factors.

The ANOVA displayed a significant main effect of age $F(1,30) = 16.3$, $p<0.0005$, and a main effect of phase $F(2,60) = 46.4$, $p<0.0005$. There was also a significant age*phase interaction $F(2,60) = 10.1$, $p<0.0005$. There was no main effect of genotype $F(1,30) = 0.23$, $p=0.633$. There were no further significant interactions: sample phase*genotype $F(2,60) = 0.20$, $p=0.816$; age*genotype $F(1,30) = 2.14$, $p=0.154$; age*phase*genotype $F(2,60) = 0.75$, $p=0.479$.

The significant age*phase interaction revealed a simple main effect of age: when collapsed over genotype, the amount of object exploration increased with age in sample phases 1 ($p<0.0005$) and 2 ($p=0.024$), but not 3 ($p=0.053$). There were also simple main effects of sample phase within each timepoint independent of genotype. At 8-10 months, object contact time significantly decreased between phases 1 & 3 ($p<0.0005$), but not between phases 1 & 2 ($p=0.56$) or phases 2 & 3 ($p=0.08$). At 16-17 months, there was a significant difference between exploration between all phases: 1 vs 2 $p<0.0005$; 1 vs 3 $p<0.0005$; 2 vs 3 $p=0.003$.

Altogether, the analysis of object interaction through the three sample phases of the OiP task demonstrated a significant drop in contact time from the 1st and 3rd phases when collapsed over genotype. There were no effects of genotype on contact times through the sample phases.

	OiP Sample Phase Contact Times				
		WT		*APP*$^{NL-F}$	
	Age/ Months	8 - 10	16 - 17	8 - 10	16 - 17
Sample Phase 1	**Mean**	**60.5**	**90.9**	**61.9**	**85.0**
	SD	18.5	24.3	16.0	24.2
Sample Phase 2	**Mean**	**55.7**	**76.6**	**60.5**	**64.0**
	SD	23.0	23.4	26.2	20.3
Sample Phase 3	**Mean**	**49.1**	**61.4**	**52.2**	**55.7**
	SD	12.9	19.0	21.9	13.8

Table 4.3 Mean contact times (seconds) for both WT & *APP*$^{NL-F}$ in the object in place task at the two age points. Standard deviation of the means is also shown. N=16 for all groups.

Test Phase Contact Times

The exploration of objects in either the same or different spatial locations was averaged for each group and these means are shown in figure 4.9. The table reveals that at 8-10 months, both genotypes spent more time with the objects that had switched locations. In contrast, at 17 months of age, only the WT exhibited this preference. All of the groups were normally distributed (Shapiro-Wilke test $p>0.05$), and there was homogeneity of variance (Levene's test $p>0.05$).

The data were analysed by a repeated measures ANOVA with age, genotype and object type (novel vs familiar object-place association) as factors. There was a significant age*genotype*object interaction $F(1,30) = 6.4$, $p=0.017$. There were also significant object*genotype $F(1,30) = 32.1$, $p<0.0005$, and age*object interactions $F(1,30) = 11.8$, $p=0.002$. There was no significant age*genotype interaction $F(1,30) = 2.2$, $p=0.150$. There was a significant main effect of object type $F(1,30) = 139.3$, $p<0.0005$ and no other significant main effects were reported: age $F(1,20) = 0.21$, $p=0.649$; genotype $F(1,30) = 2.82$, $p=0.103$.

Tests for simple main effects following the significant age*genotype*object type interaction revealed that both genotypes exhibited a significant preference for interaction with objects in novel locations at 9 months of age ($p<0.0005$). However, at 17 months, only the WT mice showed this difference ($p<0.0005$; APP^{-NL-F} : $p=.496$). Furthermore, aged APP^{-NL-F} mice interacted with the objects in novel spatial locations less than the aged WT mice ($p<0.0005$). The two genotypes displayed no differential exploration of objects in the familiar arrangement at this aged timepoint ($p>0.5$) and there was no effect of genotype on the amount of exploration of either pair of objects at 9 months ($p<0.5$). APP^{-NL-F} mice had significantly less interaction with objects in a novel object-place association at 17 versus 9 months ($p=0.026$). There was no significant simple main effect of age for the APP^{-NL-F} exploring familiar object-place associations ($p=0.060$), and no effect on either object category for the WT ($p=0.473$ for novel, 0.062 for familiar).

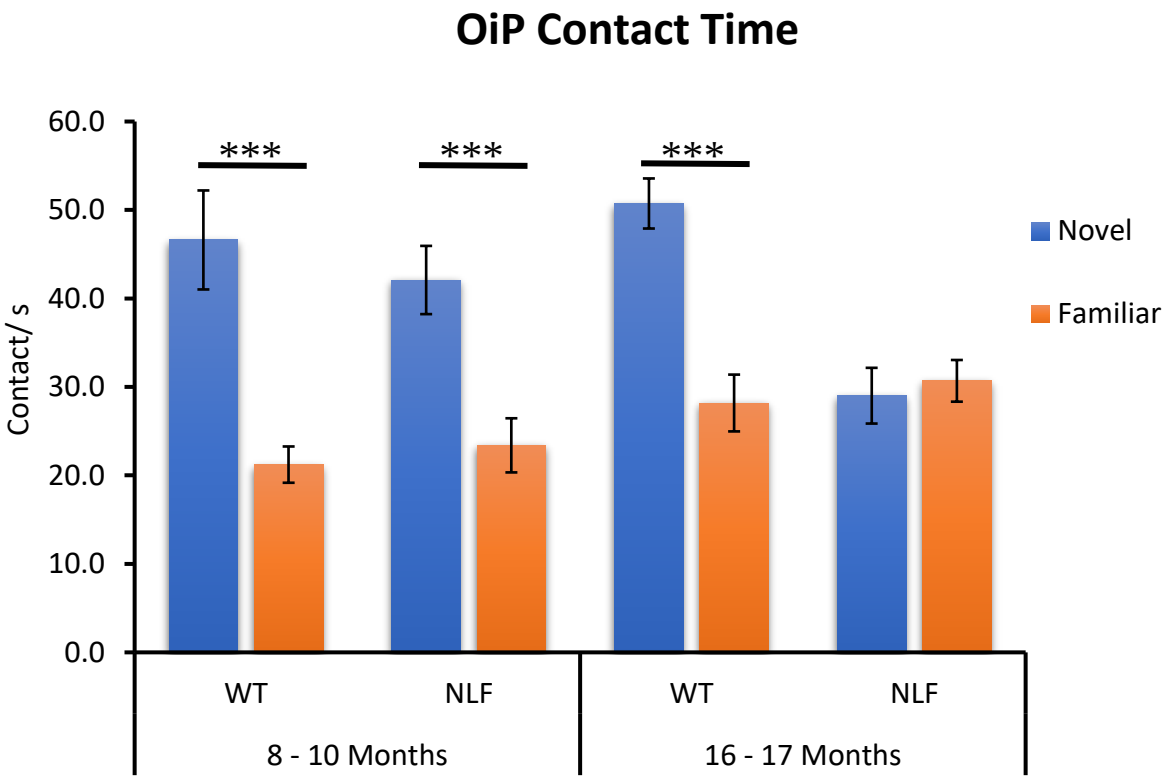


Figure 4.9: Mean contact times (seconds) of aged WT and *APP*$^{NL-F}$ mice in the object-in-place (OiP) test phase at two age-points. Contact with objects that had either remained in the same or had been switched into different positions is shown. Error bars show SEM. N=16 for all groups. ***p<0.001, mixed measured ANOVA.

Discrimination Ratios

Discrimination ratios (DRs) were calculated from each mouse's exploration of objects observed in either novel or familiar arrangements, these were then averaged for each genotype at each timepoint and are shown in figure 4.10. All four datasets had normal distributions (S-W p>0.05), and were analysed by repeated measures ANOVA. There was a significant age*genotype interaction, $F(1,30)=14.0$ $p=0.001$, a significant main effect of age $F(1,30) = 21.4$, $p<0.0005$ and a significant main effect of genotype $F(1,30) = 37.3$, $p<0.0005$.

Analysis of simple main effects revealed that, at 9 months old, there was no difference in performance of the two genotypes ($p=0.267$). However, at 17 months, WT mice showed a significantly higher preference for objects in a novel location compared to the *APP*$^{NL-F}$ group ($p<0.0005$). In addition, there was no simple main effect of age on the WT group's

discrimination ratios (p=0.534), while the performance of *APP*$^{-NL-F}$ mice significantly deteriorated with age (p<0.0005).

One-sample t-tests confirmed that at 9 months of age, both groups discriminated significantly above chance (WT: t(15)=9.6, *APP*$^{-NL-F}$: t(15)=7.6, both p<0.0005). But at 17 months old, only the WT mice discriminated above chance t(15) = 9.3, p<0.0005, *APP*$^{-NL-F}$ mice failed to discriminate t(15) = -1.381, p=0.188.

Analysis of these data shows that, at 9 months old, both genotypes exhibited preference for exploring objects in new locations compared to those objects which were presented in the same place as during the sample phases. WT mice maintained this ability at 17 months. However, the *APP*$^{-NL-F}$ mice developed an age-dependent deficit in the ability to discriminate object-place associations.

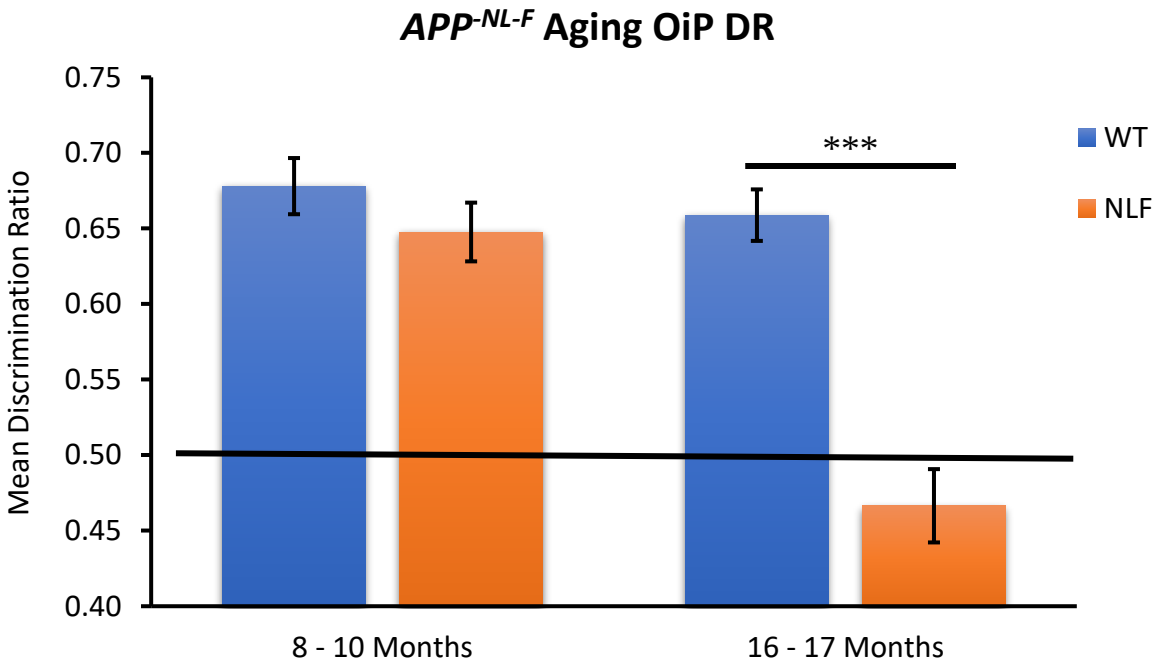


Figure 4.10: *APP*$^{-NL-F}$ mice display an age-dependent deficit in object-in-place memory. Mean discrimination ratios (DR) calculated from the contact times of WT and *APP*$^{-NL-F}$ mice with objects that had either switched spatial locations or remained in the same place during the test phase of the object-in-place (OiP) task. While there was comparable performance at 8-10 months-of-age, *APP*$^{-NL-F}$ mice showed significantly worse discrimination at 16-17 months-of-age compared to WT controls. Error bars represent SEM. N=16 for all groups. ***p<0.001, Mixed measures ANOVA.

4.6 Experiment 6: Object Location Memory in *APP*$^{-NL-F}$ Mice

▪ 4.6.1 – Introduction

Experiments 5 and 6 revealed an age-dependent deficit in memory for object place associations, while recognition of object identity remained intact. In order to characterise the specific nature of the deficit, the mice underwent further tests of associative and non-associative recognition. Considering the failure to recognise novel object-place associations, it was hypothesised that the *APP*$^{-NL-F}$ mice were demonstrating hippocampal dysfunction. Therefore, they were tested on two further hippocampal dependent tasks investigating Object Location and Temporal Order memory with the prediction that their performance in both tasks would be significantly worse than WT controls. Since the Temporal Order (TO) task required the capacity to encode object identities over a single sample session and Izumi *et al* reported an age-dependent deficit using a similar protocol, a second object novelty experiment was performed (Izumi et al. 2018). Successful performance in this task was a prerequisite before conclusions could be drawn concerning the TO results. In order to ensure that any changes detected in performance of the aged *APP*$^{-NL-F}$ mice were due to age and not genotype, a separate cohort of young mice were also assessed on these three tasks.

▪ 4.6.2 – Methods

Subjects & Design

Experiments 7a, 8a and 9a tested the same cohort of male *APP*$^{-NL-F}$ and WT cage mates as described in experiments 5 & 6. However, one WT mouse died before performing these tasks and so the group sizes were 16 (*APP*$^{-NL-F}$) and 15 (WT). Experiments 7b, 8b and 9b involved a separate cohort of *APP*$^{-NL-F}$ (n=9) and WT (n=9) mice that were 4 months of age. These mice were tested alongside the aged cohort to provide evidence of performance in these tasks at a younger time-point. All mice performed the novel location task then the object novelty with 4-hour delay and finally the temporal order task, having a rest day between the tests.

Procedure

Object Location

Object Location recognition was assessed via the same 3x 10-minute sample phase protocol as the ON & OiP tasks. However, this protocol used 2 identical objects positioned in adjacent corners, 25 cm apart. During the 5-minute delay before the 10-minute test phase, one object was moved to the corner diagonally opposite the familiar positioned object, as shown in figure 4.1. The novel spatial location was counterbalanced for all corners.

ON with 4-Hour Delay

The temporal order (TO) procedure relied upon information about objects after only one sample phase and after a 4-hour delay. Given published evidence that *APP*$^{-NL-F}$ mice may be sensitive to object memory deficits at long delays, the same mice were tested on an object recognitions paradigm with the same parameters. Mice were placed in the arena to explore two identical objects for 10 minutes. After a delay of 4 hours spent in their home cages, the mice were returned to the arena which contained one object from the sample phase and a novel object.

Temporal Order

The ability of mice to discriminate the temporal order of objects involved a two-stage sampling procedure, as shown in figure 4.1. Mice were presented with two identical objects for 10 minutes in sample phase A. They were then returned to their home cage for an interval of 1 hour after which they were returned to the arena and were presented with two new identical objects in sample phase B (which lasted 10 minutes). The animals were then returned to their home cage for an interval of 3 hours, after which the mice returned to the arena for a 10-minute test phase. In the test phase, the mice were presented with one object from each sample phase. WT mice were expected to show a preference for exploring the object seen least recently, i.e., the object seen in sample phase A. Two object sets (two pairs of identical objects) were counterbalanced between the ON and TO tasks, as well as the identity and spatial location of the novel / less recently encountered object

4.6.3 – Experiment 6a Results

Sample Phase Contact Times

Table 4.4 shows the average contact time with both identical objects through the three sample phases for the aged cohort during the object novel location task. The data was analysed by a repeated measures ANOVA with sample phase as a within subjects factor and genotype as the between subjects factor. There was a significant main effect of phase $F(2,58) = 3.76$, $p<0.029$ but no effect of genotype $F(1,29) = 0.16$, $p=0.693$ and no significant phase*genotype interaction $F(2.58) = 0.29$ $p=0.75$. The main effect of phase revealed a significant drop in contact time between phases 1 & 2 ($p=0.045$), but not between phases 1 & 3 ($p=0.096$) and 2 & 3 ($p=1$), when both genotypes were combined.

	OL Sample Phase Contact Times		
		Aged WT	**Aged *APP*$^{NL-F}$**
Sample Phase 1	**Mean**	**37**	**36**
	SD	11	12
Sample Phase 2	**Mean**	**32**	**28**
	SD	20	13
Sample Phase 3	**Mean**	**30**	**30**
	SD	20	13

Table 4.4: Mean contact times (seconds) for aged mice through the sample phases in the object location task. Standard deviation of the mean (SD) is also shown.

Test Phase Contact Times

Inspection of Figure 4.11 suggests that, while both genotypes preferentially interacted with the object in a novel location, *APP*$^{NL-F}$ mice showed lower overall contact with the objects than WT mice. The Shapiro-Wilke test revealed that not all datasets were normally distributed ($p<0.05$). This was remedied by log10 transformation of both groups. A repeated measures ANOVA with object location (within subjects) and genotype (between subjects) as factors revealed a significant main effect of object location (novel vs familiar): $F(1,29) = 65.0$, $p<0.0005$. There was no effect of genotype $F(1,29) = 1.2$, $p=0.28$ or significant interaction $F(1,29) = 0.01$, $p=0.92$. Together this analysis shows that both groups showed a strong preference for exploration of the object that had been moved to a novel spatial location.

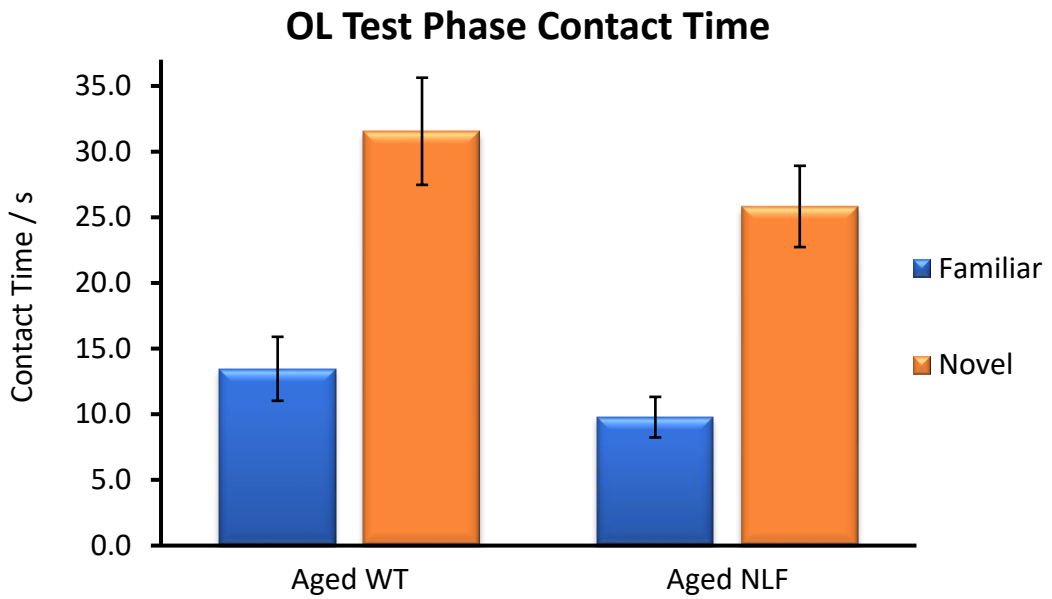


Figure 4.11: Mean contact times of aged mice in the test phase of the object location (OL) task. Contact (seconds) with the object in either a novel or familiar location in the test phase is depicted alongside the SEM. WT n=16, *APP*$^{NL-F}$ n=15. N=9 for both groups.

Discrimination Ratios

Figure 4.12 shows the average discrimination ratios calculated from the preference of aged mice to explore the object in a novel location. The distribution of all groups was normal and an independent t-test was carried out to compare them. There were no significant differences t(29) = 0.06, p=0.953. One sample t-tests showed that both genotypes interacted significantly more with the object in a novel location: aged WT t(14)=7.1; aged *APP*$^{NL-F}$ t(15)=5.6; both p<0.0005. Collectively, these analyses show that aged *APP*$^{NL-F}$ and WT mice successfully and equally discriminated between the objects that had or had not moved to a new spatial location and there was no effect of genotype.

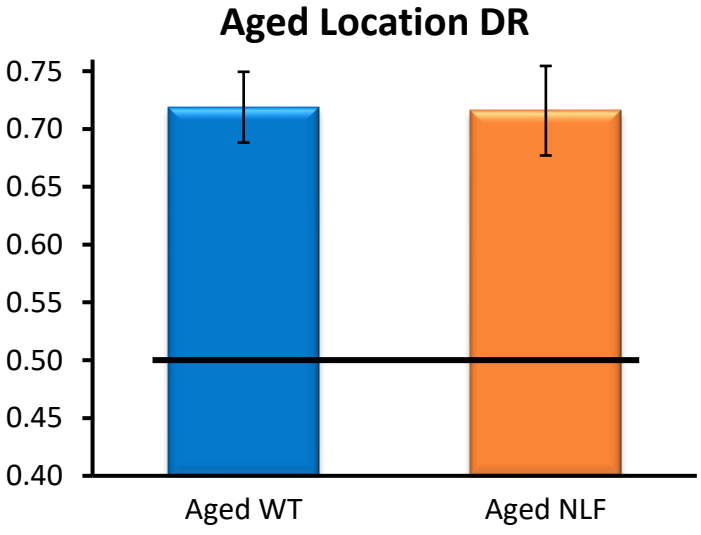


Figure 4.12: Aged *APP*$^{NL-F}$ mice show intact object location memory. Mean discrimination ratios (DR) of aged WT (n=16) and *APP*$^{NL-F}$ (n=15) mice in the object location (OL) task. Error bars show SEM.

4.6.4 – Experiment 6b Results

Sample Phase Contact Times

Table 4.5 shows the average contact time with both identical objects through the three sample phases for 4-month-old mice in the object novel location task. Some of the datasets violated the assumption of normal distribution (S-W $p<0.05$), and this violation was repaired by log10 transformation of both groups for all phases. The data was analysed by a repeat measures ANOVA with sample phase as a within subjects factor. There was a significant main effect of phase $F(2,32) = 17.0$, $p<0.0005$. There was no significant main effect of genotype $F(1,32) = 0.001$, $p=0.977$ or significant phase*group interaction $F(1,32) = 0.35$ $p=0.704$. The main effect of phase revealed a significant drop in contact time between phases 1 & 2 and 2 & 3 ($p<0.0005$), but no difference between phases 2 & 3 ($p=0.45$).

	OL Sample Phase Contact Times		
		WT	***APP*$^{NL-F}$**
Sample Phase 1	**Mean**	**62**	**57**
	SD	30	7
Sample Phase 2	**Mean**	**51**	**43**
	SD	32	15
Sample Phase 3	**Mean**	**42**	**39**
	SD	23	8

Table 4.5: Mean contact times (seconds) by 4-month-old mice with both objects through the sample phases in the object location task. Standard deviation of the mean (SD) is also shown. n=9 for both groups.

Test Phase Contact Times

Figure 4.13 depicts how 4-month-old *APP*$^{NL-F}$ and WT mice explored objects that had either moved to a novel position or remained in the same spatial location they inhabited during the sample phases. The Shapiro-Wilke test revealed that not all datasets were normally distributed. This was remedied by log10 transformation ($p<0.05$). A repeated measures ANOVA with object location (within subjects) and genotype (between subjects) as factors revealed significant main effects of object location $F(1,16) = 73$, $p<0.0005$ but not genotype $F(1,16) = 0.233$, $p=0.636$. There was no significant interaction $F(1,29) = 0.139$, $p=0.715$. Together this analysis shows that both genotypes showed a strong preference for exploration of the object that had been moved to a novel spatial location.

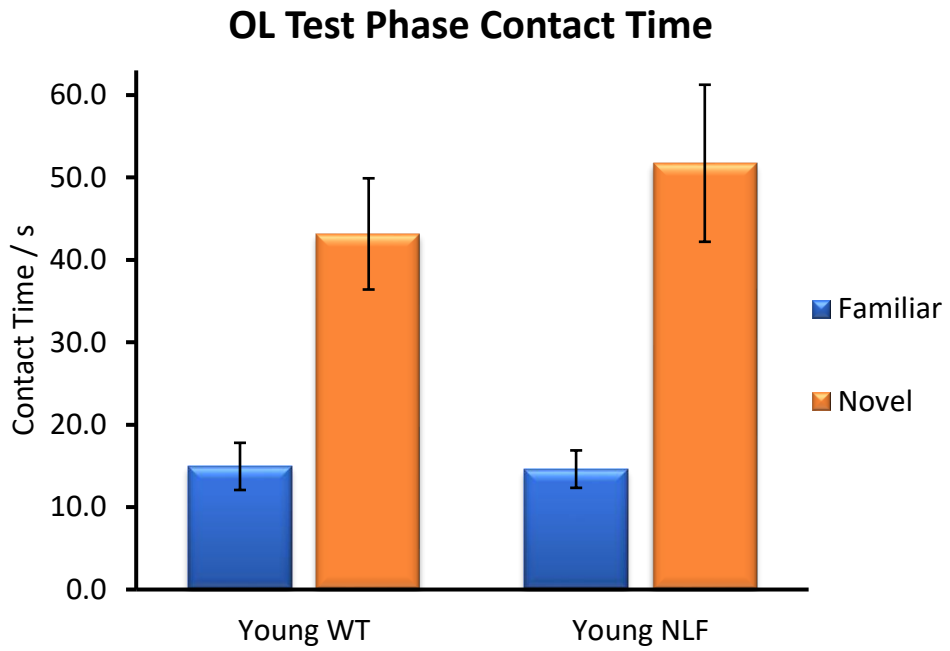


Figure 4.13: Mean contact times for 4-month-old WT and *APP*$^{NL-F}$ mice in the object location (OL) task. Contact (seconds) with the object in either a novel or familiar location is depicted alongside the SEM. N=9 for both groups.

Discrimination Ratios

Figure 4.14 shows the average discrimination ratios calculated from the preference of 4-month-old mice to explore the object in a novel location. The distribution of groups was normal and an independent t-test was carried out to compare them. There were no significant differences $t(16) = -0.482$,$p=0.759$. One sample t-tests showed that both genotypes interacted significantly more with the object in a novel location: young WT $t(8) = 7.6$; young *APP*$^{NL-F}$ $t(8) = 6.9$; both $p<0.0005$. Collectively, these analyses show that at 4 months of age *APP*$^{NL-F}$ mice successfully discriminated object that had moved to a new spatial location to an equal level as WT controls.

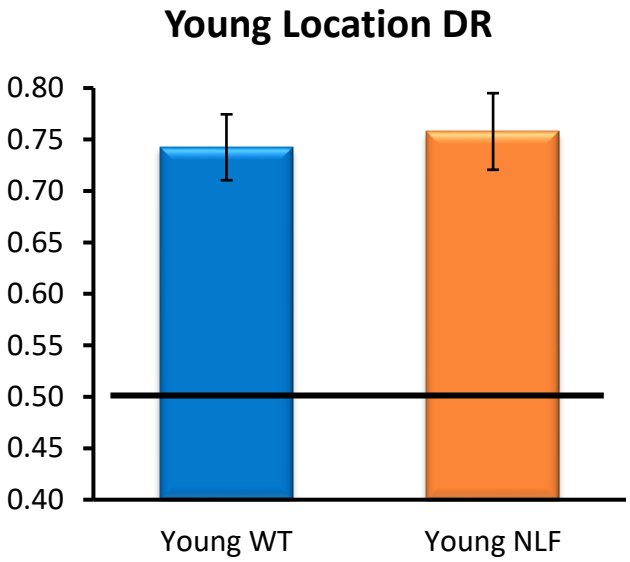


Figure 4.14: 4-month-old *APP*$^{NL-F}$ mice display comparable performance in the object location task to WT controls. Mean discrimination ratios (DR) for 4-month-old WT and *APP*$^{NL-F}$ in the object location task. Error bars show SEM, n=9 for both groups.

4.7 Experiment 7: Object Novelty with 4-Hour Delay

4.7.1 – Experiment 7a Results

Sample Phase Contact Times

The temporal order task required recognition of an object after a single sample phase and 4-hour delay. The mean contact time of the aged *APP*$^{NL-F}$ & WT mice in the sample phase (table 4.6) were analysed by an independent t-test comparing the two genotypes. The t-test returned no significant difference t(29) = 0.171, p=0.866.

4Hr ON Sample Phase Contact Times			
		Aged WT	**Aged *APP*$^{NL-F}$**
Sample Phase 1	**Mean**	**47**	**45**
	SD	21	15

Table 4.6: Mean contact time (seconds) of aged WT and *APP*$^{NL-F}$ mice in the sample phase of the 4-hour object novelty task, alongside the standard deviation (SD). n=16 (WT), n=15 (*APP*$^{NL-F}$).

Test Phase Contact Times

Contact with the novel or familiar object was averaged in each group and is presented in figure 4.15. The data were transformed by square root in order to restore any violations of normality (S-W $p>0.05$), and analysed by repeat measures ANOVA with mouse group and object type as factors. The analysis revealed a significant main effect of object type $F(1,29) = 48.9$, $p<0.0005$. There was no effect of group $F(1,29) = 0.003$, $p=0.958$ and no interaction $F(1,29) = 0.024$, $p=0.878$.

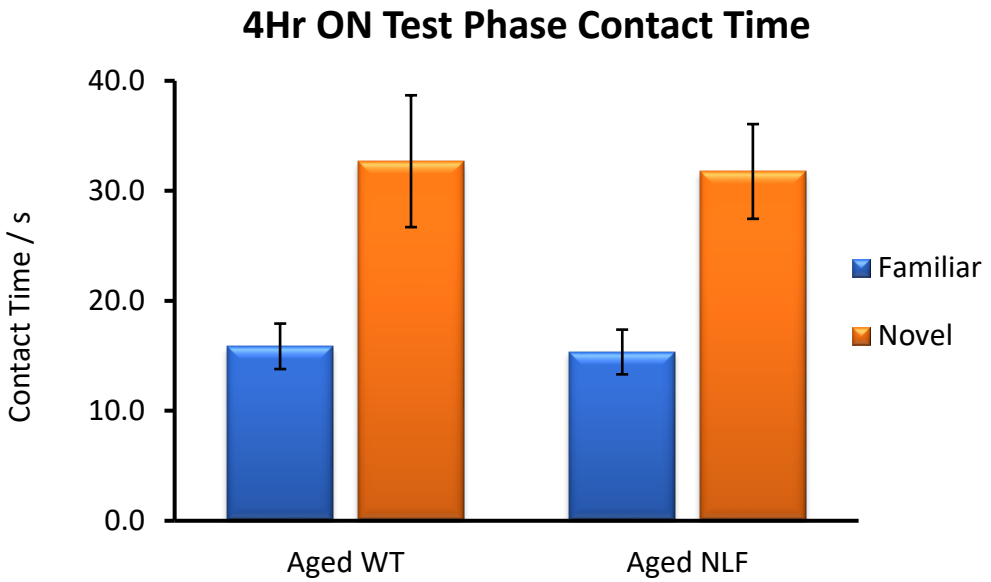


Figure 4.15: Mean contact times (seconds) of aged WT and *APP*$^{NL-F}$ mice in the object novelty (ON) task with 4-hour delay test phase. WT n=16, *APP*$^{NL-F}$ n=15. Error bars represent SEM.

Discrimination Ratios

The contact times were converted to a discrimination ratio to take into account the differences between groups. These are shown in figure 4.16. The two groups were compared by an independent t-test which found no significant difference $t(29) = -0.59$, $p=0.561$. One-sample t-tests revealed that both genotypes discriminated the novel object significantly greater than at chance level (0.5). WT $t(14) = 5.1$ $p<0.0005$; *APP*$^{NL-F}$ $t(15) = 7.4$, $p<0.0005$. Collectively, these analyses show that at 17 months of age, *APP*$^{NL-F}$ mice maintained the ability to discriminate a novel from familiar object 4 hours after one sample phase and did so at a comparable level to WT controls.

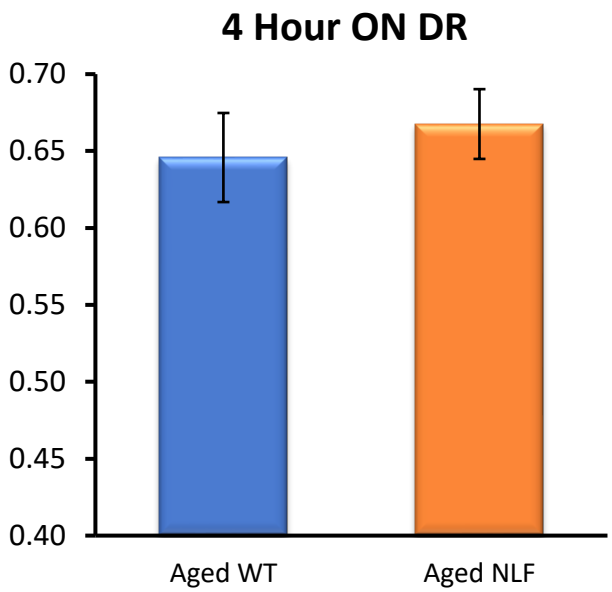


Figure 4.16: Aged *APP*$^{NL-F}$ mice display comparable performance in the 4-hour object novelty task to WT controls. Mean discrimination ratios (DR) for aged WT and *APP*$^{NL-F}$ mice in an object novelty (ON) task following a 4-hour delay. Ratios were calculated from the contact time when investigating a novel or familiar object. WT n=16, *APP*$^{NL-F}$ n=15. Error bars represent SEM.

4.7.2 – Experiment 7b Results

Sample Phase Contact Times

A separate cohort of 4-month-old *APP*$^{NL-F}$ and WT were assessed on the object novelty task with 4-hour delay. Table 4.7 shows the mean contact time in the sample phase. Both groups were transformed by square root to ensure normal distributions. The two groups were compared by independent t-test, which revealed no significant difference t(16) = -0.24, p=0.811.

4Hr ON Sample Phase Contact Times			
		Young WT	**Young *APP*$^{NL-F}$**
Sample Phase 1	**Mean**	**61**	**67**
	SD	20	40

Table 4.7: Mean contact time (seconds) for 4-month-old mice in the sample phase of the 4-hour object novelty task, alongside the standard deviation (SD) . n=9 for both groups.

Test Phase Contact Times

Contact with the novel or familiar object in the test phase was averaged in each group and is presented in figure 4.17. The data were analysed by repeat measures ANOVA with mouse group and object type as factors. The analysis revealed a significant main effect of object type $F(1,16) = 24.5$, $p<0.0005$. There was no effect of group $F(1,16) = 0.019$, $p=0.891$ and no interaction $F(1,16) = 0.053$, $p=0.821$. This analysis shows that both genotypes preferentially explored the novel object.

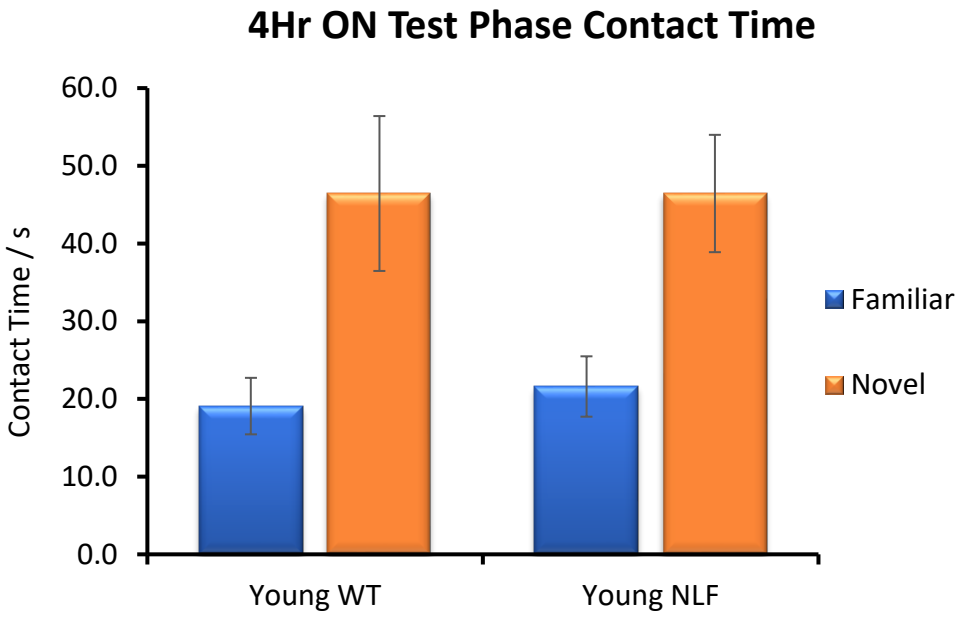


Figure 4.17: Mean test phase contact times (seconds) of 4-month-old WT and *APP^{NL-F}* mice in an object novelty (ON) task following a 4-hour delay. Contact with either the familiar or novel object is shown. N=9 for both groups. Error bars represent SEM.

Discrimination Ratios

The contact times for 4-month-old mice were converted to discrimination ratios. These are shown in figure 4.18. Data were analysed by independent t-test $t(16) = 0.241$ $p=0.812$. One-sample t-tests revealed that all four groups discriminated the novel object significantly greater than at chance level (0.5). WT $t(8) = 5.1$, $p=0.001$; *APP^{NL-F}* $t(8) = 5.3$, $p=0.001$. These analyses show all that young *APP^{NL-F}* and WT mice displayed the ability to significantly discriminate between novel and familiar objects after a single sample phase and 4-hour delay.

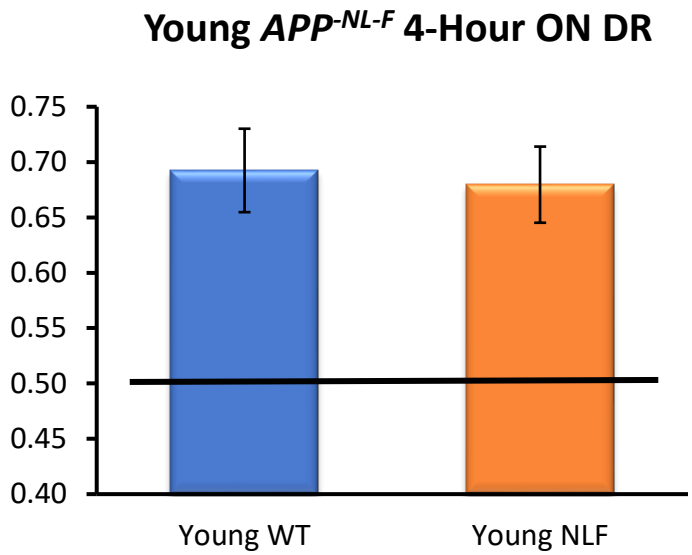


Figure 4.18: 4-month-old *APP*$^{-NL-F}$ show comparable performance in the 4-hour object novelty task to WT controls. Mean discrimination ratios (DR) for 4-month-old WT and *APP*$^{-NL-F}$ investigating a novel or familiar object following a 4-hour delay. N=9 for both groups. Error bars represent SEM.

4.8 Experiment 8: Temporal Order Memory in *APP*$^{-NL-F}$ Mice

▪ 4.8.1 – Experiment 8a Results

Sample Phase Contact Times

The temporal order task consisted of two samples phases, with object pair A in sample phase A and pair B in phase B. The order in which the pairs of objects appeared was counterbalanced. The average exploration of both objects was calculated for the aged WT and *APP*$^{-NL-F}$ mice during each sample phase and is presented in table 4.8. The datasets were not all normally distributed, so were transformed by square rooting in order that this assumption was no longer violated (S-W $p>0.05$).

The data was analysed by a repeat measures ANOVA with genotype as the between subjects factor and sample phase as a within subjects factor. There were no main effects of phase $F(1,29) = 1.20$, $p=0.282$, or genotype $F(1,29) = 0.86$, $p=0.360$, and no phase*genotype interaction $F(1,29) = 0$, $p=0.991$.

TO Sample Phase Contact Times			
		Aged WT	**Aged *APP*$^{-NL-F}$**
Sample Phase A	**Mean**	**47**	**41**
	SD	22	23
Sample Phase B	**Mean**	**43**	**36**
	SD	25	14

Table 4.8 Mean contact times (seconds) of aged WT and *APP*$^{-NL-F}$ mice in the sample phases of the temporal order (TO) task. Standard deviation of the mean (SD) is also shown. WT n=16, *APP*$^{-NL-F}$ n=15

Test Phase Contact Times

In the temporal order test phase, mice explored one object that had previously been observed in sample phase A (object A) and one from phase B (object B). The exploration of each object is shown in figure 4.19. The data suggested that the aged WT preferentially contacted the object they had seen first (in sample phase A), however this was not the case for the aged *APP*$^{-NL-F}$ group. The Shapiro-Wilke test revealed that not all datasets were normally distributed. This was remedied by square root transformation ($p<0.05$).

A repeated measures ANOVA with object (within subjects) and genotype (between subjects) as factors revealed a significant main effects of object $F(1,29) = 6.29$, $p=0.018$; but not group $F(1.29) = 1.79$, $p=0.191$. There was a significant object*genotype interaction $F(1,29) = 8.93$, $p=0.006$. Tests for simple main effects following the object*genotype interaction reported that WT mice exhibited significantly greater contact with object A compared to object B ($p=0.001$), while the *APP*$^{-NL-F}$ group failed to do so ($p=0.728$).Furthermore, the *APP*$^{-NL-F}$ group had significantly reduced exploration of object A compared to the WT ($p=0.001$) but there was no difference in contact with object B ($p=0.644$).

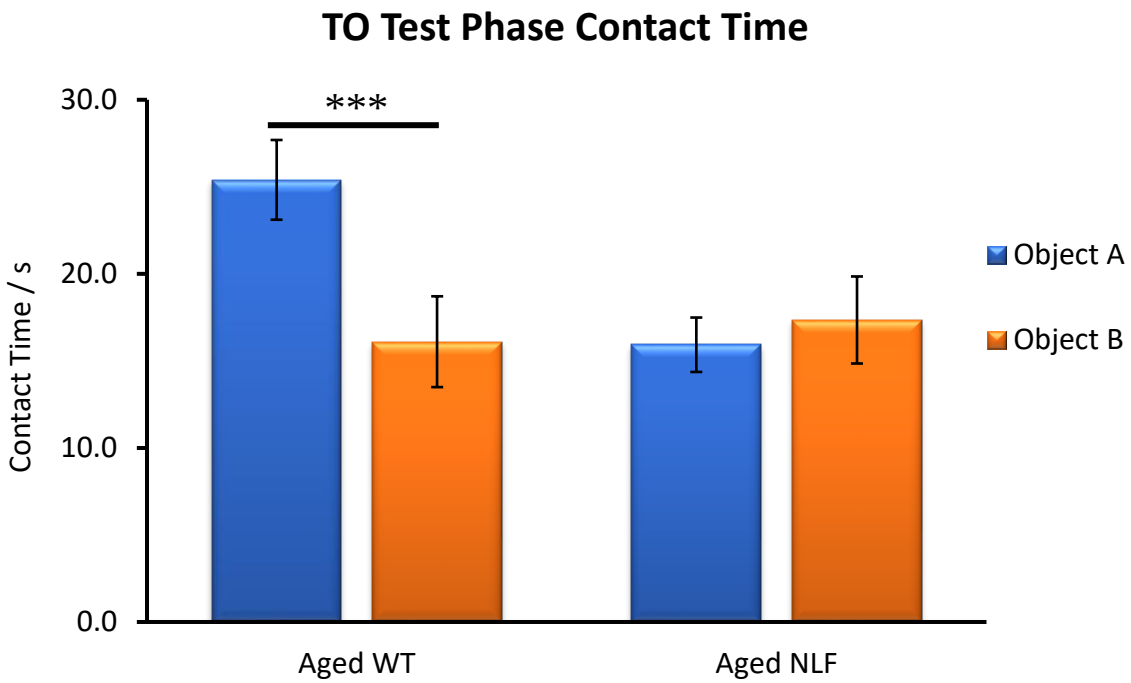


Figure 4.19 Mean contact time (seconds) aged WT and *APP*$^{NL-F}$ mice for objects A or B in the temporal order (TO) test phase. Objects A and B were presented in sample phases A and B respectively. Error bars represent SEM. WT n=16, *APP*$^{NL-F}$ n=15. ***p<0.001 mixed measured ANOVA.

Discrimination Ratios

Discrimination ratios (DR) were calculated to determine the preference of each group to interact with the object from sample phase A. Figure 4.20 shows the average DRs calculated from the preference of each group to explore the object observed first (object A). The groups were analysed by an independent t-test which revealed a significant difference between the groups t(29) = 2.86 p=0.008. One sample t-tests showed that the aged WT mice discriminated object A from object B at a rate significantly above chance (DR = 0.5): t(14)=- -3.7, p=0.002. However, the aged *APP*$^{NL-F}$ group did not discriminate t(15)= -0.16, p=0.872. Collectively, these analyses suggest the aged *APP*$^{NL-F}$ mice failed to recognise the sequential order in which the objects had originally been presented.

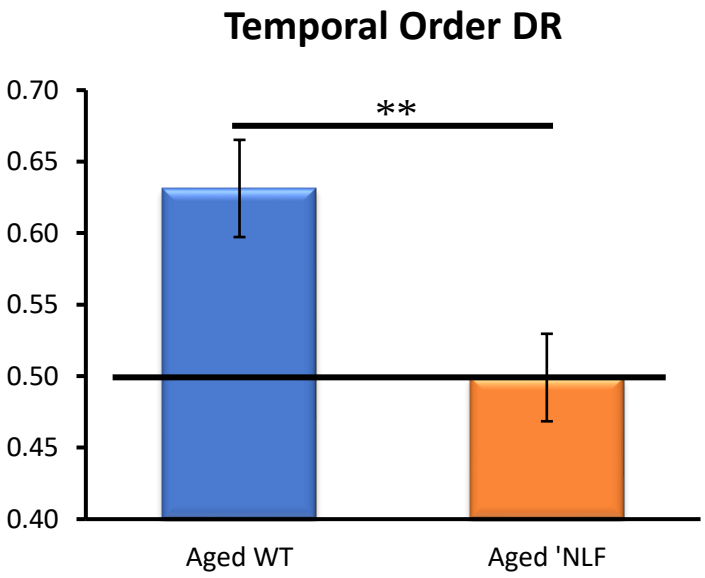


Figure 4.20: Aged *APP*$^{NL-F}$ mice exhibit a deficit in temporal order memory. Mean discrimination ratios (DR) for aged WT and *APP*$^{NL-F}$ mice. Greater than 0.5 indicates preference for the object presented less recently. Error bars show SEM. WT n=16, *APP*$^{NL-F}$ n=15. **p<0.01, independent samples t-test.

4.8.2 – Experiment 8b Results

Sample Phase Contact Times

A separate cohort of *APP*$^{NL-F}$ and WT mice were tested on the TO task at 4 months of age. The mean contact time in the sample phases is presented in table 4.9. The datasets were not all normally distributed, so were transformed by square rooting in order that this assumption was no longer violated (S-W p>0.05). The data was analysed by a repeat measures ANOVA with sample phase and genotype as factors. There was no main effect of sample phase F(1,16) = 0.339, p=0.568, no main effect of genotype F(1,16) = 1.37, p=0.259 and no phase*genotype interaction F(1,16) = 1.37, p=0.259.

TO Sample Phase Contact Times			
		Young WT	**Young *APP*$^{NL-F}$**
Sample Phase A	**Mean**	**72**	**58**
	SD	25	31
Sample Phase B	**Mean**	**70**	**67**
	SD	32	27

Table 4.9: Contact time (seconds) by 4-month-old WT and *APP*$^{NL-F}$ mice in the sample phases of the temporal order task. Standard deviation of the mean (SD) is also shown. n=9 for both groups.

Test Phase Contact Times

Figure 4.21 shows the average contact times with both objects in the test phase. Both groups were normally distributed as shown by the Shapiro-Wilke test ($p<0.05$). A repeated measures ANOVA with object (within subjects) and genotype (between subjects) as factors revealed a significant main effect of object $F(1,16) = 39.4$, $p<0.0005$; but no main effect of group $F(1,16) = 0$, $p=0.985$. There was no a object*group interaction $F(1,16) = 0.27$, $p=0.613$.

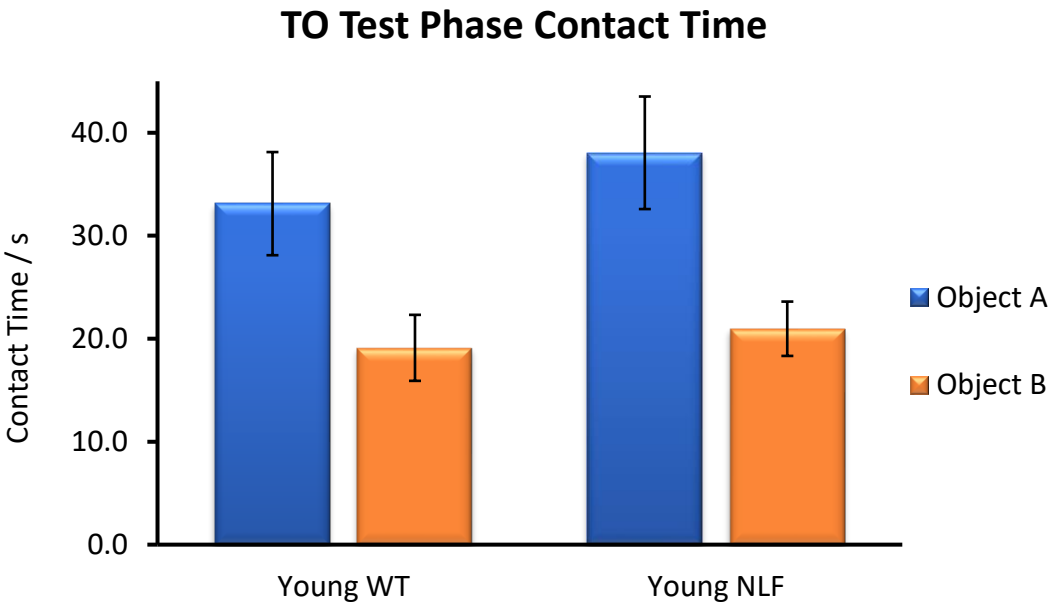


Figure 4.21: Mean contact time (seconds) for 4-month-old WT and *APP*$^{NL-F}$ mice in the temporal order (TO) test phase. Objects A and B were previously presented in either sample phase A or B, respectively. N=9 for both groups. Error bars represent standard error.

Discrimination Ratios

Figure 4.22 shows the average Discrimination ratios for the 4-month-old mice in the temporal order task. The scores of both groups were transformed by a reflect and log10 calculation in order to satisfy the assumption of normal distributions. The data were analysed by an independent t-test which found no difference between the groups: $t(16) = 0.008$, $p=0.994$. One sample t-tests showed that both genotypes discriminated between objects at greater than the chance level of 0.5 (WT: $t(8) = 4.9$, $p=0.001$; *APP*$^{NL-F}$: $t(8) = 6.0$, $p<0.0005$).

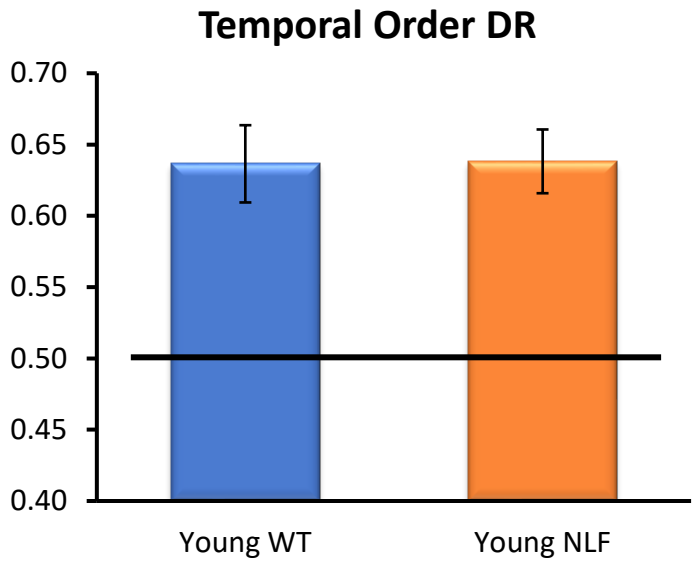


Figure 4.22: *APP*$^{NL-F}$ display comparable temporal order memory to WT controls at 4-months of age. Mean discrimination ratios (DR) for WT and *APP*$^{NL-F}$. The predicted score of random chance (0.5) is highlighted by the black line. N=9 for both groups. Error bars show SEM.

4.9 Chapter Discussion

The aim of this chapter was to present the first study investigating how the knocked-in Swedish and Iberian mutations affect multiple object recognition tests in the *APP*$^{NL-F}$ mouse model. Experiment 3 revealed no differences in anxiety or locomotive behaviours, which then permitted analysis of cognitive domains without potential confounding behaviours. Experiments 4 and 5 demonstrated that, while aged *APP*$^{NL-F}$ mice have intact memory for object identity, they failed to discriminate novel object-place associations: a novel phenotype in this model. The results suggest that while successful processing of object information was maintained up to 17 months of age, the mice exhibited age-related cognitive dysfunction specific to the hippocampus. Following this result, experiment 6 assessed non-associative, hippocampal dependent recognition in the object location task and discovered intact memory performance. This result was particularly intriguing in comparison to the object-in-place data as it helps to describe the specific nature of hippocampal pathology in the *APP*$^{NL-F}$ mice. Finally, experiments 7 and 8 revealed a deficit in another hippocampal dependent aspect of associative recognition memory, temporal order, in the knock-in mice. Memory for the sequential order in which objects are presented is another facet of episodic memory and the failure of the *APP*$^{NL-F}$ mice in this task recapitulates a well-characterised symptom of AD patients.

The only previous study assessing object recognition memory in the *APP*$^{-NL-F}$ model reported a deficit in object novelty performance in 12 month old mice (Izumi et al. 2018). The current study did not replicate this result and in fact reported intact novelty discrimination up to 17 months of age. The protocol used in experiment 4 contains many discrepancies compared to that used by Izumi and colleagues, particularly the delay involved (5 minutes vs 24 hours), the sampling procedure (3- vs 1 10-minute sessions) and the object array (4 different objects vs 2 identical). Furthermore, the group tested female mice which were not habituated to the arena and objects as in this study. The protocol in experiment 7 is more comparable to the published work, involving the one sample phase with two identical objects. Although the 4-hour delay in experiment 7 represents a long-term memory retrieval, the extended duration described by Izumi *et al* may be the determining factor in the reported deficit. We did not test memory at 24-hour interval but it would be interesting to determine if this could be replicated in our laboratory. It is also worth mentioning that Izumi *et al*'s results involved *APP*$^{-NL-F}$ mice which had been subjected to daily oral administration of distilled water between 9 & 12 months, as the vehicle control group in their experiment. The increased stress induced by this treatment may have affected performance (Baker and Kim 2002).

A study in the *APP*$^{-NL-G-F}$ mouse also reported an ON deficit. The triple mutation in the APP gene of this mouse induces a more aggressive phenotype, which may result in significant amyloid pathology in the cortex and thus impact on perirhinal cortex function (Mehla et al. 2019). In fact, a common feature of reports of ON deficits in AD mouse models is multiple combined mutations or transgenes, such as in the 3xTg, 5xFAD, APP/PS1 and J20 models (Oddo et al. 2003, Joyashiki, Matsuya, and Tohda 2011, Webster, Bachstetter, and Van Eldik 2013, Mucke et al. 2000, Citron et al. 1997). Therefore, the greater number of mutations and/or increased expression of APP and thus more aggressive pathology may cause perirhinal cortex dysfunction and ON deficits in these models. However, this was clearly not the case in the *APP*$^{-NL-F}$ mice, as the current study reports intact object recognition using two different protocols.

This chapter reports a novel phenotype: an age-related deficit in the *APP*$^{-NL-F}$ mice's ability to recognise object-place associations. The concurrent and comparable ON recognition test is vital for dissociating the detail of the deficit. Successful detection of novelty demonstrates intact memory processes for object identity and validates the deficit in the OiP task being caused by a specific deficit in associative memory integrating spatial

information with object identity. The lack of a deficit in the object location (OL) task in aged *APP*$^{-NL-F}$ mice is thus intriguing. The latter finding suggest that *APP*$^{-NL-F}$ have encoded some property(ies) of the topography of the object array; ie. they were able to detect when the spatial organisation had changed. While this task is known to be sensitive to hippocampal cell loss, *APP*$^{-NL-F}$ undoubtedly have a more subtle impact on hippocampal function, which is further evidenced by the available literature. While the original paper by Saito *et al* described spatial working memory deficits measured by reduced spontaneous alternation in the y-maze, it reported no effects in the MWM, further suggesting a subtle effect on hippocampal function (Saito et al. 2014). Subsequent literature has failed to replicate the original deficit in alternation (Masuda et al. 2016, Whyte et al. 2018).

Effects of genotype were not observed on basal locomotive or exploratory activity, as has been seen in other models of AD (Evans et al. 2019). This meant that there were no potential confounding effects of one genotype getting more contact with objects in the sample phases thus receiving greater potential for encoding information. This strengthens the conclusion that differences observed in exploration by the aged *APP*$^{-NL-F}$ group were due to deficits in memory processing.

A similar pattern of visuo-spatial recognition memory deficits has been described in Tg2576 mice that also express the Swedish mutation. Aged Tg2576 mice exhibited intact recognition of object identity and for when familiar objects were moved to a previously unoccupied location, but not for when two objects switched spatial location (Good & Hale 2007). They also display place cell activity- although the quality of information encoded by place cell is considerably compromised (Cacucci et al., 2008). This may also explain the slower learning in the water maze, and the loss of recognition of more subtle aspects of spatial memory (Zhao et al. 2014). While place cell activity has not been examined in *APP*$^{-NL-F}$ mice, the current data suggest that these mice are able to encode at least some spatial properties of the landmark array. In this way, the 17-month-old mice exhibited intact memory for the individual components of object identity and place. However, they lacked the ability to combine these dimensions into configural memories in the OiP task. As the *APP*$^{-NL-F}$ mice show a similar pattern of deficits as the Tg2576 and the models share the Swedish mutation, the effect on place cells may be common between them.

This study also presents the first evidence of a deficit in the *APP*$^{-NL-F}$ mouse's processing the temporal order of objects. Before discussing this observation, it is important

to first acknowledge the *APP*$^{-NL-F}$ mice were able to carry out a simple object novelty discrimination over the 4-hour delay. The data presented here indicate that *APP*$^{-NL-F}$ mice are able to discriminate familiar/novel objects with either a short delay (5-min) or a relatively long delay of 4 hours. Therefore, one can assume that in the temporal order task, the *APP*$^{-NL-F}$ mice had encoded both objects sets during the sample phases. Importantly, the aged *APP*$^{-NL-F}$ group failed to show any discrimination between early and recently presented objects. This is the first time that such a deficit has been reported in APP mice. Hale & Good (2005) reported that Tg2576 mice demonstrated intact recency discrimination using a protocol involving a 2-minute delay for both inter-phase intervals. Bonardi *et al* (2016) also reported intact recency in 5-month old APPswe/PS1dE9 mice alongside a deficit in OiP performance. Their protocol comprised a 24-hour delay between sample phases A and B, and then a 5-minute delay between the second sample and test phases. In contrast, the 1- and 3- hour inter-phase intervals used in the current study involved a larger separation between exposure and test, thereby reducing relative differences in memory strengths of the two object pairs. In this way, while the previous protocols tested a recency effect, the current study assessed memory for temporal order. The fact that the *APP*$^{-NL-F}$ mice exhibited decreased exploration through the 3 sample phases of the ON and OiP tasks at 16-17 months arguably shows that they also have intact recency memory.

The pattern of deficits in different recognition paradigms described above provides the basis for considering how the *APP*$^{-NL-F}$ mutation impacts the neural circuitry underpinning object memory (c.f., Barker & Warburton – 2011). Barker & Warburton showed that deficits in associative recognition memory tasks were caused by lesions of either the medial prefrontal cortex (mPFC) or HPC, or alternatively by disconnection of the PRC-HPC-mPFC circuit in both hemispheres. As already discussed, the fact that *APP*$^{-NL-F}$ mice maintain recognition of objects moving to previously unoccupied locations indicates that hippocampal function remains intact to some extent. Furthermore, successful discrimination of object novelty after a 4-hour delay suggests hippocampal NMDA receptor function was unimpaired considering evidence that long term (>3 hour) object recognition memory depends on these receptors (Baker and Kim 2002). There is currently no specific data concerning amyloid accumulation in the mPFC in *APP*$^{-NL-F}$ mice. However, altered connectivity involving the hippocampus and prefrontal regions has been detected as young as 3 months of age in both the *APP*$^{-NL-G-F}$ and *APP*$^{-NL-F}$ knock-in models (Latif-Hernandez et al. 2017, Shah et al. 2018). Shah et al., 2018 reported that *APP*$^{-NL-F}$ mice showed impaired reversal learning in the MWM at 3 and 7 months of age, alongside

BOLD analyses revealing hypersynchronous functional connectivity between the hippocampus and frontal cortex at 3 months, but hyposynchrony at 7 months of age. The same group has already reported this age-dependent switch from hyper- to hyposynchronous functional connectivity in transgenic models (Shah et al. 2016, Shah et al. 2013). The effects in *APP*$^{-NL-F}$ mice appeared far earlier than the cognitive deficits in the current study and were not examined beyond 7 months of age. However, detection of aberrant functional connectivity prior to overt cognitive symptoms mirrors what is seen children with a FAD mutation and this effect may actually be a compensatory neurobiological reaction for Aβ-induced dysfunction (Quiroz et al. 2015). Relating this fMRI evidence to mechanisms of cognitive effects is difficult, although cortical hyperconnectivity has also been correlated to a decline in reversal learning in mPFC-lesioned mice (Latif-Hernandez et al. 2016). Deficits in reversal learning are the only *APP*$^{-NL-F}$ phenotype that has currently been replicated in more than one study (Masuda et al. 2016, Shah et al. 2018). There is evidence that this cognitive flexibility is dependent on network level communication within the mPFC-hippocampal circuit (de Bruin et al. 1994, Latif-Hernandez et al. 2016). Intriguingly, a similar mechanism has been shown to be vital to the associative recognition tasks failed by the *APP*$^{-NL-F}$ mice in the current chapter (Barker and Warburton 2011). Multiple studies revealing two phenotypes that are be underpinned by the same mechanism provides strong evidence for any putative neurobiological pathology in this mouse model. Further analysis concerning amyloidosis or synaptic plasticity within the medial prefrontal cortex would be vital in determining to what extent this region is affected in the *APP*$^{-NL-F}$ model.

Individual and combinatorial lesions of distinct brain regions have been shown to impact performance of specific object recognition paradigms and this is summarised in table 4.1. However, recent evidence has presented the entorhinal cortex (EC) as vital for associative recognition memory. Despite the speculative proposition of EC dysfunction as being causative of the results described in the current study, it is worth discussion. The EC integrates multiple aspects of recognition memory required for associative recognition tasks and is also one of the most vulnerable regions of the cortex to early AD pathology (Witter et al. 2017, Gomez-Isla et al. 1996). Evidence for the involvement of the EC in recognition memory is present across species. Manual segmentation within the EC of humans revealed that its volume significantly predicted object-in-place performance (Yeung et al. 2019). *In vivo* electrophysiological recordings in rats revealed distinct cell

types in the lateral EC that responded to either objects or spatial locations in which objects were previously present (Tsao, Moser, and Moser 2013). These studies present evidence that function of the EC may underpin associative recognition memory performance. Recent reports have shown that APP-KI mice do exhibit aberrant synaptic and oscillatory mechanisms in that brain region, supporting this hypothesis. Nakazono *et al* (2017) published results showing that gamma oscillations are impaired in APP-KI mice due to reduced gamma to theta cross frequency coupling. This cross frequency coupling has been shown to predict working memory performance (Axmacher et al. 2010) and is a hypothesised mechanism for information transfer in the EC-hippocampal circuit during associative learning (Lisman 2005, Igarashi et al. 2014). Another recent study identified hyperexcitability in the EC of APP^{-NL-F} mice, along with a reduced expression of parvalbumin expressing interneurons (Petrache et al. 2019). This excitatory-inhibitory imbalance can lead to excitotoxicity and neurodegeneration, as evidenced by the measured reduction of EC pyramidal cell density compared to age matched controls. A similar finding was identified recently in transgenic Tg2576 mice which demonstrated degenerated pyramidal EC neurons that usually innervate the CA1 of the hippocampus (Yang et al. 2018). Optogenetic reactivation of these synapses was enough to rescue the spatial memory decline in these animals. Neurodegeneration within the EC of APP^{-NL-F} mice could certainly provide a mechanism for their failure of associative recognition memory following a study by Wilson and colleagues (2013). They reported that lesions of the lateral entorhinal cortex in rats impaired recognition of object-place, place-context and object-place-context associations, while object novelty and object location performance remained intact (Wilson et al. 2013).

In conclusion, these experiments describe a novel cognitive phenotype in the APP^{-NL-F} mouse model. This study has revealed an intriguing pattern of deficits across a battery of object recognition tests. The APP^{-NL-F} mice display intact memory for object identity and novel location, but fail the tasks involving encoding associations of specific objects with location or time. These associative tasks are related to episodic memory, the decline of which is a hallmark of the early stages of Alzheimer's Disease. The cause of specific associative memory failure remains unclear but may be due to disruption of the mPFC-perirhinal-hippocampus or hippocampus-entorhinal circuits, as both of these mechanisms are critical for associative memory performance.

Chapter 5 –

Working Memory Assessment in *APP*$^{-NL-F}$ Mice

5.1 Chapter Overview

A hallmark of Alzheimer Disease symptoms is a decline in spatial working memory capabilities. Therefore, investigations into the pathology exhibited by genetically engineered rodent models of the disease have predominantly involved assessment of this aspect of memory. This chapter introduces navigational strategies underpinning spatial memory as well as the various methods used to test it in rodents. While there is extensive literature concerning age-related decline in spatial working memory in transgenic mouse models, there is limited evidence of the effect in knock-in mice. Experiments in this chapter assessed spatial working memory in a task was based on foraging behaviour and permitted spatial and non-spatial versions, depending on the availability of extra or intra-maze cues. The spatial version is sensitive to hippocampal cell loss and amyloid pathology in a transgenic mouse model.

APP^{-NL-F} mice were tested on the foraging task at two age points in a longitudinal design. They exhibited a significant decline in spatial working memory performance with age. There were no effects in the non-spatial task, which was run alongside the spatial version in a counterbalanced manner. This result augments the results of chapter 4, showing that the deficit in processing of spatial information extends to a working memory format in these mice. This result is consistent with published data of a deficit in *APP*$^{-NL-F}$ mice in spontaneous alternation measured in a Y-maze.

5.2 Chapter Introduction

Chapter 4 analysed performance of the *APP*$^{-NL-F}$ mice in a battery of object recognition tasks relevant to Alzheimer's Disease. The mice exhibited an age-dependent deficit in associative recognition memory, failing to discriminate novel object-place associations. In order to investigate whether such cognitive deficits translated across multiple tests with different sensory, motivational and motor requirements, the mice were tested in a foraging based working memory task. This task was used to assess the use of both spatial and non-spatial (cue-related) information on foraging behaviour. The spatial version of this task has previously been shown to be sensitive to hippocampal lesions, and amyloid pathology in the PDAPP transgenic mouse model (Evans et al. 2018).

Working memory has previously been defined as a memory for aspects of object, stimulus or location, held within the short term period of a single trial and not recalled in subsequent trials (Olton, Becker, and Handelmann 1979, Honig 1978). David Olton described this form of memory after having designed the Radial Arm Maze (RAM) to assess spatial working memory (SWM) performance. The RAM has a central platform with eight arms extending outwards, each baited with a reward. The original paper described how rats would learn to visit each arm once, to collect all rewards as efficiently as possible. The authors established that the rodents were navigating independent of intra-maze cues (e.g. odour) or any specific order (Olton, Becker, and Handelmann 1979). The rats were apparently utilising "working memory" to avoid arms they had already visited and exploring the others to find unclaimed rewards during that session (Dudchenko 2004).

Animals are able to perform spatial working memory tasks in the same way they naturally navigate through their environment: by generating a "cognitive map" of their surroundings (Collett and Graham 2004, Bjerknes et al. 2018). The cognitive map can be accessed in distinct navigational strategies. For example, allocentric navigation involves integration of distinct visual landmarks to encode their locations relative to one another. Alternatively, an animal might model the position of objects or environmental cues relative to itself, described as egocentric processing. This contributes to the animal's conscious awareness of its own movement relative to a previously known position, which is another navigational strategy known as Path Integration. The memory required to use these strategies is believed to be incorporated in the hippocampal and entorhinal networks, specifically by hippocampal place cells and grid cells in the medial entorhinal cortex (McNaughton et al. 2006, O'Keefe et al. 1998, Morris, Hagan, and Rawlins 1986, Wills et al. 2010). The medial temporal lobe is well characterised to be susceptible to early AD pathology and it follows that deficits in navigation are a consistent hallmark of patients' symptoms (Graham 2017, Hort et al. 2007). More specific assessments revealed patients have a deteriorating ability to use path integration or allocentric navigational strategies as an early stage indicator of cognitive decline (Vlček and Laczó 2014, Mokrisova et al. 2016).

Spatial working memory tasks such as the radial arm maze described above have revealed that transgenic models of Alzheimer Disease pathology perform worse than control animals (Hsiao et al. 1996, Morgan et al. 2000). In fact, models exhibit similar deficits to patients in tests designed to be analogous between the two species (Laczo et al.

2010, Lee et al. 2015). A review of 10 mouse models by Webster *et al* (2014) identified that dysfunction of spatial working memory manifests earlier than other cognitive phenotypes, mirroring the pattern of symptom onset in patients (Webster et al. 2014). This accumulation of evidence demonstrates that tests of spatial working memory are relevant to investigate to what extent models of amyloid pathology recapitulate the cognitive symptoms of the disease. Various other tasks have been used to test Spatial Working Memory (SWM) in rodents. These include the Morris Water Maze (MWM), Barnes Maze and alternation in a T or Y maze. They all require the subject to encode spatial information about the environment and express natural and efficient navigation performance. The MWM involves a rodent swimming in a circular pool to find a camouflaged platform, navigating via extra-maze cues (Morris 1981). Versions of this task have been used extensively to test spatial learning and memory in rodents, and are known to be dependent on hippocampal function (Morris, Hagan, and Rawlins 1986).

We have used a recently published foraging-based task which was adapted from one used to test pigeons (Pearce et al. 2005). It has been shown to be sensitive to excitotoxic hippocampal lesions and amyloid pathology in the transgenic PDAPP mouse model (Evans et al. 2018). The task uses 6 pots arranged in a circular pattern similar to a radial arm maze, within an arena that was surrounded by extra-maze stimuli to enable spatial navigation. Each pot was baited with liquid reward and the mouse allowed to freely explore, foraging in the pots until every reward had been consumed, or the time limit was passed. Any return visit to a previously foraged pot was scored as an error, meaning the animal must utilise spatial working memory to distinguish which pots had been visited from their spatial location. A major advantage of this task was that animals did not have to undergo specific training more than simply associating the pots with liquid reward, which took place within its home cage. The task also does not involve aversive stimuli, relying on the rodent's natural exploratory and foraging behaviour. By drawing a curtain around the arena to obstruct view of the extra-maze cues and using individually patterned pots, one can manipulate this task to assess non-spatial working memory. Evans *et al* reported that performance of hippocampal lesioned mice was unaffected compared to sham surgery control mice in this version of the task (Evans et al. 2018).

Currently there are a limited number of studies demonstrating a robust spatial working memory in the APP knock-in mice developed by Takaomi Saido and colleagues. In fact, only their original paper has examined aged APP^{-NL-F} mice. They reported reduced

spontaneous alternation in the Y maze, which manifested at 18 months (Saito et al. 2014). While other groups have performed SWM tests with the *APP*$^{-NL-F}$ model, none aged mice beyond 12 months (Izumi et al. 2018, Shah et al. 2016). A summary of the current literature in which SWM tests have been used to assess performance of the *APP*$^{-NL-F}$ knock-in mice is presented in figure 1.3. Therefore, this study aimed to be the first to measure SWM proficiency in aged *APP*$^{-NL-F}$ mice since their original publication. In this experiment, *APP*$^{-NL-F}$ mice were tested alongside WT at 9-10 months and 16-17 months of age in spatial and non-spatial versions of the foraging task to characterise any age dependent or independent effects on performance.

5.3 Experiment 9: Spatial Working Memory in *APP*$^{-NL-F}$ Mice

▪ 5.3.1 – Introduction

Experiments 10 & 11 present assessment of working memory performance in the APP^{-NL-F} mouse model was carried out by using a foraging-based task, following a protocol adapted from that published by Evans *et al* (2018). APP^{-NL-F} and wild-type mice were split into two groups and each performed both spatial and non-spatial versions of the task in a counterbalanced order. The aim of this experiment was to investigate the performance of APP^{-NL-F} mice on this spatial working memory task, in order to see whether the deficit in visuospatial associative memory could be measured across multiple tasks. Results of this experiment will be compared to those from the non-spatial version of the task in order to dissociate spatial and non-spatial components. We hypothesised that the performance of APP^{-NL-F} mice on the spatial task would deteriorate with age and they would demonstrate an increased number of errors compared to wild-type mice, due to published studies identifying an age dependent deficit in SWM. Non-spatial performance was not expected to be sensitive to differences in genotype, consistent with results observed in novel object recognition.

▪ 5.3.2 – Methods

Subjects

The mice tested in this experiment were the same as those described in experiments 5 to 9. They were male APP^{-NL-F} (n=16) and WT (n=16) mice, tested at both 9-10 and 16-17 months of age.

Apparatus

White ceramic pots (9 cm diameter, 4.5 cm height, ProCook Ltd, UK) were used to contain the liquid reward (figure 5.2, A). Wooden platforms (3x4 cm) were used to raise the pots off the floor. Initial training was undertaken in the animal's home cage (48 x 15 x 13 cm, (L x W x H)), before the rest of the training and testing took place using the same arena and test room described in Chapter 2, including the same extra-maze cues. However, a 1 cm deep layer of wood chipping was spread over the floor throughout the foraging trials, matching the appearance of the home cage. All test trials were recorded using a USB camera connected to a laptop.

Design

Mice underwent the same testing protocol at two age points: 9-10 months & 16-17 months old. This protocol was 17 days long and is detailed in figure 5.1. Mice were water deprived to approximately 90% of their pre-experiment weight throughout the whole protocol. Water was made available for 2 hours after their training trials each day.

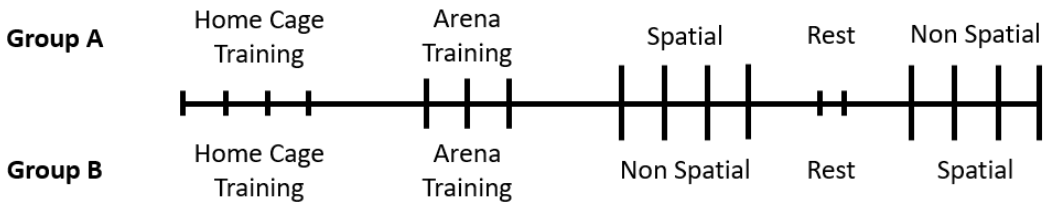


Figure 5.1: Experimental design for the foraging tasks. Each vertical line represents a separate day of training, testing or resting. Note that while training and rest days were consistent between groups, the order of test received was counterbalanced.

Procedure

Home Cage Training: Initial training involved the mice forming an association of the pots with a liquid reward. Once water deprived, the pots were filled with water and placed in the home cage for 4 hours (figure 5.2 A). On day 1, pots were placed on the floor of the cage, but were raised on the wooden platforms for days 2-4, in which the provided water level decreased. This ensured the mice learned to climb onto the pot and drink from the bottom, as they would in the test trials.

Test Arena Training: During days 5-7, mice were habituated to the arena and trained to find liquid rewards of 1:3 sweetened condensed milk (Nestle) solution prepared in water. Two pots were placed in the arena opposite each other, 40 cm apart, with locations varying each day to prevent development of any spatial bias during testing (figure 5.2, B).

On day 5, 120 μL of the solution was placed in the centre of the bottom of the pots and the mice were placed in the arena and allowed to explore until they had consumed the reward from both pots. After this, the mouse was removed and put back in the home cage, before the pots were cleaned with 70% ethanol. The same procedure was repeated for each mouse on days 6 and 7, however a 30 μL reward was used. By day 7 all mice were successfully consuming both rewards within 3 minutes.

Spatial Foraging Test: Mice received one test trial on each of day of test phase 1 or 2 (either days 8 to 11 or days 14-17). They performed either the spatial or non-spatial foraging tasks in a counterbalanced design, with no testing on days 12 and 13. The spatial task continued to use the same white pots and extra-maze cue layout, but involved 6 pots arranged in a circular shape, each 20 cm apart and containing 30 μL of the milk solution (figure 5.2, C). Mice were placed in the arena, always facing the same direction, and allowed to explore until the reward had been foraged from all six pots. Mice were replaced in their home cage; the pots were cleaned and fresh rewards placed in each of them. All test trials were recorded via a USB camera and saved onto an external hard drive.

Non-Spatial Foraging Task: A second group of mice performed the non-spatial task concurrently to the first group performing the spatial version. In this task, a black curtain was drawn around the arena to hide the extra-maze. The six pots were identical size and shape but each had a unique pattern on the external wall (figure 5.2, D). They were arranged in a circular pattern, and their individual locations changed throughout the four days of testing. Mice were transferred into the arena via the cardboard tubes so that they were always in the centre of the pots, facing the same wall. They were removed after the reward had been consumed from all six pots or 10 minutes has passed. The pots were wiped with 70% ethanol wipes and the reward was replenished. All trials were recorded as above.

Scoring: Foraging behaviour was described as when the mouse climbed upon the sides of the pot and directed its head down to find and consume any reward there. The order in which pots were foraged was recorded, and an error was counted when a mouse returned to a pot which it had already visited in that trial. The number of errors was averaged for each mouse across their four trials of each test, before a mean number of errors was calculated for each group in each version of the task. These group means were statistically analysed as described in chapter 2.

Measurement	Definition
Error	A mouse returning to a pot in which the reward has already been consumed.
Neighbouring Error	A mouse making an error by visiting the pot adjacent to the one it has just foraged in.
Distal Error	A mouse making an error by visiting a pot one or more pots away from the one it has just foraged in.
Perseverative Error	A mouse immediately returning to the pot it has just foraged in.
Chaining Response	A mouse visiting three or more consecutive pots that are immediately adjacent to one another in one direction.
Initiation	The time taken for the mouse to forage in the first pot of a session.

Table 5.1: Definitions of the different measurements used to assess performance in the foraging-based working memory task.

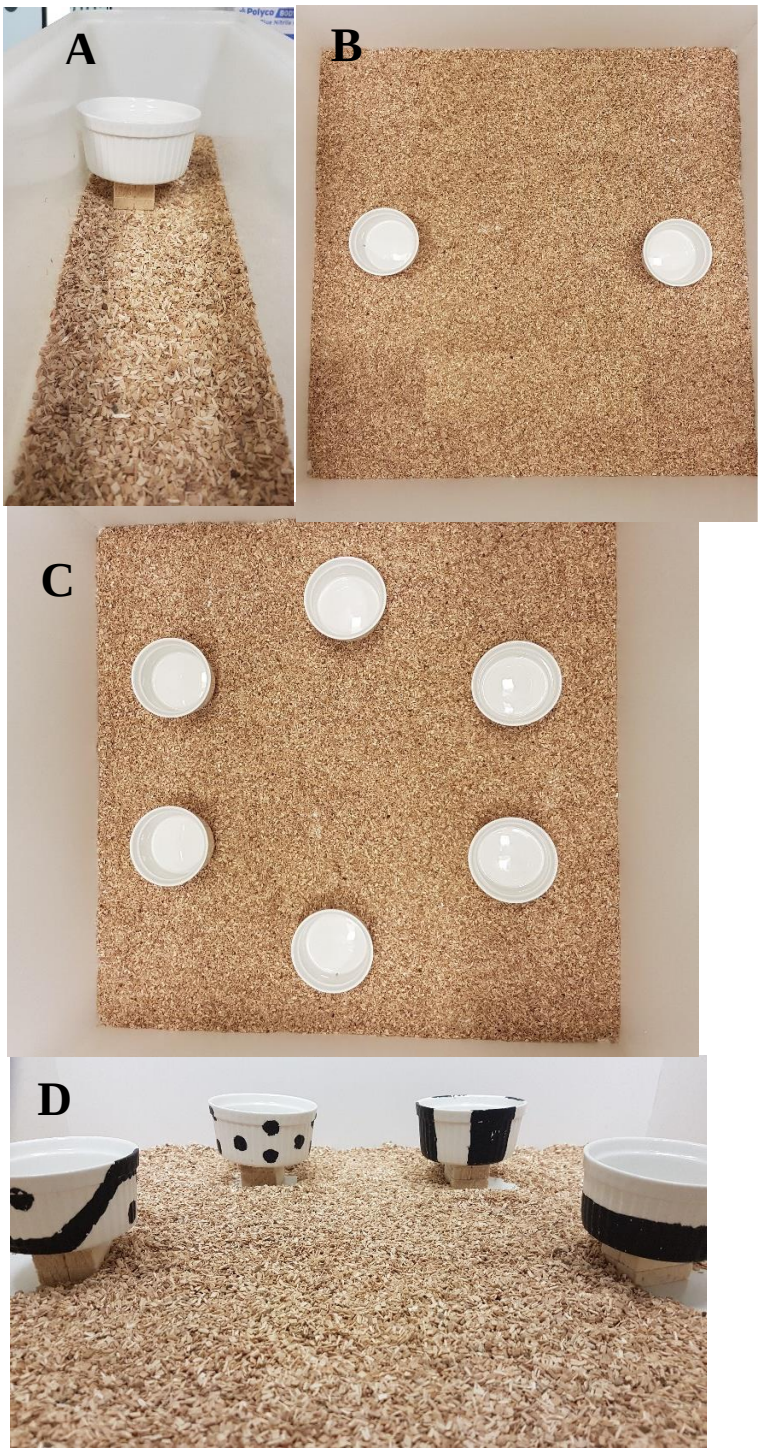


Figure 5.2: Photographs of the ceramic pots used throughout the foraging experiment.
(A) Position of the pots in the initial home cage training phase. (B) In the arena training phase, two pots were placed opposite each other. (C) Arrangement of the six pots in the foraging task test phase. (D) Distinctly patterned pots used in the non-spatial foraging task experiment, arranged in their test positions with the curtain drawn around the arena.

5.3.3 – Experiment 9 Results

Spatial Foraging Task

The number of errors scored by the WT and *APP*$^{-NL-F}$ mice was recorded at two age-points and is presented in Figure 5.3. Visual inspection of figure 5.3 suggested that WT performance improved with age, while the opposite occurred for the *APP*$^{-NL-F}$. All datasets were normally distributed (Shapiro-Wilke test $p>0.05$), and therefore were eligible for a repeated measures ANOVA, with age as a within subjects' factor and genotype as the between subjects' factor. The ANOVA revealed no main effects of age or genotype, but a significant age*genotype interaction $F(1,30) = 7.15$, $p=0.012$.

Tests for simple main effects following the significant interaction revealed that the *APP*$^{-NL-F}$ mice made significantly more errors at the 16-17-month aged timepoint relative to their test at a younger age ($p=0.039$). There were no other significant simple main effects: age on WT (9-10 vs 16-17 months) $p=0.116$; genotype at 9-10 months (WT vs *APP*$^{-NL-F}$) $p=0.122$; genotype at 16-17 months (WT vs *APP*$^{-NL-F}$) $p=0.109$.

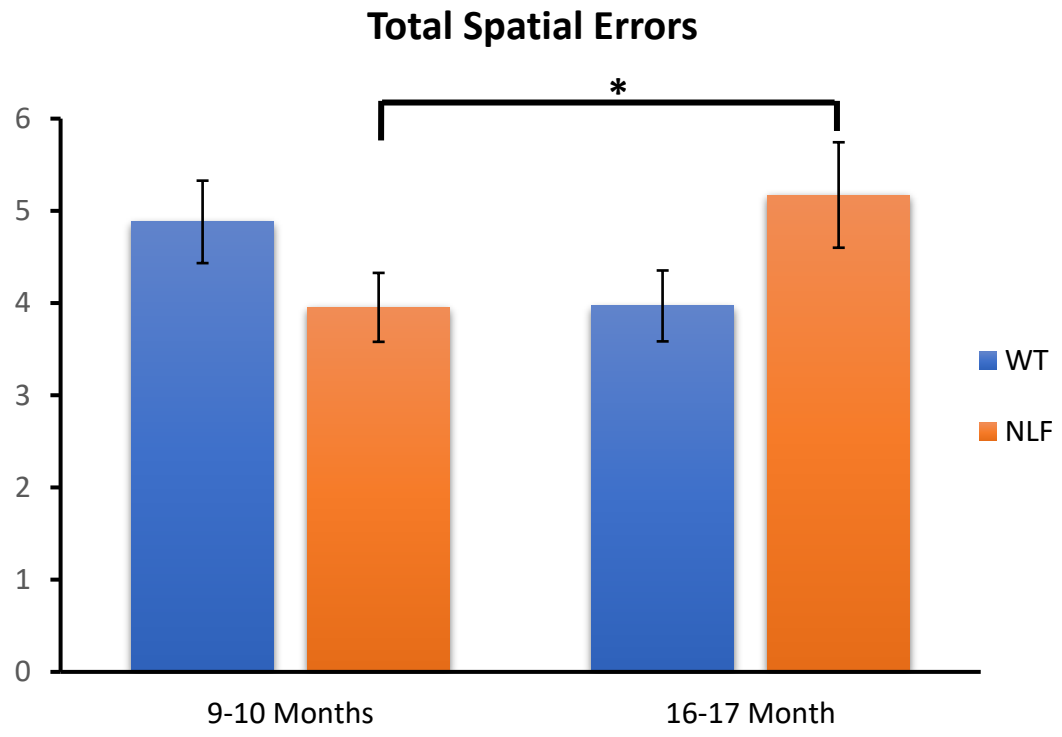


Figure 5.3: *APP*$^{-NL-F}$ mice have an age-dependent decline in the spatial foraging task. Total errors by WT and *APP*$^{-NL-F}$ mice at both 9-10 and 16-17 months of age. Error bars represent standard error of the mean. N=16 for all groups. ***** = $p<0.05$

While the number of basic errors gives an indication of spatial working memory performance of the mice at both age-points, other parameters were recorded in order to assess other aspects of behaviour such as engagement, compulsivity and foraging strategy. Table 5.2 presents the data for the initiation time, chaining responses and perseverative errors that are defined in table 5.1. The datasets for the initiation times were transformed by log10 transformation to render them all normally distributed. They were analysed by a repeated measures ANOVA with age as a within subjects' factor and genotype as a between subjects' factor. The ANOVA revealed no main effect of age $F(1,30) = 0.83$, $p=0.370$; no main effect of genotype $F(1,30) = 0.22$, $p=0.638$ and no significant interaction $F(1,30) = 0.618$, $p=0.438$.

The datasets for the number of chaining responses were all normally distributed and analysed in by the same ANOVA test. Similarly, there was no main effect of age $F(1,30) = 1.3$, $p=0.257$; no main effect of genotype $F(1,30) = 0.34$, $p=0.566$ and no significant interaction $F(1,30) = 0.057$, $p=0.812$.

The number of perseverative errors were analysed by non-parametric tests due to the large presence of zeros in the data. The Mann-Whitney U test was used to compare the two genotypes at each age-point and revealed no significant effect at 9-10 months ($U = 138$, $z = 0.39$, $p=0.724$); or 16-17 months of age ($U = 107$, $z = -0.841$, $p=0.445$). The effect of the within subjects' factor (age) was analysed by exact sign tests which revealed no significant median difference (0.0) in the number of perseverative errors for either the WT ($z = -0.40$, $p=0.690$) or the *APP*$^{NL-F}$ mice ($z = -0.289$, $p=0.774$).

Foraging strategy was assessed by comparison of whether the mice were more likely to make errors in pots that were either neighbouring or distal to the pot that had just been foraged in. The data was expressed as a ratio of error location : total errors so as to negate the effect of differences in the total number of errors made, and this is presented in figure 5.4. All data groups exhibited normal distributions, so they were analysed by a repeated measures ANOVA with error location and age as within-subjects' factors, while genotype was a between subjects factor. The ANOVA reported no significant main effects: error location $F(1,30) = 3.16$, $p=0.086$; age $F(1,30) = 0.97$, $p=0.333$; genotype $F(1,30) = 1.1$, $p=0.300$. There were also no significant interactions: age*error location $F(1,30) = 2.2$, $p=0.147$; age*genotype $F(1,30) = 0.0$, $p=1.0$; error location*genotype $F(1,30) = 0.297$, $p=0.590$; age*error location*genotype $F(1,30) = 0.014$, $p=0.906$.

Measurement	Genotype	9-10 Months		16-17 Months	
		Mean	SD	Mean	SD
Initiation /s	WT	**20**	18	**19**	22
	APP^{NL-F}	**15**	10	**22**	22
Chaining Responses	WT	**0.88**	0.51	**1.04**	0.48
	APP^{NL-F}	**0.98**	0.50	**1.09**	0.58
Perseverative Errors	WT	**0.27**	0.25	**0.38**	0.43
	APP^{NL-F}	**0.33**	0.27	**0.30**	0.48

Table 5.2: Comparison of the mean initiation time, number of chaining responses and perseverative errors recorded in the spatial foraging task. Results are presented of both genotypes at 9-10 and 16-17 months of age. Standard deviation is also shown. n=16 for all groups.

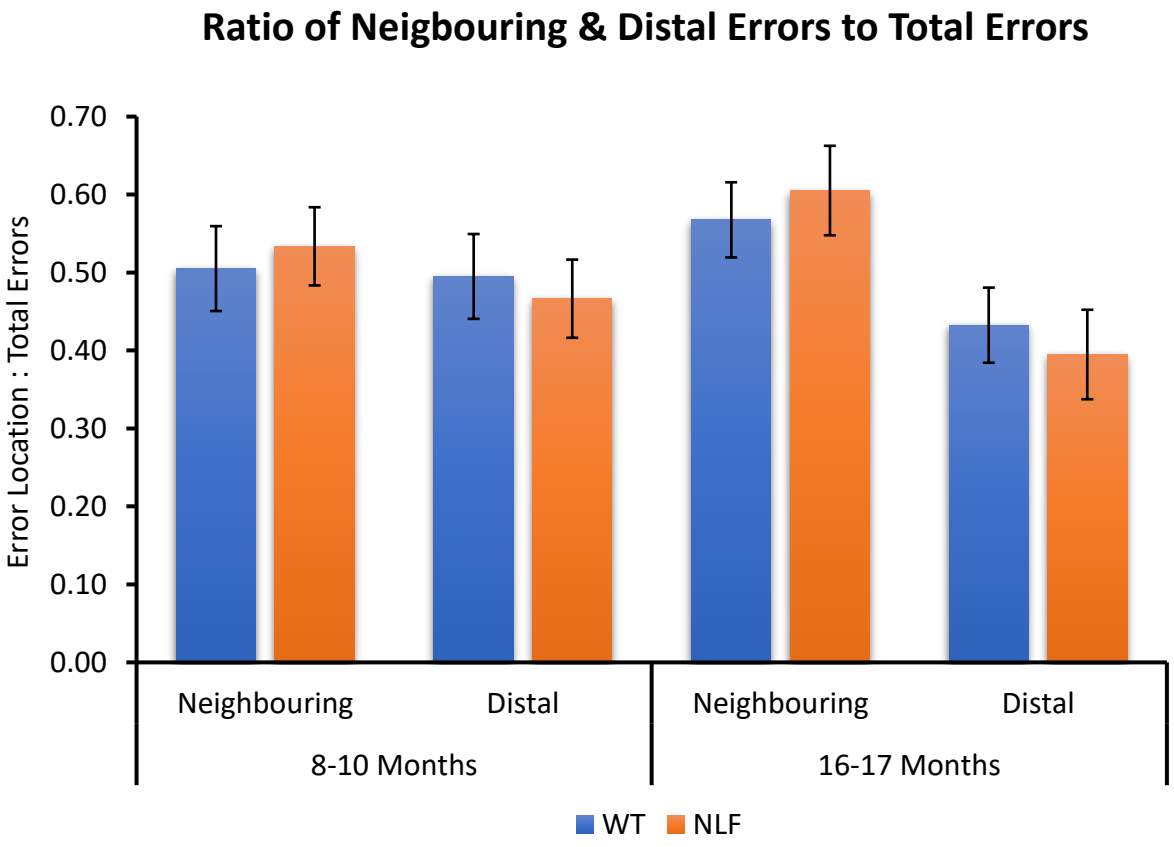


Figure 5.4: Comparison of the locations of errors made by *APP*$^{NL-F}$ and WT mice in the spatial foraging task. The proportion of both neighbouring and distal errors is presented at 9-10 and 16-17 months of age. Error bars represent SEM.

5.4 Experiment 10: Non-Spatial WM in *APP*$^{NL-F}$ Mice

▪ 5.4.1 – Experiment 10 Results

Non-Spatial Foraging Task

The non-spatial foraging task assessed hippocampal independent working memory performance, as each pot was individually patterned. Inspection of the data in figure 5.5 indicated that both groups maintained a consistent number of total errors throughout the two timepoints. The data were normally distributed as checked by the Shapiro-Wilke test ($p>0.05$), and were analysed by a repeated measures ANOVA with age as the within subjects' factor and genotype as the between subjects' factor. Results of the test revealed no significant main effect of age $F(1,30) = 0.089$, $p=0.767$; or genotype $F(1,30) = 0.01$, $p=0.921$; and no significant interaction $F(1,30) = 0.001$, $p=0.973$.

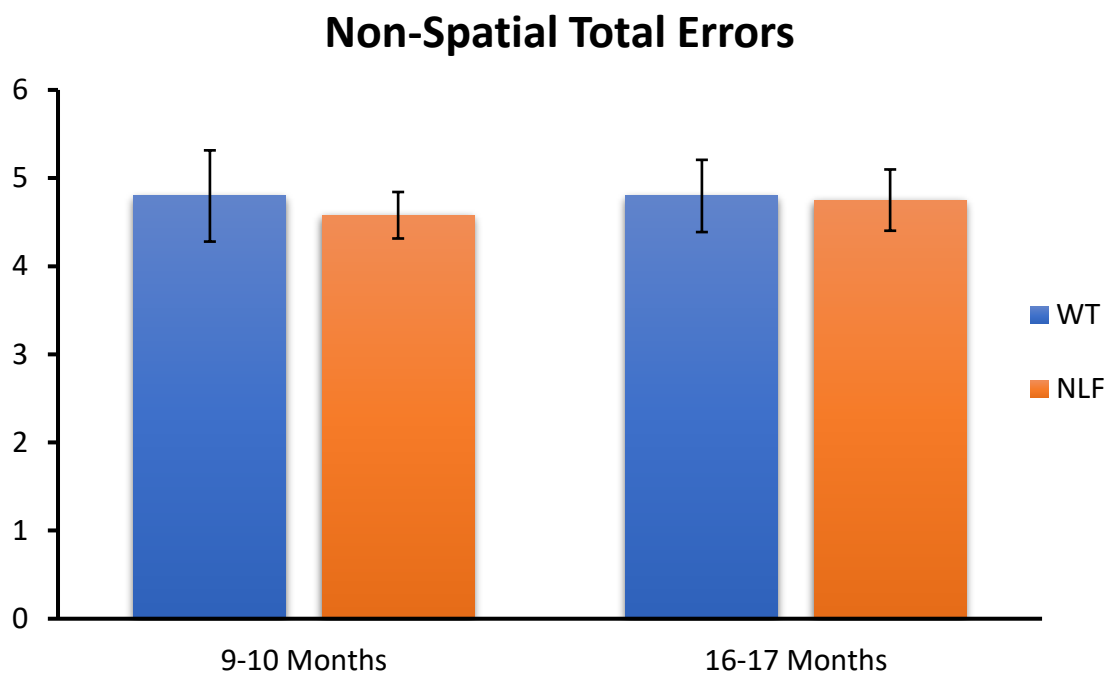


Figure 5.5: *APP*$^{NL-F}$ mice displayed comparable performance to WT controls in the non-spatial foraging task. Total errors scored in the non-spatial foraging task by WT and *APP*$^{NL-F}$ mice at 9-10 and 16-17 months of age. Error bars represent standard error of the mean. N=16 for all groups.

Table 5.3 presents the data for the time required to initiate engagement with the task as well as the number of chaining responses and perseverative errors. The distribution of the initiation times was not normal in all groups, and so the data was transformed by log10 transformation to ensure that p>0.05 in the SW test. The data were analysed by a repeated measures ANOVA with age as a within subjects' factor and genotype as a between subjects' factor. The ANOVA revealed no main effect of age $F(1,30) = 0.38$, $p=0.540$; no main effect of genotype $F(1,30) = 0.32$, $p=0.579$ and no significant interaction $F(1,30) = 0.13$, $p=0.717$.

The presence of zeros in the number of chaining responses resulted in the data being analysed by non-parametric tests. The two genotypes were compared by Mann-Whitney U tests which revealed no significant differences at 9-10 months ($U = 92$, $z = -1.38$, $p=0.184$); or 16-17 months of age ($U = 139$, $z = 0.407$, $p=0.696$). The within subjects' effect of age was assessed by exact sign tests. There was no significant difference in the median number of chaining responses in 9-10 and 16-17-month-old WT mice (0.0), $z = 0.0$, $p=1.0$. There was also no significant median difference between the two ages for *APP*$^{-NL-F}$ chaining responses (0.25), $z = 1.1$, $p=0.267$.

The number of perseverative errors were also analysed by non-parametric tests. The Mann-Whitney U test revealed no significant differences between the two genotypes at 9-10 months ($U = 143$, $z = 0.57$, $p=0.590$); or 16-17 months of age ($U = 146$, $z = 0.681$, $p=0.515$). An exact sign test revealed no statistically significant median difference (0.125) in the total number of perseverative errors made by the WT mice at the two age-points ($z = 0.87$, $p=0.388$). There was also no significant median difference (0) between the two ages for the *APP*$^{-NL-F}$ mice ($z = 0.0$, $p=1.0$).

The proportions of neighbouring and distal errors are presented in figure 5.6. All datasets were normally distributed and were analysed by a repeated measures ANOVA with error location and age as within-subjects' factors, while genotype was a between subjects factor. The ANOVA reported a significant main effect of error location $F(1,30) = 4.6$, $p=0.04$. There was no significant effect of age $F(1,30) = 0.0$, $p=1.0$ or genotype $F(1,30) = 2.1$, $p=0.154$. There were also no significant interactions: age*error location $F(1,30) = 0.001$, $p=0.971$; age*genotype $F(1,30) = 0.0$, $p=1.0$; error location*genotype $F(1,30) = 0.004$, $p=0.953$; age*error location*genotype $F(1,30) = 0.028$, $p=0.868$.

Measurement	Genotype	9-10 Months		16-17 Months	
		Mean	SD	Mean	SD
Initiation (s)	WT	**14**	9	**17**	14
	APP^{NL-F}	**17**	11	**18**	12
Chaining Responses	WT	**1.0**	0.14	**0.95**	0.10
	APP^{NL-F}	**0.75**	0.09	**0.97**	0.08
Perseverative Errors	WT	**0.27**	0.27	**0.34**	0.39
	APP^{NL-F}	**0.39**	0.49	**0.46**	0.46

Table 5.3: Comparison of the mean initiation time, number of chaining responses and perseverative errors recorded in the non-spatial foraging task. Results are presented of both genotypes at 9-10 and 16-17 months of age. Standard deviation is also shown.

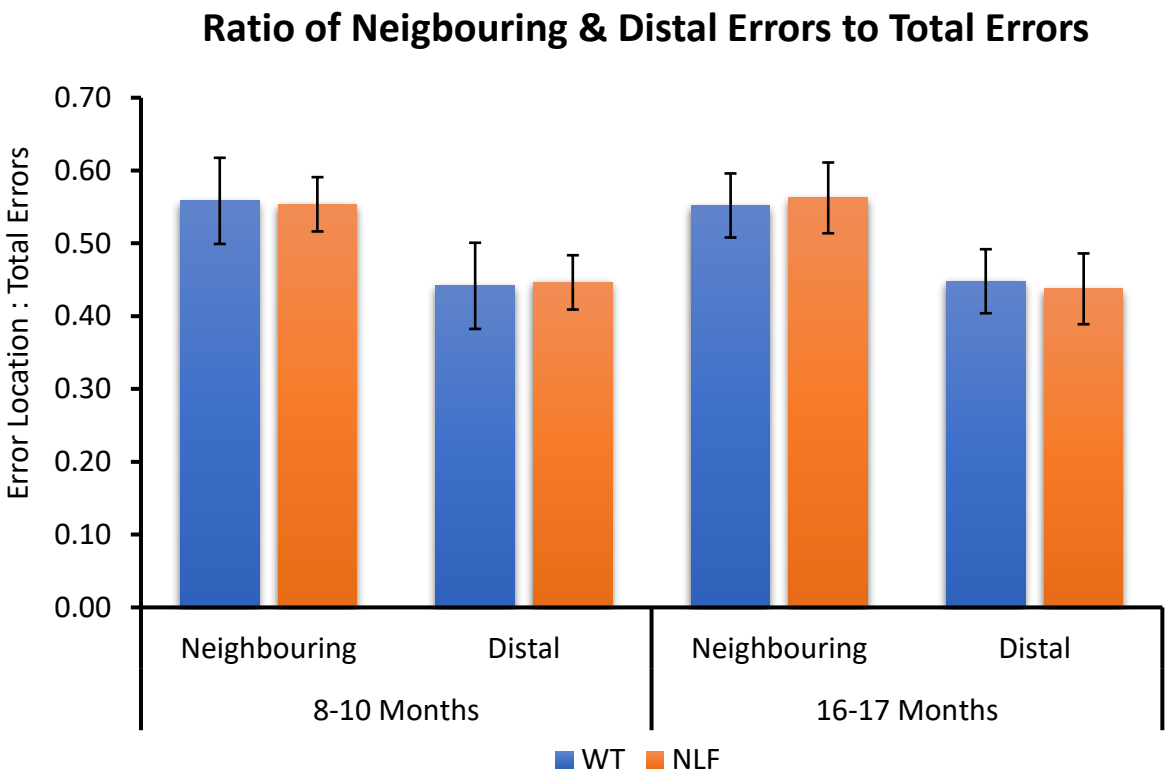


Figure 5.6: Comparison of the locations of errors made by *APP*$^{NL-F}$ and WT mice in the non-spatial foraging task. The proportion of both neighbouring and distal errors is presented at 9-10 and 16-17 months of age. Error bars represent SEM.

5.4 Chapter Discussion

Experiments within this chapter assessed working memory in the *APP*$^{NL-F}$ knock-in mouse model via a foraging-based paradigm. By manipulating the existence of intra and extra maze cues, spatial and non-spatial working memory was examined. The results revealed an age-dependent deterioration in the *APP*$^{NL-F}$s in the spatial version of the task in the absence of significant genotypic differences. There were no effects of age or genotype in the non-spatial task. This result is consistent with the age-related decline in SWM performance of transgenic PDAPP mice in this task, while a previous study has also reported a decline in *APP*$^{NL-F}$ mice (Saito et al. 2014, Evans et al. 2018, Evans et al. 2019). While the original paper by Saito *et al* presented deficits in Y-maze spontaneous alternation at 18 months, subsequent reports have not assessed mice aged beyond 12 months (Izumi et al. 2018, Shah et al. 2018). Consistent with these studies, we observed that *APP*$^{NL-F}$ mice exhibited performance comparable to WT at 9-10 months of age. To our knowledge this is the first experiment to replicate the deficit in spatial working memory presented by Saito and colleagues.

Although there is a very limited literature with which to compare the results of the *APP*$^{NL-F}$ mice, the *APP*$^{NL-G-F}$ knock-in model has garnered greater interest. However, results of SWM assessment have proved controversial. The spontaneous alternation deficit identified at 6 months of age by Saito *et al* (2014) was not replicated by Whyte *et al* (2018), who suggested the phenotype may not be robust to changes in environmental factors. One group used the Barnes maze to assess SWM in the *APP*$^{NL-G-F}$. This is a circular platform with numerous holes around the perimeter, one of which leads to the "target box", a dark pocket that becomes the animal's refuge to escape from the open platform, due to their natural preference for a dark environment (Barnes 1979). Male *APP*$^{NL-G-F}$ were tested at 4, 6 and 8 months and exhibited similar performance to WT at 4 & 6, but developed subtle deficits in learning the spatial location of the target box at 8 months (Sakakibara et al. 2018). Testing was not carried out at older age points, potentially because performance of WT deteriorates with age (Barnes and McNaughton 1985). The Morris water maze has been a more popular tool for examining SWM, with four groups using it to tested *APP*$^{NL-G-F}$ mice. Two of these groups observed no effect of genotype with mice aged 6 and 11 months respectively (Whyte et al. 2018, Latif-Hernandez et al. 2017). However, two groups published data showing reduced learning and memory efficiency. Mehla *et al* measured this at 6 and 12 months of age, while the Sakakibara group only tested 24 month old *APP*$^{-}$

$^{NL\text{-}G\text{-}F}$ mice (Mehla et al. 2019, Sakakibara et al. 2019). The pattern of discrepant results is potentially due to the use of different protocols or environmental factors, but a more compelling argument could be made for disparity in sex, age and the nature of control groups used. For example, while most groups tested male mice against C57Bl/6 controls, the Latif-Hernandez group measured female *APP*$^{-NL\text{-}G\text{-}F}$ mice versus *APP*$^{-NL}$ mice as controls, which express the Swedish mutation and do not develop amyloid pathology despite increased soluble beta-amyloid (Salas et al. 2018, Sakakibara et al. 2019). The use of this control line may have limited the ability to detect a deficit related in amyloid production.

While the current experiment presents an age-related deficit, it is important to note the comparison of errors made by the *APP*$^{-NL\text{-}F}$ and WT groups was non-significant. The significant age*genotype interaction was driven by the significant within-subjects comparison of the *APP*$^{-NL\text{-}F}$ group at the two ages. The current literature does not include experimental designs where this comparison is reported, which may suggest the explanation for the apparent lack of cognitive effects in the *APP*$^{-NL\text{-}G\text{-}F}$ studies. The interaction included a non-significant tendency for the WT to have more errors than the *APP*$^{-NL\text{-}F}$ mice at 9-10 months, and for this to reduce with age. Their numerical improvement with age can be rationalised simply as an effect of repeated testing, as prior experience in the radial arm maze can improve performance (Hall and Berman 1995). Furthermore, the WT mice tested by the Evans group seemed to make fewer errors at 14-16 months than 10-12, although this was not part of their formal analysis (Evans et al. 2018). As for the non-significant trend for increased errors by WT mice at 9-10 months it is tempting to speculate, considering the context of this thesis, that this is an effect of marginally increase levels of beta-amyloid, which has been shown to allow improved spatial memory performance in the water maze (Puzzo et al. 2008). The *APP*$^{-NL\text{-}F}$ mice express the Swedish mutation at endogenous levels, which would lead to a gradual increase in beta-amyloid generation. There will be a point where the *APP*$^{-NL\text{-}F}$ hippocampi contain a concentration that is increased by a picomolar magnitude, as was applied in the experiment by Puzzo and colleagues, and it is possible that this experiment occurred within that window. Further evidence for this theory comes from the original paper by Saito *et al*, in which spontaneous alternation was compared between *APP*$^{-NL\text{-}F}$, *APP*$^{-NL=G\text{-}F}$ and WT at 6 months of age. The *APP*$^{-NL\text{-}F}$ performance appears superior to that of the WT, however this apparent effect was either not analysed nor discussed (Saito et al. 2014). *APP*$^{-NL\text{-}F}$ performance also exceeded that of WT controls in place preference reversal learning during

Intellicage experiments (Masuda et al. 2016). This comparison is purely speculative, but provides a hypothesis to account for the disparity between the genotypes at 9-10 months and strengthens the result of the mixed measures ANOVA. Further assessment of the *APP*$^{-NL-F}$ model at this age would be interesting in order to evaluate whether the trend observed in the current study was real. Collectively, the interaction of age and genotype in the spatial version of this foraging task represents an age-dependent decline in spatial working memory for the *APP*$^{-NL-F}$.

One drawback with using an appetitive reward is that water deprivation must be used to ensure engagement and motivation to complete the task. This allows performance to become susceptible to individual variations in body weight or hunger (Vorhees and Williams 2014). In this way this foraging task is similar to the RAM, in that ideally all subjects would need to be equally motivated to consume the food reward. No variations in body weight have been reported for the knock-in mice, but there is evidence that motivation decreases with amyloidosis, especially in the striatum (Hamaguchi et al. 2019). In these experiments, we measured no differences in engagement (time until the first pot was foraged) between the genotypes, indicating that motivation to complete the task was consistent between them. It is worth mentioning that the foraging-based tasks used in this chapter may be a more reliable measure of working memory in mice than other tasks such as the MWM, in which forced swimming induces a high stress response, as measured by release of glucocorticoids (Aguilar-Valles et al. 2005). Elevated stress levels may confound results by affecting cognitive performance independent of baseline hippocampal function (Francis et al. 1995, Lupien and McEwen 1997). Furthermore, chronic stress exacerbates amyloid pathology and expedites the age of onset of cognitive deficits (Green et al. 2006, Jeong et al. 2006, Srivareerat et al. 2009). The potential of these effects to confound results has theoretically been limited in this study due to the avoidance of the MWM task; although in absence of a specific comparison of stress response induced by both tasks, this cannot be concluded for certain.

The design of this working memory task allows analysis of foraging strategies, as well as the dissociation of performance when using extra or intra maze cues. Meanwhile, the publication of transgenic PDAPP performance on this task by Evans *et al* (2018) permits direct comparison of second-generation AD model performance to that of a first-generation model, as well as hippocampal lesioned animals. PDAPP mice scored a similar number of errors as WT littermates at 6-8 & 10-12 months, but significantly increased

errors at 14-16 months old. Unlike the study by Evans and colleagues, this experiment did not reveal any age-dependent and independent differences in strategy used by the AD model compared to WT controls. PDAPP mice exhibited significantly more chaining behaviour, and a greater likelihood of going from one pot to neighbouring pots rather than distal ones from 6 months old (Evans et al. 2018). This effect is not uncommon and has been reported in animals with hippocampal lesions or transgenic models of amyloid pathology in the radial arm maze and Morris water maze (Olton and Werz 1978, Huitron-Resendiz et al. 2002). Considering the *APP*$^{-NL-F}$ mice did not demonstrate altered foraging strategy, and the PDAPP effect was age-independent, it suggests that it was caused by APP overexpression, rather than the accumulation of amyloid pathology. Recent studies using the *APP*$^{-NL-F}$ have highlighted such mechanisms, for example chronically elevated activation of the calpain-calpastatin pathway (Saito et al. 2016). Calpains are known to play an important role in synaptic activity and aberrant manipulation could influence working memory processes (Briz and Baudry 2017). In fact, inhibition of calpains in the APP/PS1 transgenic mouse model rescued spatial working memory performance, giving further evidence that deficits in transgenic mice may be due to artefacts induced by the genetic methodology employed to generate them (Trinchese et al. 2008). This further validates the *APP*$^{-NL-F}$ model which was generated to eradicate artefacts of overexpression and therefore be a more reliable tool to model mechanisms and pathology of Alzheimer's Disease.

The current study also identified no differences in perseverative behaviour (repeated responses without reward) in this experiment, which defined it as a mouse immediately returning to a pot it has just left. Meanwhile, 14-16-month old PDAPP mice accumulated a significantly greater number of these errors than their WT counterparts (Evans et al. 2018). The authors corrected for perseverative errors when reporting the total errors made by both genotypes in their study, in order to eradicate any potential confounding effects of this behaviour. However, as the *APP*$^{-NL-F}$ did not demonstrate this difference to controls, there was no need to apply this correction to the data in this chapter. It is worth noting that the measurement of perseverative behaviour in this study is similar to the compulsivity tests performed by Masuda *et al* in the IntelliCage characterisation of the knock-in AD strains. That paper reported significantly increased compulsivity from 8months (Masuda et al. 2016). Increased returns to the same pot / arm / location have been associated with hippocampal lesions previously, as well as transgenic models of Alzheimer's (Jarrard 1983, Daumas et al. 2008, Honey et al. 2007, Evans et al. 2018).

Therefore, perseverative behaviour may reflect the degree of dysfunction; while *APP*$^{-NL-F}$ mice have a comparatively mild pathology manifesting in compulsivity not being consistently observed across multiple test paradigms.

In conclusion, this chapter presents the first replication of a spatial working memory deficit in the *APP*$^{-NL-F}$ mouse model. While there were no effects of age or genotype on the non-spatial version of the foraging task, experiment 9 revealed a significant decline in performance between 9-10 and 16-17 months of age in *APP*$^{-NL-F}$ mice. Furthermore, the lack of genotypic differences in engagement, foraging strategy or perseverative behaviour further validates knock-in models of amyloid pathology as more reliable reconstructions of familial Alzheimer's Disease than transgenic lines.

Chapter 6 –

Biochemical Characterisation of Aged *APP*$^{-NL-F}$ Mice

6.1 Chapter Overview

Following behavioural characterisation of the APP-NL-F mice, biochemical analyses were undertaken to investigate potential changes in hippocampal receptor expression that may underpin the cognitive impairment reported in chapter 4. *APP*$^{-NL-F}$ mice accumulated soluble and insoluble Aβ42 with age. This was specifically due to the Swedish and Iberian mutations present in their endogenous APP gene, and not due to increased expression of APP, validating the rationale of using these mice as second-generation, non-transgenic models. *APP*$^{-NL-F}$ mice also demonstrated an age-dependent increase in the inflammatory cytokine IL-1β which indicates an increased immune response with age.

6.2 Chapter Introduction

The genetic techniques involved in engineering the *APP*$^{-NL-F}$ knock-in mice provides an opportunity to characterise the biochemical progression of amyloid pathology in the absence of abnormal APP overexpression seen in first-generation APP transgenic mice. The current literature concerning the impact of the Swedish and Iberian knock-in mutations on neuronal function is limited. However, the age-related accumulation of amyloid species, as well as deposition of amyloid plaques, was reported in the initial study by Saito *et al* (2014). They observed plaques detectable in the cortex and the hippocampus from 15 months of age. The proportion of the cortical and hippocampal area that displayed plaque deposition was 10% and 7% respectively in 24-month-old mice. Meanwhile, Saito *et al* also reported augmented inflammatory responses around plaques in aged *APP*$^{-NL-F}$ mice, as reactive gliosis was detectable by immunofluorescence analysis due to increased reactivity of antibodies to GFAP (glial fibrillary acidic protein) and IbaI, which are markers of astrocytosis and microgliosis. Excessive engagement of inflammatory pathways is a common feature of transgenic APP mouse models as well as human patients and is thought to be a downstream effect of amyloid pathology (see section 1.3). Although amyloid accumulation in humans leads to tau pathology, this is not usually observed in murine models of FAD expressing mutations in APP alone. Saito *et al* (ibid.) reported no tau hyperphosphorylation but did not publish the data. Due to the dearth of studies involving aged cohorts of *APP*$^{-NL-F}$ mice, it would be interesting to investigate any presentation of the biochemical hallmarks of both human AD and transgenic mouse models for comparison with the results of the Saito group.

The decrease in immunoreactivity of the synaptic marker PSD95 that is common to APP transgenic models and has been recapitulated in aged *APP*$^{-NL-F}$ mice, indicating reduced density of synaptic connections (Saito et al. 2014, Zhang, Song, et al. 2015). However, little is known about the impact of the APP-KI on glutamate receptor dynamics that may underpin the cognitive decline of *APP*$^{-NL-F}$ mice reported in chapters 4 and 5. Increased production of Aβ has been linked to changes in synaptic plasticity in the hippocampus related to increased removal of glutamate receptors from the synapse, and despite electrophysiological techniques not being performed in the current study, an impairment in hippocampal LTP induction was previously observed in 6-month-old *APP*$^{-NL-F}$ mice (Zhang et al. 2016). Dephosphorylation of GluA1 results in internalisation of AMPA receptors, and a reduction in pGluA1 has been reported in the *APP*$^{-NL-G-F}$ knock-in model alongside a hippocampal LTP impairment (Moriguchi et al. 2018). Therefore, one may hypothesise that the aged *APP*$^{-NL-F}$ mice would exhibit similar alterations in AMPA receptor dynamics.

The aim of this experiment was to characterise biochemical features in the hippocampi of aged *APP*$^{-NL-F}$ mice that may relate to any underlying pathology of the model and offer a mechanism for the behavioural changes reported in chapters 4 and 5. The proposed analysis included age-related changes in the concentration of soluble and insoluble Aβ species, as well as inflammatory markers. The hypothesis of this experiment was that *APP*$^{-NL-F}$ mice would demonstrate increased Aβ accumulation with age in the absence of APP overexpression, as well as augmented detection of inflammatory markers compared to WT controls. Decreased activation of NMDA and/or AMPA receptors in the hippocampus, which are known to impact visuospatial memory, were also expected to elucidate putative mechanisms by which the accumulation of Aβ may have led to the observed decline in cognitive performance.

6.3 Experiment 11: APP Processing in *APP^{NL-F}* Mice

▪ 6.3.1 – Introduction

The aim of this experiment was to characterise the expression levels of proteins involved in APP metabolism and assess any accumulation of Aβ species with age in *APP^{NL-F}* mice. Due to the specific nature of the knock-in method leading to expression of the Swedish and Iberian APP mutations, it was hypothesised that Aβ (particularly Aβ42) would accumulate and aggregate into insoluble plaques with age, in the absence of changes to APP expression.

▪ 6.3.2 – Methods

Subjects, Design

The samples analysed in this experiment were hippocampi isolated from the same cohort of WT and *APP^{NL-F}* mice reported in chapters 4 and 5. However, the eldest five *APP^{NL-F}* mice were not included in this *ex vivo* analysis as they were involved in a surgical pilot study. Four *APP^{NL-F}* mice from another cohort that had undergone identical behavioural testing were culled at 16 months of age and included in this experiment resulting in an n of 15 for the aged *APP^{NL-F}* group. Aged WT mice (n=16), 4-month old WT (n=9) and 4-month old *APP^{NL-F}* (n=9) were compared to the aged *APP^{NL-F}* group. Animals were culled between 72 and 96 hours following their final behavioural test and the hippocampus removed and flash-frozen in liquid nitrogen. Biochemical procedures including protein extraction, ELISA and Western blots were carried out by Chiara Favero under my supervision, and analysis of all data was carried out by myself. Protein was extracted from these hippocampi as described in chapter 2. The left hemisphere was utilised to produce tissue homogenate, while synaptosome preparations were obtained from the right hemispheres. ELISA and Western blot protocols have been described in chapter 2 unless otherwise stated.

Il-1β and TNFα ELISA

ELISA kits quantifying the concentration of inflammatory markers IL-1β (#DY401) and TNFα (#DY410) were obtained along with the ancillary reagent kit 2 (#DY008) from R&D Systems (Minnesota, USA). Reagents and protocols were consistent for the identification of both markers, unless specified. The capture antibodies were reconstituted in 0.5 mL sterile PBS and diluted to the working concentration (8 µg/mL for

anti-IL-1β and 1.33 μg/mL for anti-TNFα). 100 μL of the diluted capture antibody was loaded onto 96-well plates and left at RT overnight. The next morning, wells were aspirated and washed 3 times with diluted wash buffer before being filled with 300 μL diluted reagent diluent and incubated for a minimum of 90 minutes. Following another plate wash cycle, 100 μL of either serially diluted protein standards or samples of hippocampal homogenate were loaded onto the plate before a 2-hour incubation. The plate was then washed and 100 μL detection antibody was loaded for another 2-hour incubation (200 ng/mL anti-IL-1β or 75 ng/mL anti-TNFα). The detection antibody was then washed off before 100 μL diluted streptavidin-HRP solution (1:40) was loaded into the wells for 20 minutes. Colour reagents A and B were mixed in a 1:1 ratio and 100 μL was pipetted into each well after the streptavidin HRP had been washed out. After 30 minutes the reaction was halted by the addition of 50 μL stop solution. Absorbance was read at 450 nm and the concentrations were calculated by inputting the standard curve into GraphPad software.

▪ 6.3.3 – Experiment 11 Results

APP Metabolism

ELISA kits were utilised in order to measure the accumulation of Aβ species in aged versus young *APP*$^{NL-F}$ mice and the results are presented in figure 6.1. The figure shows Aβ40 (A) and Aβ42 (B) concentrations in the soluble fraction of the hippocampal homogenate. Both the young and aged groups exhibited normal distributions (Shapiro-Wilke test $p>0.05$) and were compared by independent samples t-tests. There was a significant increase in Aβ40 quantity $t(22) = 2.67$; $p=0.014$; as well as Aβ42 $t(22) = -13$, $p<0.0005$. The insoluble fraction (6.1 C) was assessed for Aβ42 concentration only, due to only 20% of *APP*$^{NL-F}$ mice exhibiting a detectable signal in the soluble Aβ40 ELISA (6.1 A). The distributions of young and aged insoluble Aβ42 demonstrated opposite skewness and so were compared by the non-parametric Mann-Whitney U test which revealed a significant increase in the aged mice $U = 122$, $z = 3.25$, $p=0.001$.

Western blot analysis was used to determine the expression levels of APP and BACE1 in young and aged WT mice (figure 6.2). A one-way ANOVA revealed significant differences between the groups $F(3,48) = 5.61$, $p=0.002$. Bonferroni *post-hoc* tests demonstrated that the aged WT mice had significantly greater expression of APP than both the young and aged *APP*$^{NL-F}$ groups ($p=0.034$ and $p=0.003$ respectively, figure 6.2 B).

Meanwhile, assessment of BACE1 expression by one-way ANOVA revealed no significant differences between groups F(3,48) = 0.95, p=0.426 (figure 6.2 C).

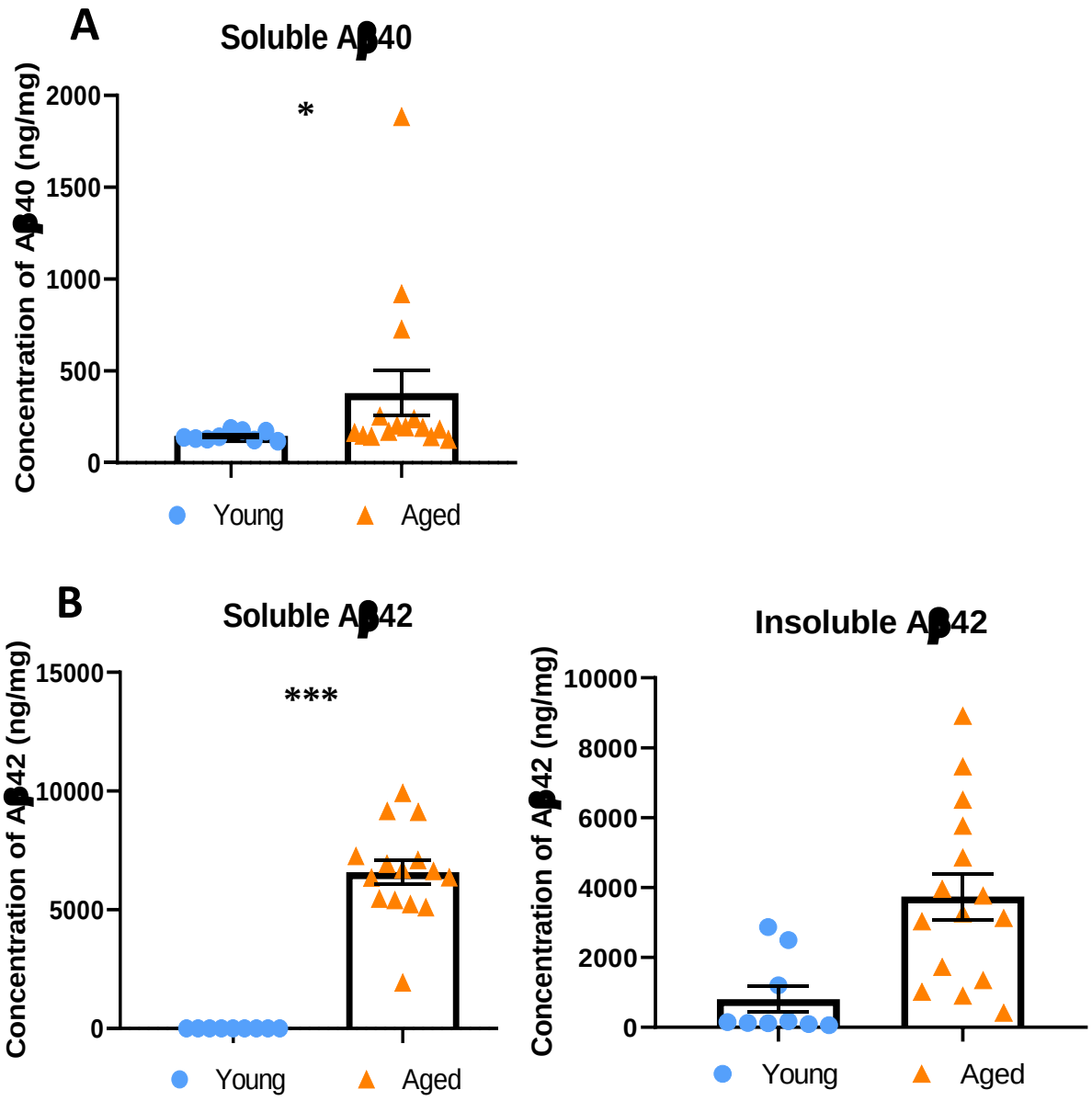


Figure 6.1: Concentration of Aβ species increases with age in the hippocampus of *APP*$^{NL-F}$ mice. ELISA results comparing the soluble levels of Aβ40 (A) and Aβ42 (B) as well as the insoluble Aβ42 (C) in hippocampi of 4-month old (n=9) or 16-17-month old (n-15) *APP*$^{NL-F}$ mice. Error bars represent SEM. *p<0.05, ***p<0.005

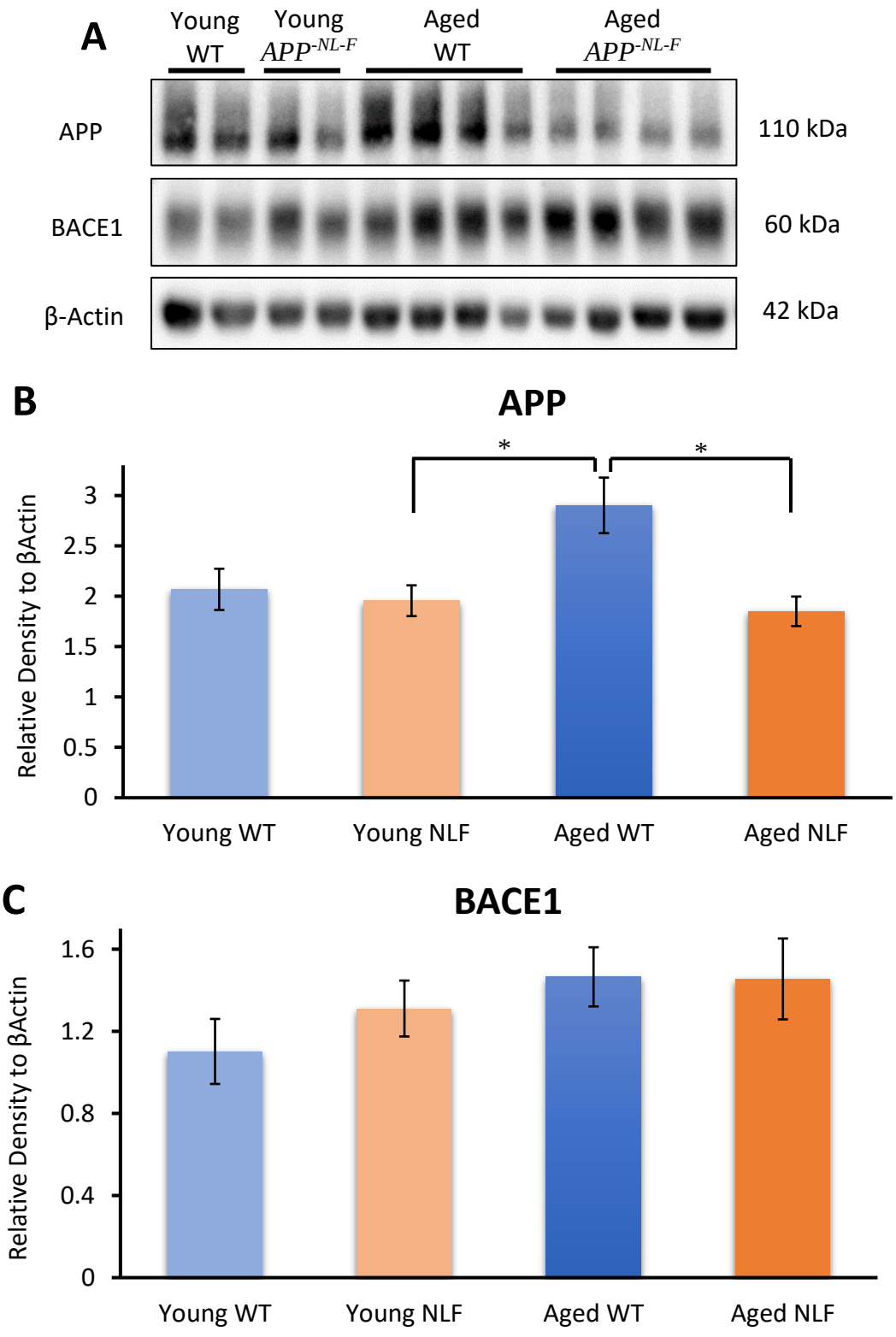
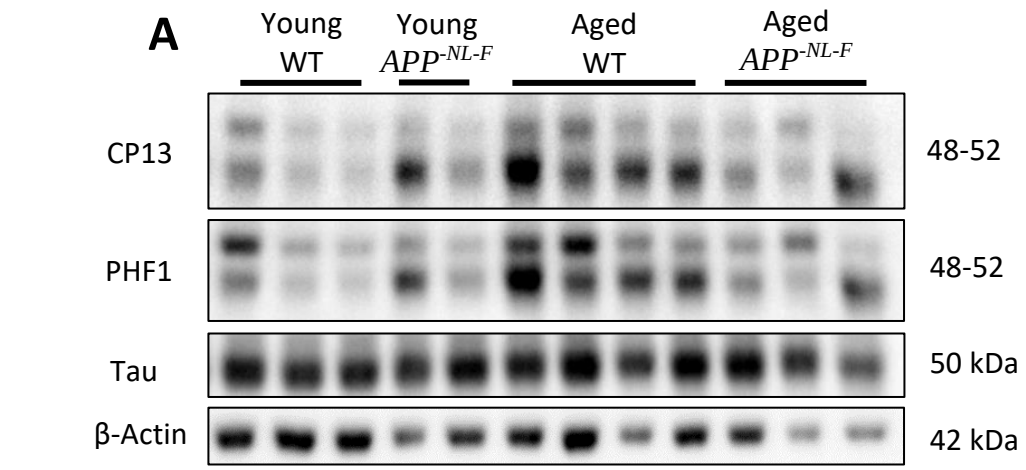


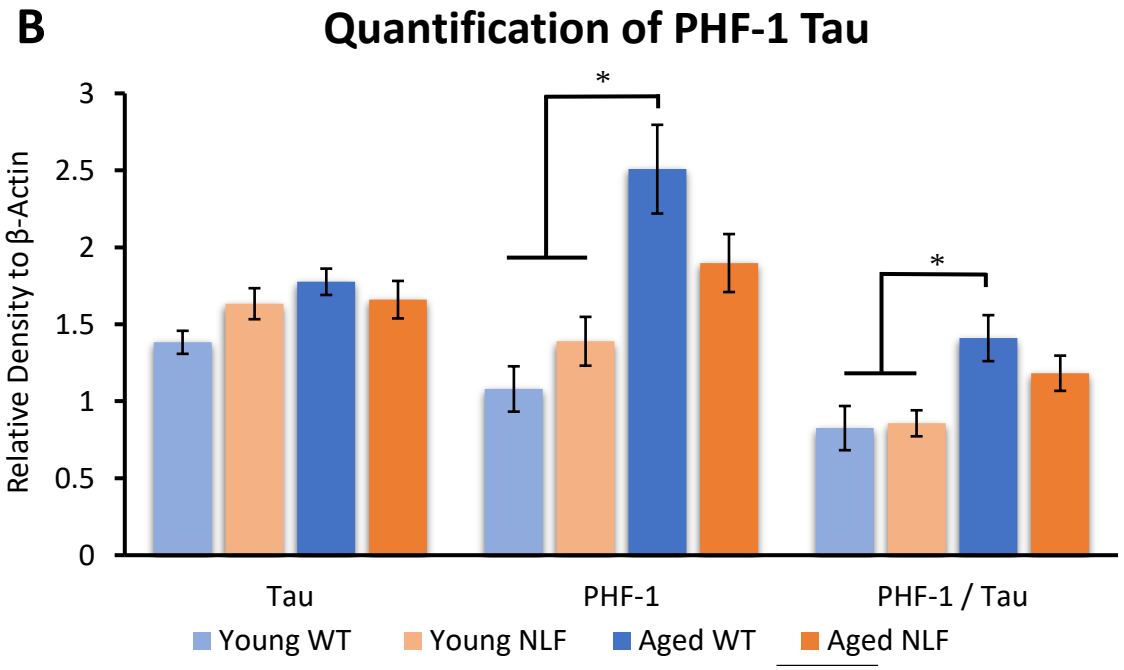
Figure 6.2: Expression levels of APP and BACE1 in the hippocampus of *APP*$^{NL-F}$ mice. (A) Representative Western blot images of the expression of APP and BACE1 in the hippocampus of young (4-month) and aged (16-17-month) WT and *APP*$^{NL-F}$ mice. **(B)** Quantification of APP expression revealed significant differences between the aged WT group and both young and aged *APP*$^{NL-F}$ groups (one-way ANOVA, *p<0.05). **(C)** Quantification of BACE1 protein expression. Young WT (n=9), young *APP*$^{NL-F}$ (n=9), aged WT (n=16), aged *APP*$^{NL-F}$ (n=15). Error bars represent SEM.

Tau Pathology

Western blot analysis was performed to characterise the expression and phosphorylation of tau protein in young and aged *APP^{-NL-F}* mice compared to WT littermates (figure 6.3). Two anti-phospho-tau antibodies were used which target epitopes that are associated with the hyperphosphorylation seen in AD patients, alongside an antibody that recognises total tau. PHF1 is a monoclonal antibody which binds tau that is phosphorylated at Ser396 and/or Ser404 (Otvos et al. 1994), while CP13 recognises phosphorylation of Ser202 (Petry et al. 2014). A one-way ANOVA revealed no differences in tau expression between the four different groups $F(3,48) = 2.53$, $p=0.069$. Inspection of the data from the PHF1 analysis revealed that the assumption of homogeneity of variance was violated, as reported by Levene's test ($p=0.009$). Therefore, a Welch ANOVA was utilised to statistically compare the groups and this revealed a significant difference in the total amount of pSer396 and pSer404 tau protein: Welch's $F(3,24.15) = 8.093$, $p=0.001$ (figure 6.3 B). Games-Howell *post-hoc* analysis revealed a significantly greater expression in aged WT mice compared to young WT ($p=0.001$) and young *APP^{-NL-F}* ($p=0.013$) mice. There was also a significant difference between the young WT and aged *APP^{-NL-F}* groups ($p=0.010$). Finally, the quantification of PHF1 densities were normalised to total tau expression to examine relative phosphorylation, and analysed by a one-way ANOVA which revealed significant differences between groups $F(3,48) = 4.90$, $p=0.005$. Bonferroni *post-hoc* analysis demonstrated significantly greater PHF1-specific phosphorylation of tau in aged WT mice compared to young WT ($p=0.012$) and young *APP^{-NL-F}* ($p=0.035$) samples (figure 6.3 B).

Quantification of the pSer202-tau expression (CP13) followed a similar pattern. There was no homogeneity of variances (Levene's test $p=0.036$) and a Welch ANOVA reported significant group differences: Welch's $F(3,23.07) = 7.18$, $p=0.001$. There was significantly greater expression of pSer202-tau in the aged WT group compared to young WT and young *APP^{-NL-F}* ($p=0.001$ and 0.032, Games-Howell *post-hoc* analysis, (figure 6.3 C). There was also a significant increase in the relative phosphorylation of tau in the aged WT group compared to young *APP^{-NL-F}* ($p=0.013$), as revealed by a significant one-way ANOVA, $F(3,48) = 4.40$, $p=0.009$ and Bonferroni *post hoc* test (figure 6.3 C).


A
Young WT
Young APP-NL-F
Aged WT
Aged APP-NL-F
CP13
PHF1
Tau
β-Actin
48-52
48-52
50 kDa
42 kDa


B
Quantification of PHF-1 Tau
Relative Density to β-Actin
3
2.5
2
1.5
1
0.5
0
*
*
Tau
PHF-1
PHF-1 / Tau
Young WT
Young NLF
Aged WT
Aged NLF

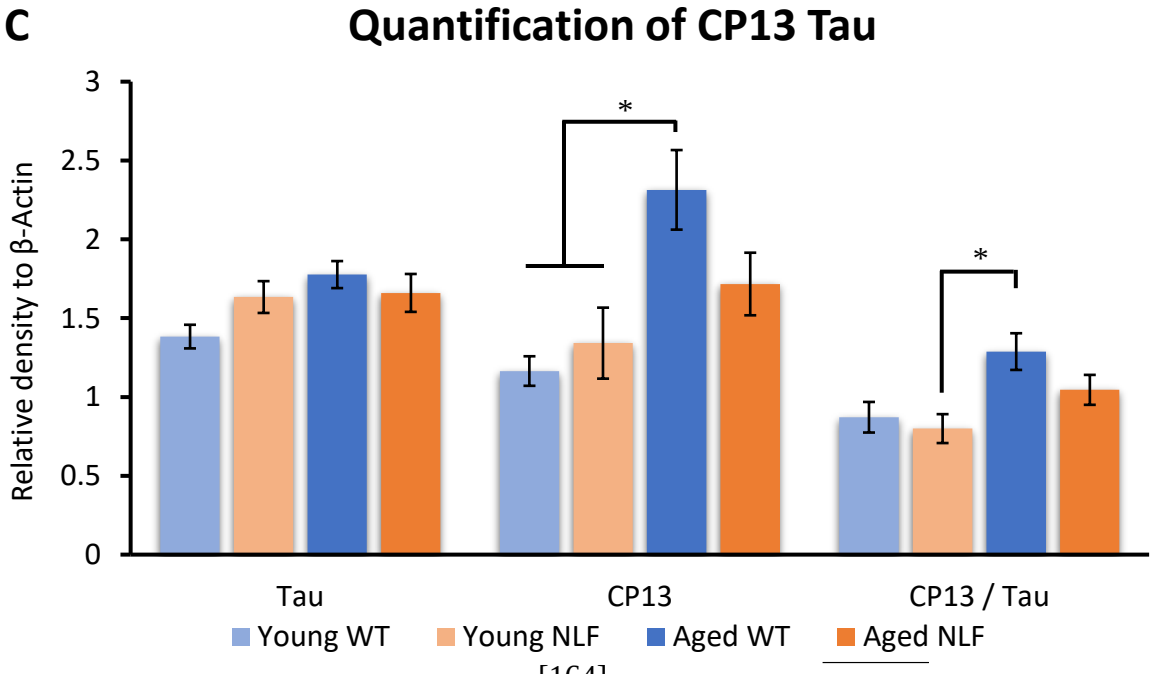
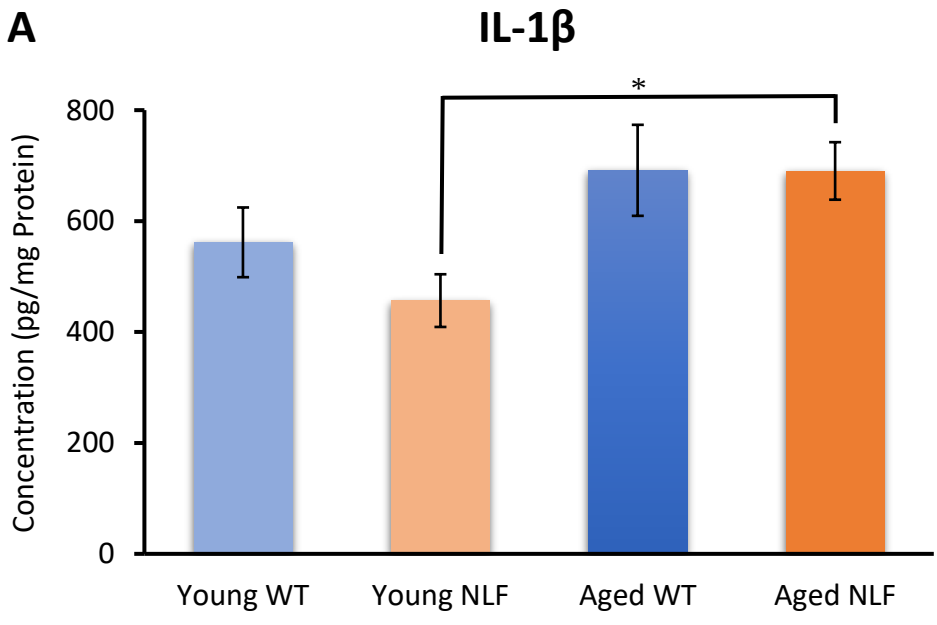

C
Quantification of CP13 Tau
Relative density to β-Actin
3
2.5
2
1.5
1
0.5
0
*
*
Tau
CP13
CP13 / Tau
Young WT
Young NLF
Aged WT
Aged NLF

Figure 6.3: Expression and phosphorylation of tau protein in *APP*$^{-NL-F}$ mice. Western blot analysis of overall tau expression, as well as the phosphorylation at multiple serine residues. **(A)** Representative Western blot images in young (4 month) and aged (16-17 month) WT and *APP*$^{-NL-F}$ mice. **(B)** Quantification of tau protein, pSer396-tau and pSer404-tau expression using the PHF-1 antibody revealed significant differences between the groups. Analysis of relative phosphorylation normalised to tau levels (PHF-1 / Tau) showed a significant increase in the aged WT mice compared to young WT and *APP*$^{-NL-F}$ (one-way ANOVA). **(C)** Quantification of pSer202-tau protein expression and relative phosphorylation showed similar effects. Young WT (n=9), young *APP*$^{-NL-F}$ (n=9), aged WT (n=16), aged *APP*$^{-NL-F}$ (n=15). Error bars represent SEM. *p<0.05

Inflammatory Markers

Analysis of the inflammatory markers IL-1β and TNF-α was performed using ELISA kits to determine any changes in concentration between young and aged WT and *APP*$^{-NL-F}$ mice (figure 6.4). A one-way ANOVA reported significant differences among the four groups $F(3,48) = 3.43$, $p=0.025$. *Post hoc* Bonferroni tests showed that aged *APP*$^{-NL-F}$ mice exhibited significantly increased levels of IL-1β compared to young (4-month-old) *APP*$^{-NL-F}$ animals ($p=0.034$, figure 6.4 A). Although this analysis indicated a specific effect for the NLF genotype, this cannot conclusion cannot be supported by the null hypothesis significance tests used. Furthermore, observation of the data suggested that there was a general increase in IL-1β expression with age across both genotypes.

Assessment of TNF-α revealed no significant differences between groups: one-way ANOVA $F(3,48) = 0.615$, $p=0.609$ (figure 6.4 B).



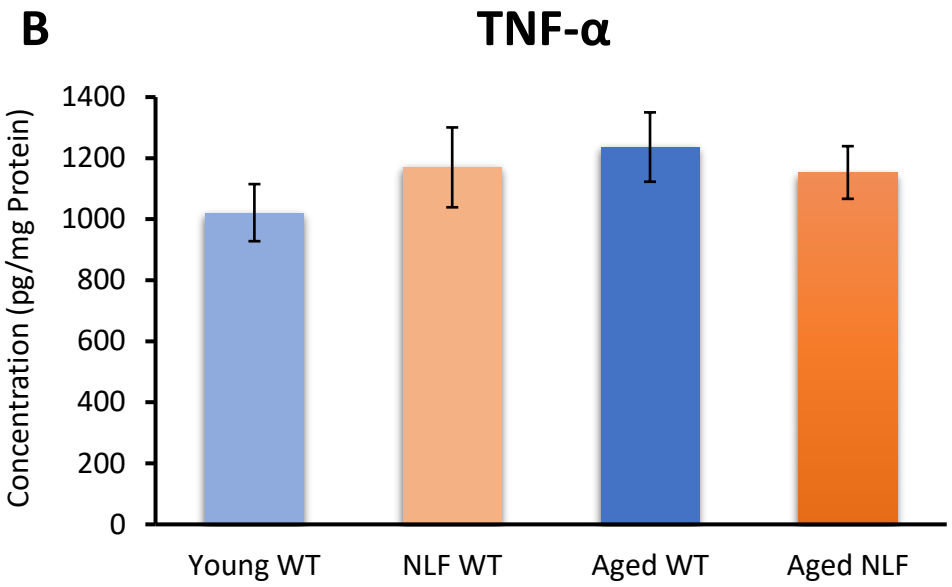


Figure 6.4: Quantification of inflammatory markers in *APP*$^{NL-F}$ mice. ELISA analysis of the concentrations of IL-1β (A) and TNF-α (B) in the hippocampi of young WT (n=9), young *APP*$^{NL-F}$ (n=9), aged WT (n=16), aged *APP*$^{NL-F}$ (n=15) mice. Error bars represent SEM. *p<0.05

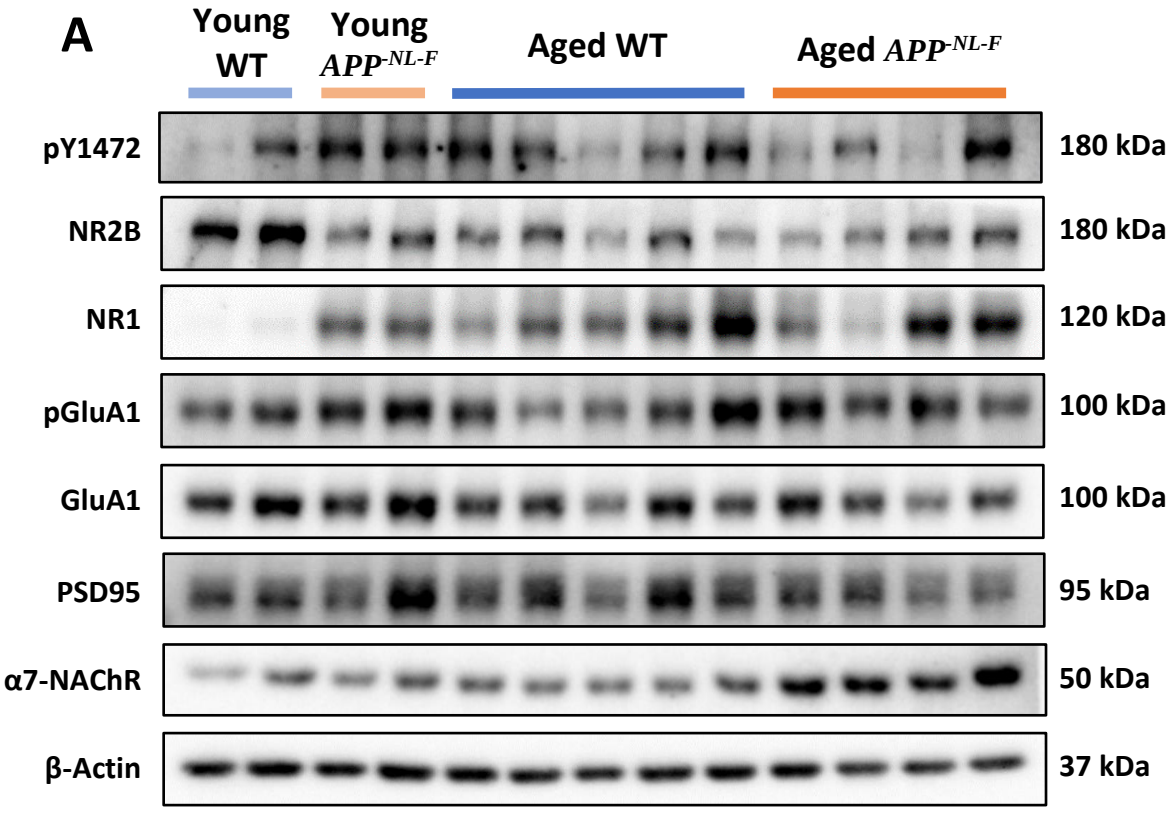
Synaptic Markers

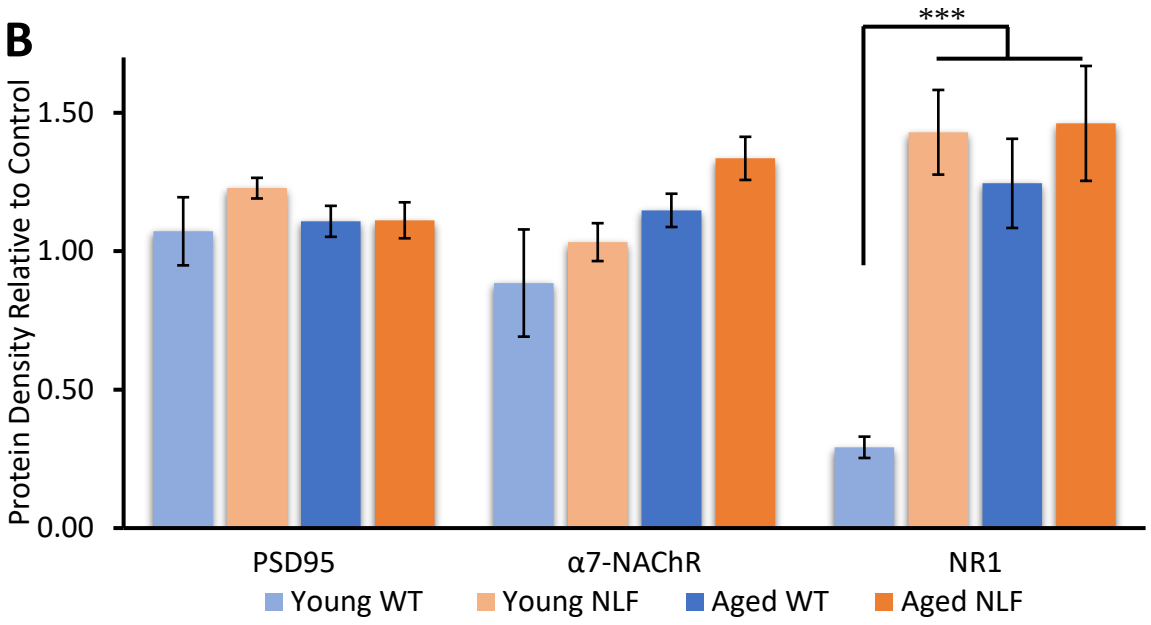
Synaptosome extractions were performed to analyse synaptic proteins in young and aged WT and *APP*$^{NL-F}$ mice by Western blot (figure 6.5). The distributions of all datasets were assessed by the Shapiro-Wilke test and transformations were performed in order to ensure normal distributions. Differences in protein expression were analysed by one-way ANOVAs with Bonferroni *post hoc* tests where applicable, unless otherwise stated. There were no significant differences in PSD95 density $F(3,45) = 0.84$, $p=0.477$, indicating that there was no difference in the number of synapses between the groups (figure 6.5 B). The assumption of homogeneity of variance was violated for the expression of α7-NAChR (Levene's test $p<0.05$), and so the groups were compared by Welch's ANOVA. Although the ANOVA test was significant: Welch's $F(3,20.3) = 4.25$, $p=0.018$, Games-Howell *post-hoc* tests did not show significant differences between groups (largest effects: young WT vs aged *APP*$^{NL-F}$ $p=0.060$, young *APP*$^{NL-F}$ vs aged *APP*$^{NL-F}$ $p=0.053$, (figure 6.5 B).

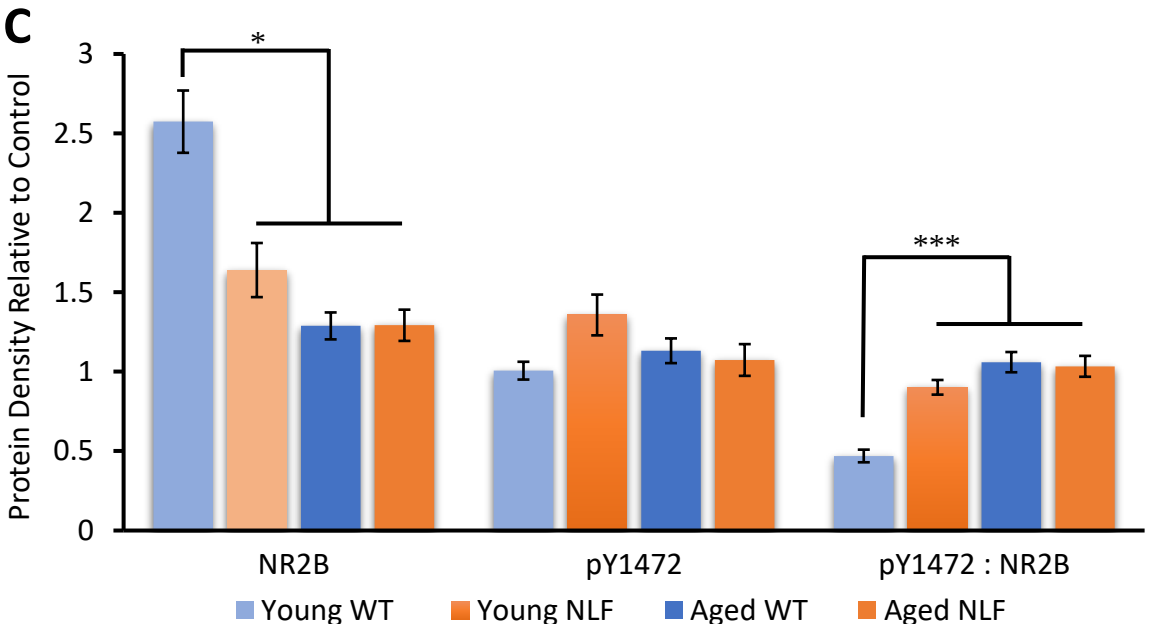
Expression of NMDA receptors (NR1) was significantly different between the groups $F(3,45) = 27.1$, $p<0.0005$, with Bonferroni *post hoc* tests revealing expression was lower in the young WT group than all three other groups ($p<0.0005$ for all, (figure 6.5 B). There was also a significant difference between the groups in expression of the NR2B

subunit F(3,45) = 10.9, p<0.0005, with the young WT group showing significantly more expression than the young *APP*$^{-NL-F}$ (p=0.044) and both aged groups (p<0.0005 for both, (figure 6.5 C). The one-way ANOVA comparing expression of the phosphorylated form of NR2B (pY1472) did not show significant effects F(3,45) = 1.9, p=0.133; however, when the relative phosphorylation was calculated by normalising to the amount of NR2B, there was a significant group effect F(3,45) = 10.2, p<0.0005. *Post hoc* tests showed that the young WT group had significantly less phosphorylation of NR2B than the other three groups (p<0.001 for all, (figure 6.5 C).

The expression of the AMPA subunit GluA1 was also assessed by one-way ANOVA, revealing no significant differences in expression of the protein F(3,45) = 1.9, p=0.142 (figure 6.5 D). There was also no significant result when comparing the amount of phosphorylated GluA1 F(3,45) = 1.3, p=0.274. However, there were significant differences between the groups when comparing the relative phosphorylation F(3,45) = 3.2, p=0.031. The young WT group had significantly less phosphorylation than the aged *APP*$^{-NL-F}$ group, as shown by Bonferroni *post hoc* tests (p=0.023, figure 6.5 D).




B
Protein Density Relative to Control
1.50
1.00
0.50
0.00
***
PSD95
α7-NAChR
NR1
Young WT
Young NLF
Aged WT
Aged NLF


C
Protein Density Relative to Control
3
2.5
2
1.5
1
0.5
0
*
***
NR2B
pY1472
pY1472 : NR2B
Young WT
Young NLF
Aged WT
Aged NLF

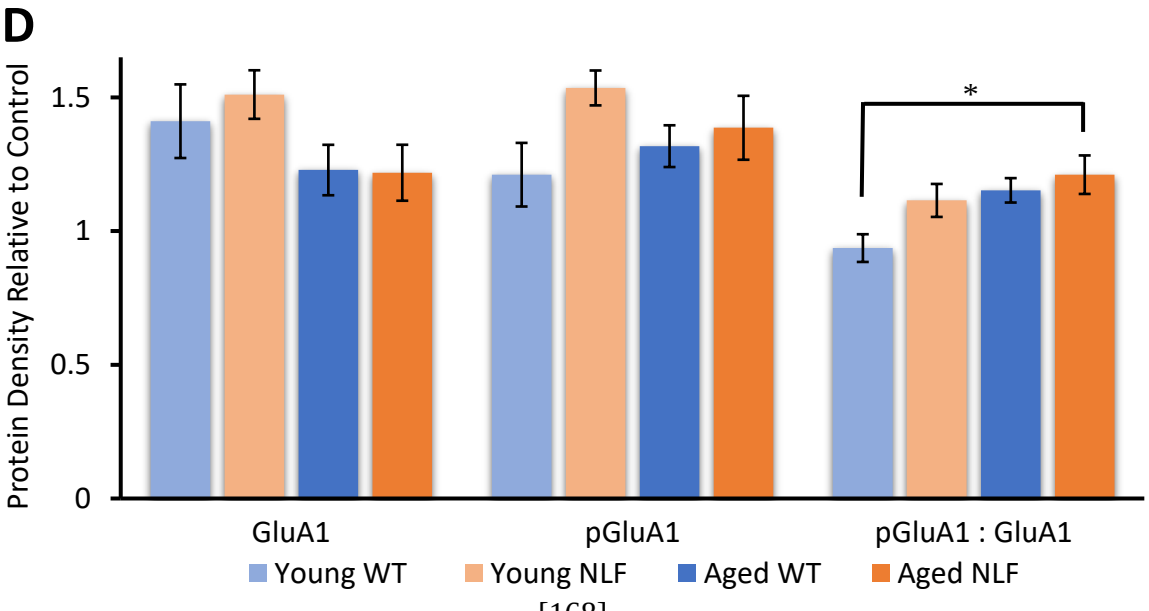

D
Protein Density Relative to Control
1.5
1
0.5
0
*
GluA1
pGluA1
pGluA1 : GluA1
Young WT
Young NLF
Aged WT
Aged NLF

Figure 6.5: Western blot analysis of hippocampal synaptosomes in *APP*$^{-NL-F}$ mice. Representative Western blot images of various synaptic markers. **(A)** Representative Western blot images in young (4 month) and aged (16-17 month) WT and *APP*$^{-NL-F}$ mice. **(B)** Quantification of protein expression for PSD95, α7 nicotinic acetylcholine receptor (α7-NAChR) and total NMDA receptor (NR1). **(C)** Quantification of the expression of NR2B and phosphorylated NR2B (pY1472), as well as the relative phosphorylation of the receptor. **(D)** Quantification of the expression of GluA1 and phosphorylated GluA1, as well as the relative phosphorylation of the receptor. Young WT n=9, young *APP*$^{-NL-F}$ n=9, aged WT n=16, aged *APP*$^{-NL-F}$ n=15. Error bars represent SEM. *p<0.05, ***p<0.001

6.3 Chapter Discussion

The main aim of this experiment was to validate the *APP*$^{-NL-F}$ mouse model as a non-transgenic model of FAD. The results confirmed that aged *APP*$^{-NL-F}$ mice demonstrated an age-dependent accumulation of both soluble and insoluble Aβ42 in the hippocampus, in the absence of increased APP expression. The ELISA data were consistent with the reports of Saito *et al* as the concentration of Aβ42 in aged *APP*$^{-NL-F}$ was over an order of magnititude greater than that of Aβ40. In fact, the original publication describing the *APP*$^{-NL-F}$ mice showed no age-dependent increase in soluble Aβ40, which may underpin why, in the current experiments, only three mice exhibited strong accumulation. The accumulation of Aβ42 was not simply due to increased amount of APP or BACE1, as aged *APP*$^{-NL-F}$ mice did not show increased expression of these proteins. In fact, aged WT mice mice actually showed significantly higher APP expression than both young and aged *APP*$^{-NL-F}$ mice. However, this seems to be driven by the non-significant increase in expression between young and aged WT mice. To my knowledge, an age-related increase in APP expression is not a phenomenon that has been reported in C57Bl/6 mice and the potential cause is unclear. However, most studies investigating APP expression examine FAD models rather than WT mice, thus it may not have been thoroughly scrutinised. Replicating this result using an ELISA to quantify APP expression levels in each group would increase the reliability of this result.

In addition to the numerical increase in APP expression, the aged WT mice also displayed significantly increased phosphorylation of tau compared to young WT and *APP*$^{-NL-F}$, consistent across two antibodies specific to separate phosphorylation epitopes. While the lack of genotypic difference was expected, due to previous data on these mice, the effect of age in WT mice was not. Similar to APP, the number of studies investigating the

phosphorylation of tau in WT mice is limited, however, one study has reported an increased expression of phosphorylated tau from 10 to 22 month old C57Bl/6 mice (Bu et al. 2018). The lack of a significant age-related increase in *APP*$^{-NL-F}$ mice is therefore interesting; it potentially demonstrates some protective mechanism offered by the amyloid accumulation in these mice, however, it more likely reveals a lack of sensitivity combined with the conservative ANOVA test, particularly in the results of the PHF-1 antibody.

Inflammatory markers were also analysed to investigate any changes in immune activation between the groups. While there were no changes in TNF-α, the IL-1β ELISA was inconclusive. Other groups have shown effects of both genotype and age on immune activation in these knock-in mice, reporting that microglial and astroglial activation increases from 6 to 18 months in *APP*$^{-NL-F}$ mice, while also being significantly greater than WT levels (Saito et al. 2014, Masuda et al. 2016). The explanation for the lack of effects observed here may be the difference in techniques used. The current experiment used ELISA analysis to quantify inflammatory markers in hippocampal homogenate. While this provides information of the general state of immune system activation, the other studies used histological techniques which permitted selective, *in situ* examination of the environment surrounding plaques. They observed an increased number of activated microglia and astroglia in close proximity to these plaques. The precision enabled by histochemical techniques is lost during the homogenisation required for ELISA analysis. Alternative studies using ELISA analysis of transgenic APP models have identified an increase in inflammatory cytokines that correlates with amyloid burden (Patel et al. 2005). It is possible that the lack of significant effects in the current experiment represents the fact that these knock-in model exhibit more subtle pathology compared to transgenic mice.

Synaptic markers and glutamate receptors were analysed with the aim of providing a mechanism for the cognitive deficits observed in the aged *APP*$^{-NL-F}$ group described in chapters 4 and 5. There was no difference in synapse density between the groups, which was inconsistent to previous studies which have reported a reduction in aged *APP*$^{-NL-F}$ mice (Saito et al. 2014, Zhang, Wu, et al. 2015). An explanation for this discrepancy may be the alternative techniques used by other studies. Zhang and colleagues showed a 26% reduction in PSD95 immunoreactivity in lysates of neuronal cultures derived from *APP*$^{-NL-F}$ mice. Saido *et al* examined brain sections, observing decreased immunoreactivity of both PSD95 and synaptophysin in close proximity to Aβ plaques. The sensitivity provided by analysing synaptic density around plaques *in situ* is lost during the preparation of synaptosomes. The

consistency of synaptic density here indicated by PSD95 immunostaining suggested that any changes in the density of other receptors were not simply due to alterations in the number of synapses. There was a significant effect of group when analysing the α7-NAChR but, although the aged *APP*$^{-NL-F}$ group showed numerically increased expression, this did not reach significance during *post hoc* analysis. Increased expression of this receptor has been observed in the brains of AD patients and aged Tg2576 mice (Chu et al. 2005, Dineley et al. 2001). However, further discussion of the role of this receptor in both the physiological and pathological roles of Aβ will be presented in chapter 8.

Analysis of glutamate receptors produced an interesting result in the young WT group which showed significantly lower overall expression of NMDA receptors, yet a significantly higher amount of the NR2B subunit. Although this result seems contradictory, the decrease in total NMDA receptors may be conveyed by expression of other subunits such as the NR2A. The young WT group also exhibited significantly lower relative phosphorylation of NR2B than all groups which was correlated with a numerical reduction in GluA1 phosphorylation. The genotypic difference in 4-month old mice is not present at 17 months and the expression of both aged groups is statistically unchanged from that of young *APP*$^{-NL-F}$ mice, potentially suggesting that this group displays an effect of premature aging. The changes in receptor dynamics of young WT mice did not result in differences in cognitive performance compared to *APP*$^{-NL-F}$ mice of the same age in the tests described in chapters 4 and 5. However, there is some evidence of functional differences in *APP*$^{-NL-F}$ mice at 3 months of age. Shah *et al* (2018) demonstrated that 3-month-old *APP*$^{-NL-F}$ mice exhibited a deficit in spatial reversal learning in the MWM compared to *APP*$^{-NL}$ control mice. This was correlated with hypersynchronous functional connectivity within the hippocampal network, revealed by resting-state functional MRI, that did not exist when mice were reassessed at 7 months of age. While further analysis is required to investigate the potential relevance of that study to the observed changes in NMDA receptor expression in the current experiment, these results indicate that abnormal APP processing may lead to changes in neuronal function even at a young age, prior to Aβ accumulation.

In summary, this chapter has investigated the biochemical changes associated with the Swedish and Iberian mutations expressed in the *APP*$^{-NL-F}$ model, as well as how these change with age. The age-dependent accumulation of Aβ40 and Aβ42 in absence of APP hyperexpression validates the model as being non-transgenic. The lack of tau pathology or extensive inflammation further present this mouse as a model of preclinical AD.

Chapter 7 –

2B3-Treatment Causes Dissociated Effects on Cognition in Aged *APP*$^{-NL-F}$ and WT mice.

7.1 Chapter Overview

Anti-amyloid therapies have been the current primary focus of research into treating AD patients. However, the results of Chapter 3 suggest that this approach may have detrimental effects on cognition in patients who lack severe amyloid accumulation. Although many preclinical studies have reported beneficial effects of clearing amyloid, these have been against the background of APP overexpression and very few have examined the effect of such interventions in healthy WT control mice. Therefore, this chapter details the effect of 2B3 administration on memory function in an aged cohort of *APP*$^{-NL-F}$ as well as aged WT controls. Experiment 12 first replicated the OiP deficit reported in experiment 5 in un-treated mice, before the two genotypes underwent chronic icv infusion of either 2B3 or a control antibody. The results showed that, while the control antibody did not alter performance of either genotype in the OiP test relative to baseline, 2B3 administration produced an intriguing dissociation in associative recognition memory in WT and *APP*$^{-NL-F}$ mice. Although the anti-APP antibody rescued performance in the *APP*$^{-NL-F}$ mice, it induced a deficit in WT mice. In Experiment 13 *ex vivo* analysis of the hippocampal tissue revealed that the 2B3 significantly reduced Aβ40 in the WT mice and Aβ42 in the *APP*$^{-NL-F}$ mice. Western blot analysis also revealed changes in glutamate receptor dynamics, with significantly lower relative phosphorylation of AMPA receptor subunit GluA1 being associated with the groups that showed intact memory performance

7.2 Chapter Introduction

Putative therapeutic treatments for AD have mainly focused on two amyloid-related strategies: small molecule inhibitors of the enzymes responsible for APP metabolism and immunotherapies that target Aβ to augment its clearance by the immune system. Despite promising results in preclinical studies, these strategies have consistently failed in clinical trials due to lack of beneficial effect on cognitive outcomes. In fact, various trials have actually reported significant worsening of cognitive decline in the groups of patients receiving higher doses of the treatments. Despite these alarming results, the development of anti-amyloid therapies has continued. The rationale, mechanisms and preclinical success of both β-secretase inhibitors and immunotherapies are summarised in section 1.4.5, and this section will evaluate the development and clinical performance of these therapies.

7.2.1 – Anti-Amyloid Immunotherapy

The concept of immunotherapy has been introduced in section 1.4.5 along with the putative mechanisms underpinning this therapeutic strategy. Briefly, immunotherapy in the context of AD involves the use of anti-Aβ antibodies to clear plaques and relieve other symptoms of amyloid pathology. The antibodies can either be exogenous (passive immunotherapy) or raised by the patient's own immune system (active immunotherapy).

Active Immunotherapy

The first *in vivo* studies of active immunotherapy involved injection of Aβ42 as an immunogen. While (Schenk et al. 1999) reported significant inhibition of amyloid pathology development following immunisation of both young and old transgenic mice, (Das et al. 2001) observed an effect only after treating young transgenic mice. Other studies have used fibrillar Aβ as the immunogen. Oddo et al (2006) reported that, although vaccination of 18-month-old 3xTg-AD mice did not reduce plaque number, the amount of insoluble Aβ or insoluble tau was reduced and cognitive performance significantly improved. This evidence gave further credence to the contemporary view that soluble, oligomeric Aβ is responsible for the synaptotoxicity in AD. Moreover, while a similar vaccine reduced the plaque burden of aged beagles, the absence of a change in oligomer concentration was accompanied by a lack of cognitive improvement (Head et al. 2008).

Active immunotherapy has also been trialled in the clinic. AN1792 (Janssen, Pfizer), which used the full-length Aβ42 peptide as the immunogen, was enrolled in a phase II trial that was interrupted following reports of meningoencephalitis in 6% of the 300 patients (Gilman et al. 2005). Long-term follow-up investigations reported significantly reduced functional decline in 25 "antibody responders" (patients in whom anti-AN1792 antibodies had been detected) compared to patients in the placebo group (Vellas et al. 2009). Meanwhile neuropathological assessment of 8 patients receiving the vaccine showed significantly increased plaque clearance in the cerebral cortex that was not associated with cognitive improvement, even in the brains with virtually complete plaque removal (Holmes et al. 2008). A further follow-up study was recently published which analysed neuropathology in 22 patients 15 years after the initial vaccination (Nicoll et al. 2019). They reported evidence of plaque clearance that had a significant inverse correlation with post-vaccination anti-AN1792 antibody production, indicating that the treatment exerted a persistent response. However, despite the beneficial effect on plaque reduction,

most patients had still progressed to severe stages of dementia, including those with extensive plaque reduction. The fact that clearance of amyloid plaques did not arrest cognitive decline indicated that plaques are not the appropriate target in patients with AD. Approaches to remove plaque burden are not suitable for late stage AD patients where there is established tau pathology and neurodegeneration.

Analysis of the AN1792 clinical trial provoked interest in the development of second-generation immunogens with shorter N-terminal fragments of the peptide. These reduced brain amyloid accumulation and enhanced memory acquisition in transgenic APP mice (Wiessner et al. 2011, Maier et al. 2006, Seabrook et al. 2007). An initial phase I trial of CAD106 (Novartis) reported a favourable safety profile and 74% of people responded to the vaccine by generating anti-Aβ antibodies (Winblad et al. 2012). In a 90-week phase IIb study enrolling 121 patients with mild AD, strong antibody responses were observed in 55% and 81% of those receiving the 150 and 450 μg dose regimes, respectively (Vandenberghe et al. 2017). There was an inverse correlation between antibody response and amyloid PET uptake but there was no difference in cognitive decline. Another vaccine, ACC-001 (Janssen/Pfizer) has demonstrated a similar safety profile and capability of provoking an antibody response, but a beneficial effect on plaque load or cognitive function has not been reported (van Dyck et al. 2016, Hull et al. 2017). Although active immunotherapy has resulted in no clinical benefit in patients diagnosed with dementia it remains a possible intervention in pre-symptomatic stages of the disease.

Passive Immunotherapy

Passive immunotherapy with anti-Aβ antibodies does not rely on stimulation of the host's immune system but results in a similar clearance of amyloid following systemic injection in PDAPP mice (Bard et al. 2000). Another study observed a rapid 1000-fold increase in plasma Aβ along with its clearance from the brain following peripheral injection of the m266 mAb (DeMattos et al. 2001). Administration of this antibody was also associated with a rapid rescue of object recognition and spatial working memory deficits (Dodart et al. 2002). Kotilinek et al (2002) published similar results with BAM-10, an Aβ N-terminal region-targeted antibody, which reduced amyloid pathology (significant reductions in both soluble and insoluble Aβ) and improved cognitive function in Tg2576 mice. Passive anti-Aβ immunotherapy has also demonstrated the potential to influence other symptoms of amyloid pathology in multiple FAD models, such as synaptic plasticity impairment, dendritic spine loss and reactive gliosis (Chauhan and Siegel 2002, 2003,

Klyubin et al. 2005, Spires-Jones et al. 2009). Antibody treatment also reduced the rate of early aggregation of hyperphosphorylated tau in 12-month-old 3xTg-AD mice (Oddo et al. 2004). However, this effect did not extend to more advanced stages of NFT pathology in older mice; further hinting at a therapeutic window that is restricted to early stage AD.

Consistent with other mechanisms of anti-AD treatments, clinical trials of passive immunotherapies have not been successful (Wisniewski and Goñi 2015). Bapineuzemab (Pfizer) was the first antibody to progress to a phase III trial, but was terminated in 2012 due to the lack of clinical benefit (Vandenberghe et al. 2016). A meta-analysis of six randomised controlled trials revealed that, although the treatment provoked no safety concerns, significantly reduced the concentration of phosphorylated tau in the CSF and reduced brain Aβ burden compared to placebo, no beneficial effect was observed in the ADAS-Cog and MMSE cognitive assessments (Abushouk et al. 2017). An alternative immunotherapy, Solanezumab (Eli Lilly), is the humanised m266 antibody targeting an epitope on the mid-region of Aβ that is only accessible in monomers. It failed its primary endpoint, which was a reduction in cognitive decline measured on the 14-item cognitive subscale of the Alzheimer Disease Assessment Scale (ADAS-cog). Consequently its development was terminated in 2017 due to insufficient evidence that it could demonstrate a meaningful benefit to patients with prodromal AD from multiple phase III trials (Siemers et al. 2016, Honig et al. 2018).

One consequence of these failed clinical trials is that it has led to a refinement of patient inclusion criteria, specifically the selection of earlier stage patients with confirmed amyloid pathology (Schilling et al. 2018). Crenezumab (Genentech/AC Immune), gantenerumab (Hoffman - La Roche) and aducanumab (Biogen/Neurimmune) are antibodies that recognise fibrillar Aβ and have all demonstrated the potential to reduce amyloid burden in AD patients in early stage clinical trials (Bohrmann et al. 2012, Adolfsson et al. 2012). A phase Ib trial of aducanumab in 166 individuals with prodromal or mild AD even reported slowing of cognitive decline (Sevigny et al. 2016). However, the termination of the phase III trials involving crenezumab and aducanumab was announced in January and March 2019 following interim analyses that the treatments would not achieve their primary endpoints (ALZFORUM 2019). While the results from the aducanumab trials have yet to be disclosed; Roche revealed that in the 13% of 813 enrolees for whom data was available, crenezumab treatment had been ineffectual (International Conference on Alzheimer's and Parkinson's Diseases 2019).

7.2.2 – Small Molecule β-Secretase Inhibitors

While interest in γ-secretase inhibitors has diminished due to off-target effects caused by their impact on alternative substrates, BACE1 has remained the premier anti-AD target since its discovery. The concept of BACE1 inhibition as a therapeutic method was strengthened by reports of genetic KO of the enzyme alleviating the neuropathology and cognitive deficits exhibited by aged transgenic models (Ohno et al. 2004). However, it is important to note that BACE1 deficiency alone caused sensorimotor impairments, spatial memory deficits and hypersensitivity to seizure, indicating that the enzyme plays an important physiological role either due to its interaction with APP or its numerous other substrates (Laird et al. 2005, Kobayashi et al. 2008). The alternative pathways in which BACE 1 has been implicated include neuron myelination, axon guidance and neurogenesis (Hu et al. 2006, Hu et al. 2013, Hitt et al. 2012). Heterozygous deletion of BACE1 resulted in the rescue of synaptic function and memory performance in 5xFAD mice (Kimura, Devi, and Ohno 2010), indicating that partial inhibition but not total eradication of BACE1 activity may reduce Aβ burden without inducing a detrimental effect on neuronal function.

Another study replicated some of the phenotype observed in BACE1 KO mice following chronic administration of two separate BACE1 inhibitors in WT mice, further demonstrating the physiological importance of the enzyme (Filser et al. 2015). Aβ levels were reduced in both the cortex and CSF to below 50% of that measured in vehicle-treated mice. LTP was reduced in hippocampal slices and dendritic spine formation was disrupted in layer V pyramidal neurons, while they also reported cognitive deficits in the Y-maze and novel object recognition. This is the only study to date that has investigated the effect of pharmacological inhibition of BACE1 on synaptic function and cognition in WT mice and it again raised the question of whether the detrimental effects were caused by the reduction of interaction with APP or its alternative substrates. Experiments in Chapter 3 sought to answer this and hinted at the former explanation. Considering the BACE1 inhibitors LY2811376, LY2886721 (both Eli Lilly) and verubecestat (Merck) have exerted beneficial effects on amyloid pathology in aged transgenic models of AD (May et al. 2011, May et al. 2015, Kennedy et al. 2016), it is possible that the impact of treatment depends on the initial severity of amyloid pathology. In fact, when Verubecestat was administered to prodromal AD patients in a clinical trial of 1454 patients, those receiving the highest dose (40 mg/day) demonstrated a significantly larger cognitive decline and were more likely to progress to AD than patients who had received placebo (Egan et al. 2019).

Several BACE1 inhibitors have progressed to clinical trials of AD patients and all failed to reduce cognitive decline. LY2811376 (Eli Lilly) was the first oral non-peptidic BACE1 inhibitor to demonstrate reduced Aβ levels in both animals and humans (May et al. 2011). LY2811376 was not progressed beyond phase I clinical trials, as chronic exposure toxicology studies revealed off-target pathology. A second-generation compound from Eli Lilly showed similar preclinical data, and progressed to a phase II trial. However, the drug was discontinued after 15 months due to potential liver toxicity (May et al. 2015). Another drug that showed promising results in rats and phase I trials was MK-3931 (Verubecestat, Merck) (Kennedy et al. 2016). This drug entered a phase 2/3 trial of 1958 patients with a diagnosis of mild-to-moderate AD. However, the trial was discontinued following a mid-term review that indicated a positive clinical effect was unlikely to be achieved (Egan et al. 2018). Moreover, verubecestat treatment was associated with adverse events such as rash, sleep disturbance, suicidal ideation, weight loss and hair colour change.

In order to selectively reduce BACE1 cleavage of APP, the present study used the antibody 2B3, which prevents Aβ cleavage by steric hindrance (Thomas *et al*, 2011). In transgenic mice overexpressing human mutant APP, icv infusion of 2B3 into the lateral ventricle significantly reduced the concentration of soluble Aβ40 and rescued an OiP associative recognition memory deficit (Evans et al. 2019). However, APP overexpression causes a number of brain changes that are not specific to Aβ, with one estimate suggesting that up to 60% of the phenotypes expressed by transgenic mice, such as increased tau phosphorylation and somatodendritic atrophy, are artefactual (Saito et al., 2014). Assessing the behavioural and biochemical impact of 2B3 in knock-in mice provides a critical test of the hypothesis that excess Aβ deposition is a driving force in cognitive decline.

In summary, while targeting amyloid remains an active area of clinical investigation, the fact that disruption of Aβ processing (even using the most clinically effective drugs) does not halt cognitive decline suggests that other factors contribute to neurodegeneration and that treatment of those with symptomatic AD will require a multi-target approach. The fact that the verubecestat trial with prodromal patients returned an even more severe outcome suggests that modulation of APP may need to be finely balanced in individuals with mild or non-existent amyloid pathology. The results of Chapter 3 support this hypothesis, and, as 2B3 has previously been shown to rescue cognitive deficits in a transgenic hAPP model of AD, the present chapter sought to concurrently examine the impact of the antibody in *APP*$^{-NL-F}$ and WT mice.

7.3 Experiment 12: Effect of 2B3 Treatment on OiP Task

7.3.1 – Introduction

The aim of this experiment was to investigate the effects of anti-APP antibody 2B3 on cognition performance depending in both WT and *APP*$^{-NL-F}$ mice. The hypothesis was that 2B3 would rescue the OiP deficit exhibited by aged *APP*$^{-NL-F}$ mice, while inducing an impairment of memory in aged WT littermates.

7.3.2 – Methods

Subjects, Design

The subjects were a cohort of 16-17 months old male *APP*$^{-NL-F}$ and WT mice. There were 24 mice of each genotype that had been housed in cages of four since weaning (two *APP*$^{-NL-F}$, two WT in each; littermates where possible). The OiP test protocol was the same as that described in Chapter 2, with the exception that animals underwent two trials of the OiP task both before and during 2B3 treatment. The control treatment was the same IgG described in Chapter 3. The concentration of 2B3 used in this experiment was 2.71 mg/mL.

Baseline OiP performance was measured for all mice, before the mice in each cage were split into the four distinct treatment groups (*APP*$^{-NL-F}$ 2B3; *APP*$^{-NL-F}$ IgG; WT 2B3; WT IgG), ensuring that DR scores within each genotype were matched as closely as possible. Object sets, and the objects that switched positions were counterbalanced both between groups and across the two surgery timepoints (pre vs post). The surgical procedure was described in Chapter 2. Briefly, osmotic minipumps (Alzet) were filled with antibody solution and attached to a cannula inserted into the lateral ventricle. Nine days following the surgery, mice underwent habituation to the empty arena before habituation to objects on days 10 and 11, which consisted of three 5-minute sessions with four different objects. OiP testing took place on days 12 and 14 and mice were culled and brain tissue collected immediately following the final test session.

7.3.3 – Experiment 12 Results

Sample Phase Contact Times

Table 7.1 shows the mean total contact time throughout the sample phases of the experiment. Visual inspection of the data indicates a decrease in exploration from sample phase 1 to 3. All the data were transformed by log10 in order to ensure that all groups

exhibited normal distributions (Shapiro-Wilke test p>0.05). The sample phase contact times were analysed by a repeated measures ANOVA with sample phase and surgery timepoint (pre- vs post-) as within-subject factors and genotype and antibody treatment as the between subjects' factors.

The ANOVA revealed significant main effects of sample phase $F(2,88) = 61$, $p<0.0005$ and genotype $F(1,44) = 5.9$, $p=0.02$. There was no main effect of surgery stage $F(1,44) = 0.55$, $p=0.461$ or antibody treatment $F(1,44) = 0.41$, $p=0.524$. There were no significant interactions: surgery*phase $F(2,88) = 0.43$, $p=0.653$; surgery*treatment $F(1,44) = 0.072$, $p=0.789$; surgery*genotype $F(1,44) = 0.089$, $p=0.767$; phase*treatment $F(2,88) = 0.33$, $p=0.720$, phase*genotype $F(2,88) = 0.098$, $p=0.907$; genotype*treatment $F(1,44) = 0.49$, $p=0.486$; phase*surgery*treatment $F(2,88) = 0.057$, $p=0.944$; phase*genotype*treatment $F(2,88) = 0.27$, $p=0.767$; surgery*genotype*treatment $F(1,44) = 0.22$, $p=0.640$; phase*surgery*genotype*treatment $F(2,88) = 2.05$, $p=0.135$.

Pairwise comparisons on the main effect of sample phase showed that there was significantly greater contact time in sample phase 1 than in both phases 2 and 3 ($p<0.0005$ for both). Sample phase 3 also showed significantly less exploration than in phase 2 ($p=0.008$). Therefore, all mice demonstrated habituation to the objects across the three sample phases. The main effect of genotype revealed that the overall *APP*$^{NL-F}$ mice explored objects significantly less than their WT counterparts and this was consistent over both surgical timepoints.

		Sample Phase Contact Times							
		Pre-Surgery				Post-Surgery			
		WT		*APP*$^{NL-F}$		WT		*APP*$^{NL-F}$	
		IgG	2B3	IgG	2B3	IgG	2B3	IgG	2B3
Sample Phase 1	**Mean**	**67**	**78**	**67**	**55**	**78**	**75**	**63**	**67**
	SD	20	25	20	15	30	31	14	27
Sample Phase 2	**Mean**	**56**	**55**	**46**	**43**	**53**	**53**	**48**	**47**
	SD	22	20	16	11	26	27	18	16
Sample Phase 3	**Mean**	**47**	**42**	**42**	**36**	**51**	**52**	**43**	**37**
	SD	13	9	16	5	21	21	15	8

Table 7.1: Mean contact times across the OiP sample phases pre- and post-infusion of 2B3 or control IgG in WT and *APP*$^{NL-F}$ mice. Contact time is measured in seconds, SD = standard deviation.

Test Phase Contact Times

The contact times for objects both in familiar or novel locations in the test phase of the object in place task are recorded in figure 7.1. The Shapiro-Wilke test reported non-normal distributions in specific datasets ($p<0.05$). Log10 transformations of all groups generated normal distributions and the data were analysed by a 2x2x2x2 repeated measures ANOVA which analysed the main effects of object-place (novel or familiar), surgery stage, genotype and treatment.

The ANOVA revealed a significant main effect of object-place $F(1,44) = 124$, $p<0.0005$. Pairwise interactions showed that overall, there was greater exploration of objects that had been switched to novel spatial locations. There were no significant main effects of surgery $F(1,44) = 0.16$, $p=0.690$; treatment $F(1,44) = 0.008$, $p=0.930$ or genotype $F(1,44) = 0.70$, $p=0.408$. There was a significant four-way surgery*object-place*genotype*treatment interactions: $F(1,44) = 18$, $p<0.0005$. There were also other significant interactions: object-place*genotype $F(1,44) = 46$, $p<0.0005$; object-place*genotype*treatment $F(1,44) = 25$, $p<0.0005$; object-place*genotype*surgery $F(1,44) = 20$, $p<0.0005$. F values were <1 for all remaining interactions: surgery*genotype; surgery*treatment; surgery*genotype*treatment; object-place*treatment; object-place*surgery; object-place*surgery*treatment.

Simple main effects analysis on the four-way interaction revealed that WT mice **preferentially explored different over same** object-place pairings before surgical treatment (both $p<0.0005$). However, after surgery, only WT mice treated with IgG but not 2B3 demonstrated this preference ($p<0.0005$ and $p=0.813$, respectively). In contrast, APP^{-NL-F} mice exhibited the opposite pattern: there was no significant difference in contact time with the objects in novel locations before 2B3 or control IgG treatment in APP^{-NL-F} mice ($p=0.553$ and $p=0.808$ respectively) or during infusion of the IgG ($p=0.750$). However, APP^{-NL-F} mice receiving 2B3 did explore the different object-place pairings significantly more than familiar ones ($p<0.0005$). Furthermore, there were no simple main effects of **treatment** prior to the surgery (all $p>0.4$). In contrast, following the surgery, there was a significant simple main effect of treatment in both genotypes on the contact with objects that had remained in familiar locations during the test phase. WT mice receiving 2B3 explored these objects significantly more than those receiving 2B3 ($p=0.013$); meanwhile APP^{-NL-F} mice showed a reduction in exploration ($p=0.009$). There were no significant

effects of treatment in either genotypes contact with objects in novel locations following surgery (WT p=0.085; *APP*$^{-NL-F}$ p=0.855).

There was also a significant simple main effect of **surgery stage** on contact time with both object types for the WT 2B3 group. These mice explored the novel object-place associations significantly less after the surgery (p=0.007) while spending more time with the familiar pairings (p=0.029). *APP*$^{-NL-F}$ mice treated with 2B3 spent less time exploring objects that had remained in the same location during treatment compared to before the surgery (p=0.004), whereas contact time with objects that had switched positions did not change (p=0.344). There were no significant simple main effects of surgery state on contact times in the IgG treated group with either object type (p>0.5). Moreover, prior to the surgery there were significant main effects of **genotype** on contact time with the objects in novel locations in both treatment groups, as WT mice showed greater contact times (IgG p=0.025; 2B3 p=0.004). There was no difference on the contact times with objects in familiar locations (IgG p=0.051; 2B3 p=0.130). Following surgery, the significant effects of genotype were evident on contact times with the familiar object-place pairings. WT IgG mice showed reduced contact compared to *APP*$^{-NL-F}$ IgG mice (p=0.023), while the opposite (increased contact) was observed in WT 2B3 versus *APP*$^{-NL-F}$ 2B3 mice (p=0.005). There was no significant differences in exploration of objects in novel locations in the WT IgG vs *APP*$^{-NL-F}$ IgG and WT 2B3 vs *APP*$^{-NL-F}$ 2B3 comparisons (p=0.051 and 0.768 respectively).

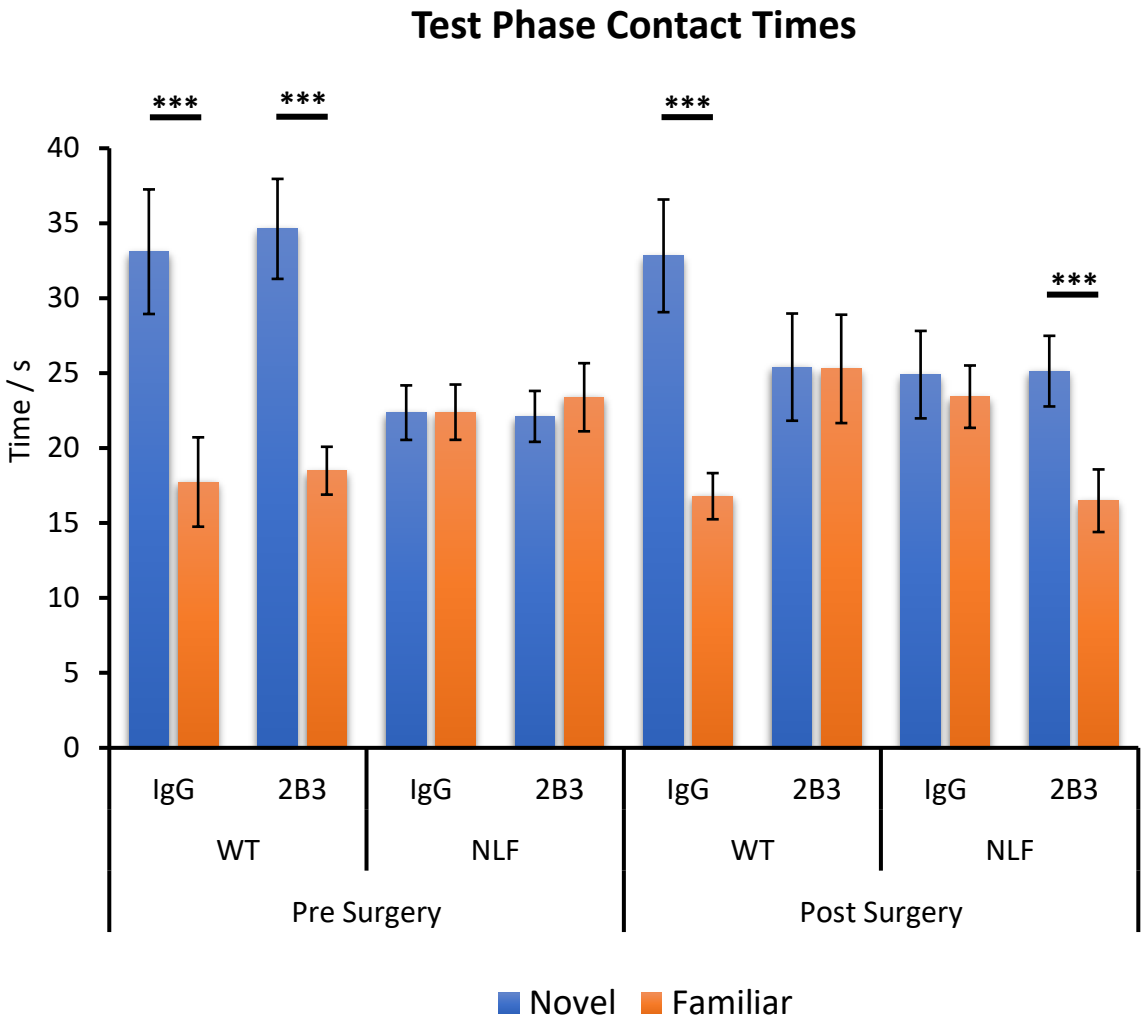


Figure 7.1: Mean contact times of WT and *APP*$^{NL-F}$ mice in the test phase of the object-in-place task pre- and post-infusion of 2B3 or control antibody. Measurement in seconds of exploration of objects in either novel or familiar locations. Error bars represent SEM, n=12 for all groups.

Test Phase Discrimination Ratio

Figure 7.2 shows the contact preferences during the test phase expressed as discrimination ratios for each group, which negates potential confounding effects of differences the decreased contact exhibited by *APP*$^{NL-F}$ mice. These DRs are displayed in figure 7.2. The datasets were all normally distributed and were analysed by a 2x2x2 mixed measures ANOVA in which surgery (pre- vs post-) was a within subjects' factor, and antibody treatment and genotype were between subjects' factors. The ANOVA revealed a significant main effect of genotype $F(1,44) = 38$, $p<0.0005$), but no main effects of surgery $F(1,44) = 0.044$, $p=0.835$; or treatment $F(1,44) = 1.4$, $p=0.240$. There was no surgery*treatment interaction $F(1,44) = 0.66$, $p=0.422$, However the surgery*genotype

F(1.44) = 24, p<0.0005; genotype*treatment F(1,44) = 23, p<0.0005; and surgery*genotype*treatment F(1.44) = 23, p<0.0005 interactions were significant.

The simple main effects analysis of the three-way interaction revealed that WT mice achieved significantly higher DRs than *APP*$^{-NL-F}$ mice prior to the surgery, as did the WT mice in the IgG group after surgery (all p<0.0005). However, the opposite pattern occurred in the 2B3 infused groups, as the *APP*$^{-NL-F}$ mice demonstrated better discrimination than WT mice (p<0.0005). Meanwhile, while there were no simple main effects of antibody treatment before surgery, for both WT (p=0.982) and *APP*$^{-NL-F}$ (p=0.745), WT mice treated with 2B3 showed worse discrimination than those receiving the control IgG (p<0.0005). In contrast, 2B3 infusion significantly improved performance in *APP*$^{-NL-F}$ mice compared to IgG treatment (p=0.001). The within subjects' factor of surgery state was only significant for the 2B3 treatment in both genotypes, as WT mice developed an impairment and *APP*$^{-NL-F}$ mice improved their DR score (p<0.0005 for both). There was no effect of surgery when either genotype received the control IgG (WT p=0.798; *APP*$^{-NL-F}$ p=0.733).

The significant treatment*genotype interaction revealed that in the control IgG condition, WT mice showed significantly greater discrimination than *APP*$^{-NL-F}$ mice (p<0.0005), there was no difference in the 2B3 condition (p=0.354). Furthermore, the WT IgG group had higher DR scores than the WT 2B3 group (p<0.0005) while the *APP*$^{-NL-F}$ IgG group achieved significantly lower scores than the *APP*$^{-NL-F}$ 2B3 group. Pairwise comparisons within the genotype*surgery interaction revealed a significant simple main effect of genotype before surgery as WT mice demonstrated higher DRs (p<0.0005), but no difference between the WT and *APP*$^{-NL-F}$ groups after treatment (p=0.549). There were significant effects of surgery in both the WT and *APP*$^{-NL-F}$ groups (p=0.001 and 0.002 respectively), as the WT mice's ability to discriminate declined while the *APP*$^{-NL-F}$ mice significantly improved.

One-sample t-tests were used to compare the group DR scores to the predicted chance score of 0.5. In the pre surgery condition, both WT groups significantly discriminated at above chance levels (IgG t(1,11) = 8.3, p<0.0005; 2b3 t(1,11) = 8.7, p<0.0005). In contrast, neither *APP*$^{-NL-F}$ group showed better than chance discrimination (IgG t(1,11) = 0.051, p=0.961; 2b3 t(1,11) = -0.38, p=0.715). However following surgery, the WT group that had been treated with 2B3 failed to discriminate same and different object-location pairings (IgG t(1,11) = 7.2, p<0.0005; 2b3 t(1,11) = -0.17, p=0.865). The

opposite pattern occurred in the *APP*$^{NL-F}$ mice, as those treated with 2B3 were able to discriminate above chance while those receiving control IgG antibody did not perform above chance (IgG $t(1,11) = 0.574$, $p=0.578$; 2b3 $t(1,11) = 6.1$, $p<0.0005$). These results were supported by live-tracking data obtained using EthoVision software. The settings used for tracking are detailed in section 2.3.3 and data is available on request.

Collectively this analysis demonstrates that icv infusion of 2B3 induced contrasting effects *APP*$^{NL-F}$ and WT mice. It caused a significant object-in-place memory deficit in WT mice, while rescuing age-dependent deficit in *APP*$^{NL-F}$ mice.

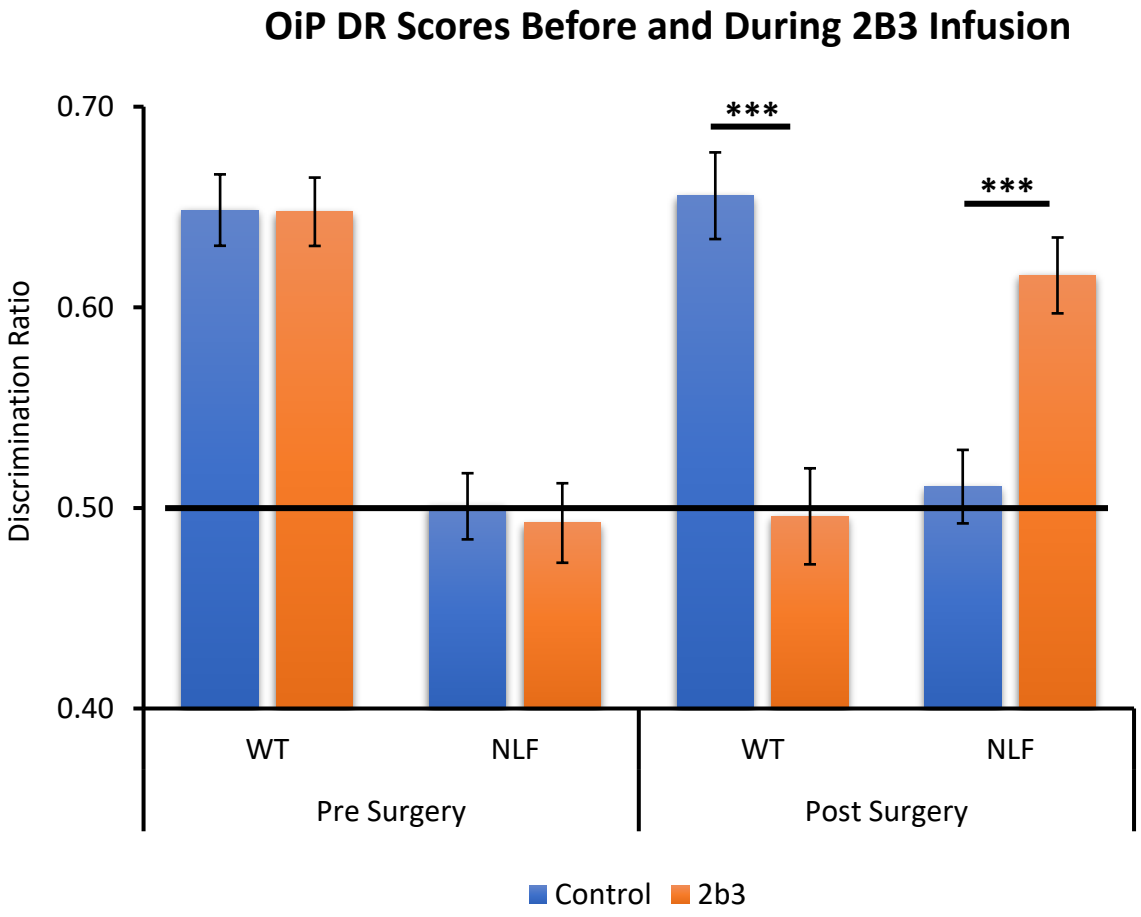


Figure 7.2: 2B3 Infusion causes dissociated effects on object-in-place (OiP) memory in WT and *APP*$^{NL-F}$ mice. Discrimination ratios calculated from contact with objects that have either switched or remained in the same spatial location. The score predicted by random performance (0.5) is highlighted by the black bar. Error bars represent SEM. N=12 for all groups. *** $p<0.0005$, 2x2x2 mixed measures ANOVA.

7.3.4 – Experiment 12 Discussion

The results of this experiment present an intriguing dissociation of impact of icv infusion of 2B3 on OiP recognition memory performance. While 2B3 significantly improved the performance of aged *APP*$^{-NL-F}$ mice, alleviating their deficit so that they were able to discriminate between objects that had or had not switched locations, the opposite effect occurred in WT littermates, as 2B3 disrupted visuospatial memory until the group were unable to discriminate. This is the first time that this dissociation of effect has been reported within one experiment. Furthermore, to our knowledge this is the first rescue of cognitive deficits by an anti-amyloid therapy reported in *APP*$^{-NL-F}$ mice. The effect of 2B3 in these knock-in mice is consistent with that observed by the Evans group, who saw OiP performance improve in aged PDAPP mice following a similar administration and test protocol (Evans et al. 2019). Moreover, the deficit in visuospatial memory observed in the pre-treatment performance of WT and *APP*$^{-NL-F}$ mice replicates the result of experiment 5 in a separate cohort of mice. This replication of the OiP deficit increases the reliability of the result and presents a protocol that is sensitive to the age-related changes in neuronal function exhibited by the *APP*$^{-NL-F}$ model. Therefore this protocol may be used by future studies in order to assess the efficacy of putative AD therapies.

There was no impact of 2B3 or control IgG on overall contact time with objects in the sample or test phases in any treatment group, demonstrating that neither treatment nor the surgery itself impacted the motivation to interact with objects. However, a significant main effect of genotype revealed that *APP*$^{-NL-F}$ mice were less likely to engage with objects in the sample phase. The reduction in contact time may present an explanation as to why these mice did not discriminate between objects in familiar or novel locations. However, this is unlikely because the different cohort of *APP*$^{-NL-F}$ mice in experiment 5 also showed a deficit in discrimination without reduced sampling. However, the reduced object contact may suggest why the DR of the 2B3 treated *APP*$^{-NL-F}$ group is numerically reduced compared to the pre-surgery WT groups.

In summary, these data reveal that 2B3 treament elicited opposite effects in *APP*$^{-NL-F}$ and WT mice, indicating that the putatitive therapeutic strategy of inhibiting BACE-dependent APP cleavage may be advantageous to patients with amyloid accumulation but detrimental to those without.

7.4 Experiment 13: *Ex Vivo* Analysis of 2B3 Treatment

7.4.1 – Introduction

The aim of this experiment was to investigate the impact of 2B3 treatment on APP metabolism and neuronal receptor dynamics in *APP*$^{NL-F}$ and WT mice. The proposed mechanism of action of 2B3 on APP metabolism involves binding to the BACE1 cleavage site and sterically inhibiting production of Aβ by the endogenous enzyme (Thomas, Liddell, and Kidd 2011). On the basis of this, one would expect that the levels of Aβ would be lower in the hippocampus of WT and *APP*$^{NL-F}$ mice administered 2B3.

7.4.2 – Methods

All of the protocols used in this chapter have been detailed in Chapter 2. The samples used in this experiment were isolated from the mice in experiment 12. There were four groups (WT control IgG; WT 2B3; *APP*$^{NL-F}$ control IgG; *APP*$^{NL-F}$ 2B3), all of which had an n of 12 mice. Mice were culled immediately following the final OiP trial and the brains were dissected and flash-frozen in liquid nitrogen. Hippocampi from the left hemispheres were processed to produce homogenate samples, while the right hippocampi underwent synaptosome extractions. Homogenate samples were used in ELISA analysis with WT mice analysed using the Mouse Aβ40 kit and *APP*$^{NL-F}$ groups compared on the human Aβ42 kit. The concentration of Aβ42 in WT mice was below detectable levels, while a similar issue with *APP*$^{NL-F}$ Aβ40 levels has been described in Chapter 6. Biochemical procedures including protein extraction, ELISA and Western blots were carried out by Chiara Favero under my supervision, and analysis of all data was carried out by myself.

7.4.3 – Experiment 13 Results

APP Processing

The effect of 2B3 on APP processing in *APP*$^{NL-F}$ mice was measured by ELISA analysis of Aβ42 concentration. There was a significant reduction in hippocampal Aβ42 in *APP*$^{NL-F}$ mice treated with 2B3 compared to those from *APP*$^{NL-F}$ mice receiving control IgG antibody, t-test $t(22) = 2.15$; $p=0.043$; see figure 7.3 A. Figure 7.3 A also shows the effect of 2B3 on Aβ42 extracted in the insoluble fraction. An independent t-test reported no effect of treatment type $t(22) = 0.97$, $p=0.344$.

The impact of 2B3 on APP processing in WT mice was assessed by ELISA measuring the concentration of Aβ40, which is presented in figure 7.3 B. There was a significant reduction in Aβ40 in the 2B3-treated samples, t-test: t(22) = 2.25, p=0.04.

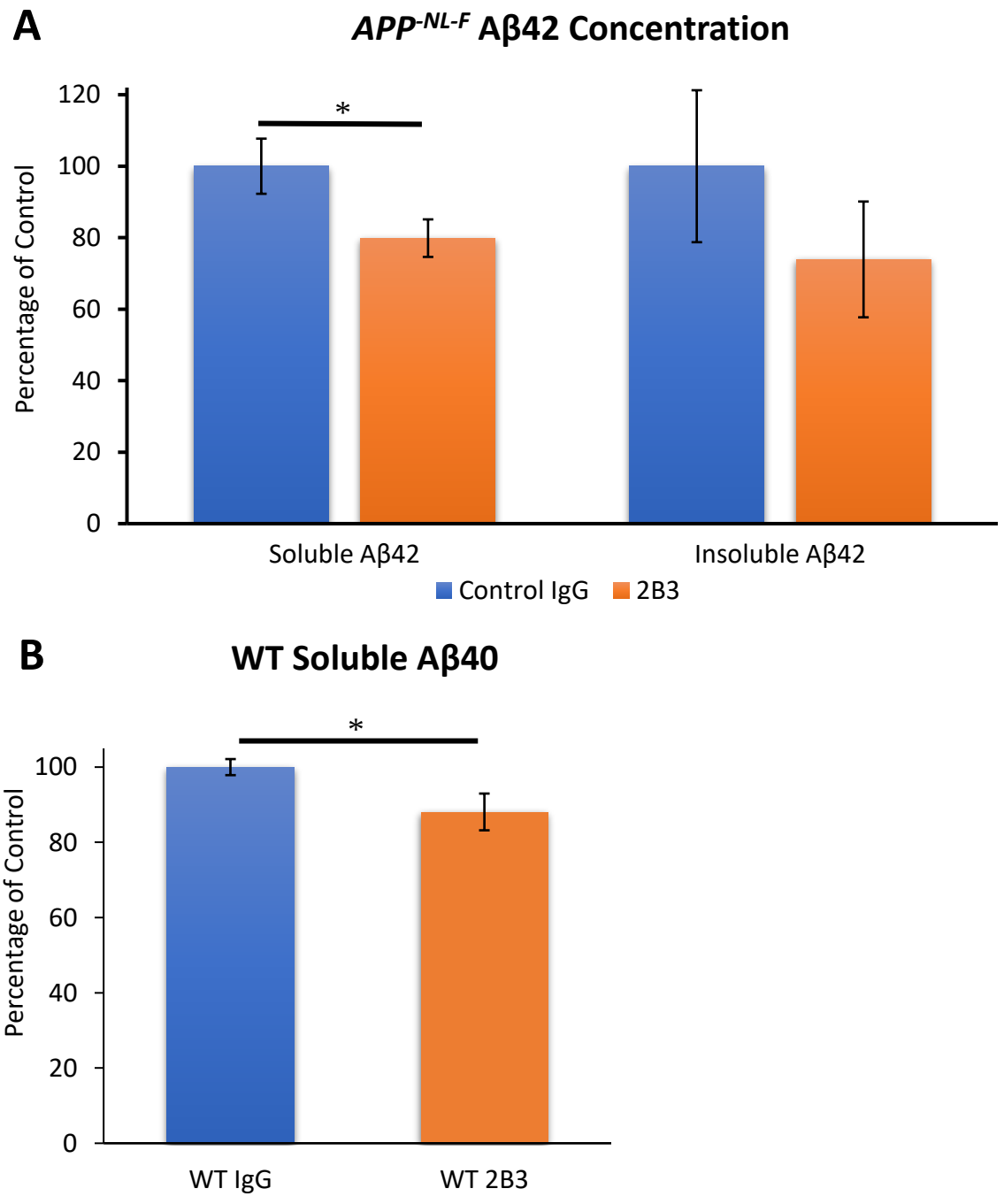


Figure 7.3: 2B3 reduced Aβ concentration in both *APP*$^{NL-F}$ and WT mice. ELISA results comparing the concentrations of Aβ in hippocampi following icv infusion of either 2B3 or a control antibody. Data were normalised to the mean concentration of the control group and analysed by independent samples t-tests. **(A)** 2B3 significantly reduced the concentration of soluble Aβ42 in aged *APP*$^{NL-F}$ mice (* $p<0.05$). There was no significant difference in the amount of Aβ42 in the insoluble fraction. N=12 for both groups. **(B)** 2B3 also significantly reduced the level of soluble Aβ40 in aged WT mice. * $p<0.05$, independent samples t-test. N=12 for both groups.

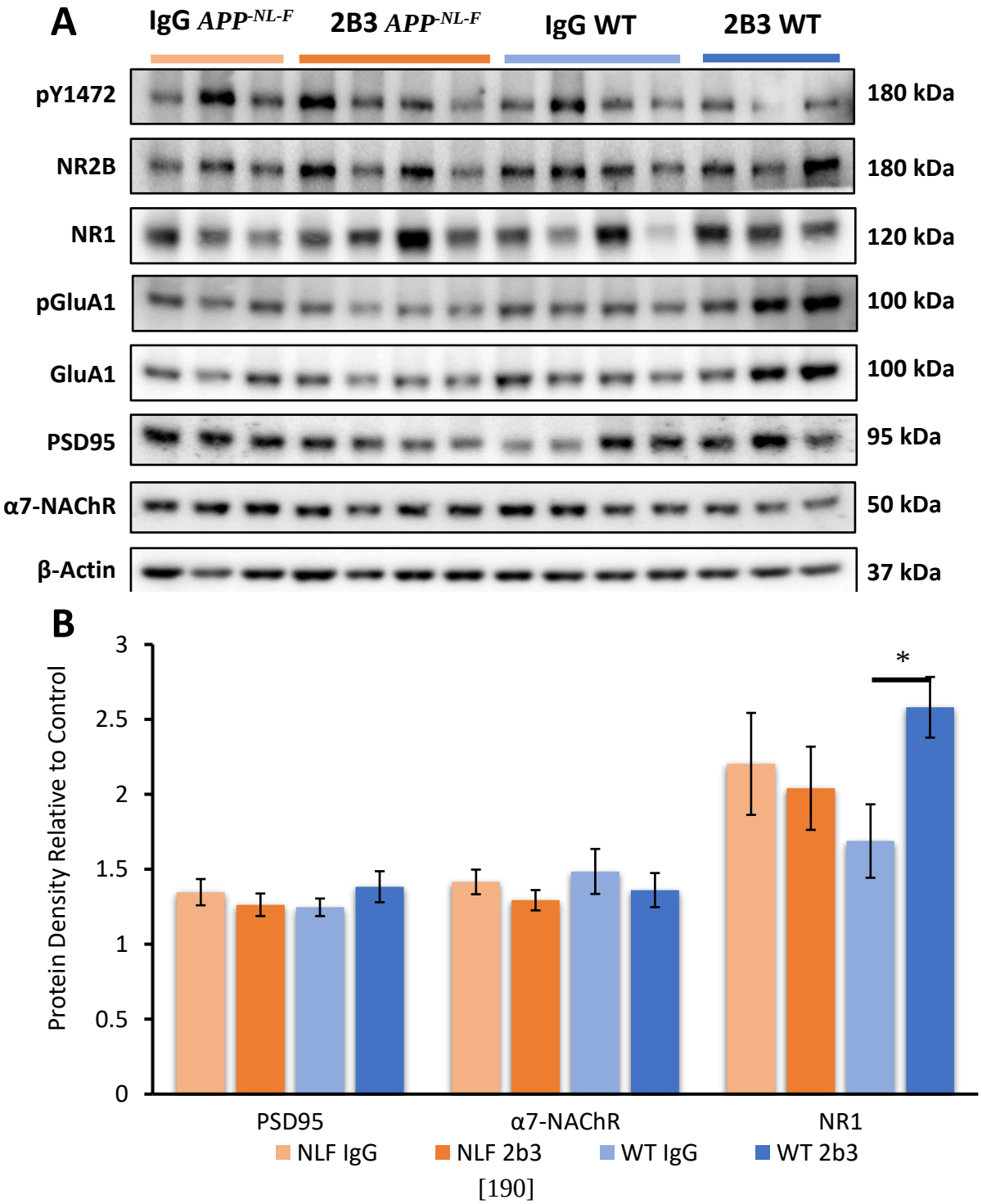
Synaptic Receptor Expression

The right hippocampi were processed into synaptosome preparations and Western blot analysis was performed to compare expression levels of synaptic markers. The distributions of all datasets were assessed by the Shapiro-Wilke test and transformed by square root if necessary, to ensure normality ($p>0.05$). The groups were compared by a two-way ANOVA with genotype and treatment as factors. The total expression of PSD95 did not show significant effects of genotype $F(3,44) = 0.018$, $p=0.893$, or treatment $F(3,44) = 0.073$, $p=0.789$ (figure 7.4 A). There was also no genotype*treatment interaction $F(3,44) = 1.6$, $p=0.217$. When comparing the expression of α7-NAChR, there were no significant main effects of genotype $F(3,44) = 0.39$, $p=0.537$, or treatment $F(3,44) = 1.2$, $p=0.27$, and no interaction $F(3,44) = 0$, $p=0.992$.

Expression of NMDA receptors was analysed (NR1, figure 7.4 B). There were no main effects of genotype $F(3,44) = 0.006$, $p=0.938$, or treatment $F(3,44) = 2.3$, $p=0.139$, however there was a significant interaction: genotype*treatment $F(3,44) = 4.3$, $p=0.044$. Pairwise comparisons on this interaction showed that there was significantly higher expression in WT mice that had been infused with 2B3 than control IgG ($p=0.015$). The NR2B subtype was also investigated (figure 7.4 C), and there were no significant effects or interactions present: main effect of genotype $F(3,44) = 0.84$, $p=0.363$; main effect of treatment $F(3,44) = 0.35$, $p=0.557$; genotype*treatment $F(3,44) = 0.18$, $p=0.677$. There was a similar lack of significant effects in the expression of the phosphorylated form of NR2B (pY1472, figure 7.4 C). main effect of genotype $F(3,44) = 3.2$, $p=0.079$; main effect of treatment $F(3,44) = 3.0$, $p=0.088$; genotype*treatment $F(3,44) = 3.325$, $p=0.075$. However, when the amount of pY1472 was normalised to the amount of NR2B (pY1472 : NR2B, figure 7.4 C), there were significant main effects of genotype $F(3,44) = 8.0$, $p=0.007$; and treatment $F(3,44) = 5.9$, $p=0.019$. There was also a significant genotype*treatment interaction $F(3,44) = 5.2$, $p=0.027$. Pairwise comparisons revealed that WT mice treated with 2B3 exhibited lower relative phosphorylation of NR2B than WT mice administered control IgG ($p=0.002$) and 2B3-infused *APP*$^{NL-F}$ mice ($p=0.001$).

AMPA receptor expression was also investigated (GluA1, figure 7.4 D). There was no main effect of genotype $F(3,44) = 0.67$, $p=0.417$, or treatment $F(3,44) = 0.61$, $p=0.437$, and no genotype*treatment interaction $F(3,44) = 0.27$, $p=0.605$. Analysis of the expression of phosphorylated GluA1 did not reveal main effects of genotype $F(3,44) = 0.44$, $p=0.511$ or treatment $F(3,44) = 0.26$, $p=0.613$. There was a significant genotype*treatment

interaction F(3,44) = 4.7, p=0.036, however no significant differences were observed in the pairwise interactions. When expression of phosphorylated GluA1 was normalised to the total amount of GluA1 (pGluA1 : GluA1, figure 7.4 D), there were no main effects of genotype F(3,44) = 0.075, p=0.786 or treatment F(3,44) = 0.065, p=0.799, and a significant genotype*treatment interaction effect F(3,44) = 10.2, p=0.003. Pairwise comparisons revealed that 2B3-treated *APP*$^{NL-F}$ mice had significantly reduced phosphorylation of GluA1 compared to control-treated *APP*$^{NL-F}$ mice (p=0.018). On the other hand, 2B3 infusion was associated with increased GluA1 phosphorylation in WT mice (p=0.043).



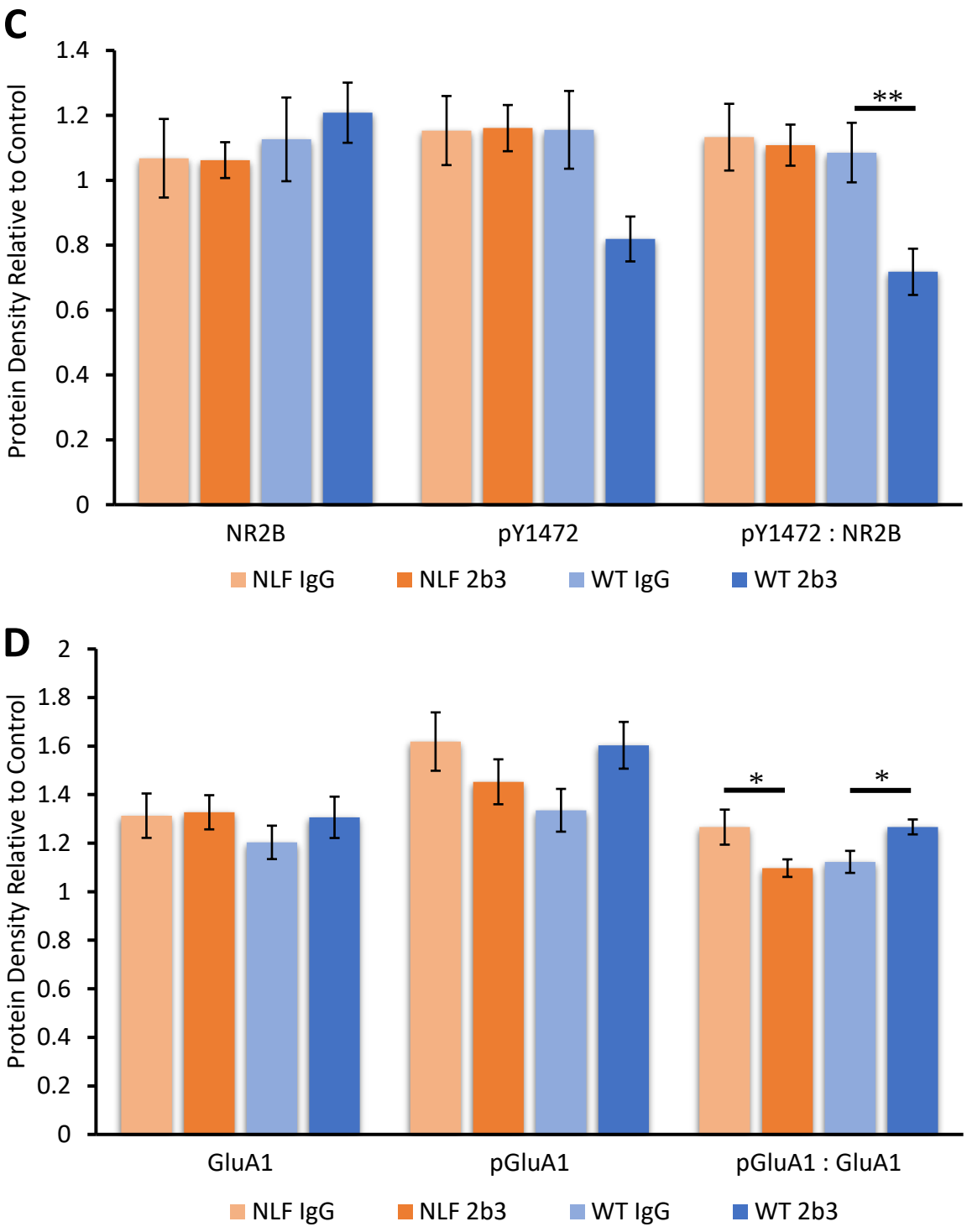


Figure 7.4: Western blot analysis of hippocampal synaptosomes.
Representative Western blot images of various synaptic markers. **(A)** Representative Western blot images in *APP*$^{NL-F}$ and WT mice treated with either 2B3 or control IgG. n=12 for all 4 groups. **(B)** Quantification of protein expression for PSD95, nicotinic acetylcholine receptor α7 (α7-NAChR) and total NMDA receptor (NR1). **(C)** Quantification of the expression of NR2B and phosphorylated NR2B (pY1472), as well as the relative phosphorylation of the receptor. **(D)** Quantification of the expression of GluA1 and phosphorylated GluA1, as well as the relative phosphorylation of the receptor. Error bars represent SEM. *p<0.05, **p<0.01

7.4.4 – Experiment 13 Discussion

This experiment demonstrated that selective inhibition of BACE-dependent APP cleavage significantly inhibited the production of Aβ42 in the hippocampus of aged *APP*$^{NL-F}$ mice. This is the first example of this strategy being used in a knock-in model of AD, as Evans and colleagues infused 2B3 into transgenic PDAPP mice. The fact that 2B3 induced a significant reduction of Aβ42 in the *APP*$^{NL-F}$ mice shows that the result of the previous study was not dependent on APP overexpression. As the knock-in model exhibits increased clinical relevance compared to transgenics, the results of this experiment support the evidence that steric hinderance of BACE-dependent APP cleavage is a potential therapeutic strategy to reduce amyloid pathology. Infusion of 2B3 in aged WT mice also resulted in significant reduction of Aβ40 concentration, consistent with the result of Experiment 2, in which the subjects were 5-months-old.

This study also investigated the effect of 2B3 on glutamate receptor dynamics in aged *APP*$^{NL-F}$ and WT mice. Although no changes were identified in the expression or phosphorylation of NMDA receptors between *APP*$^{NL-F}$ groups infused with either 2B3 or control antibody; WT mice administered 2B3 showed significantly increased NMDA expression and reduced phosphorylation of NR2B compared to control-treated mice. Although Experiment 2 showed only a numerical increase in NMDA expression following 2B3-treatment, the effect on NR2B phosphorylation was significant in both that and the current experiment. However, the impact on phosphorylation of the AMPA receptor subunit GluA1 in WT mice was opposite to that reported previously. While the result in Chapter 3 showed a significant decrease in phosphorylation, the current experiment showed a significant increase in 2B3-infused hippocampi compared to control-IgG. The reason for this is unclear and is expanded upon in section 7.5. Meanwhile, 2B3 administration resulted in significantly decreased phosphorylation of GluA1 in *APP*$^{NL-F}$ mice compared to the control group. The fact that the opposite effect is observed in the two genotypes is intriguing considering the association with memory performance reported in Experiment 12, as the two groups that showed intact OiP performance also showed significant dephosphorylation of GluA1 compared to mice that did not.

7.5: Chapter Discussion

Anti-amyloid therapies have consistently showed promise in alleviating the amyloid burden and cognitive deficits exhibited by transgenic models of AD, yet failed in clinical trials. The failure of therapies aimed at reducing amyloid burden to show clinical benefit has led to criticism of previous preclinical studies, as well as challenges to the amyloid cascade hypothesis. The artificially high levels of amyloid species in the brains of transgenic mice may represent an unphysiologically aggressive insult to neuronal function and therefore, while many potential treatments have shown beneficial results in these models, they may not be clinically relevant. For example, Evans *et al* (2019) reported that icv infusion of 2B3 reduced Aβ40 in the hippocampus and rescued OiP performance in aged PDAPP mice. However, the results of the current chapter indicate that this strategy can be effective even in absence of APP overexpression, as 2B3 reduced Aβ42 in the hippocampus and rescued the deficit in OiP memory performance of aged *APP*$^{-NL-F}$ mice. This represents the first use of this strategy in a knock-in model that expresses APP at normal endogenous levels and therefore provides more evidence in support of the amyloid cascade hypothesis and the potential for this therapeutic strategy.

However, these results also raise an inherent problem with anti-amyloid therapies: that endogenous Aβ is required for normal cognition. Amyloid-based therapies may have differential impacts on synaptic and memory processes that depend on the level of amyloid pathology. Therefore, reducing Aβ concentration in low or near normal contexts may be detrimental or not effective. Potentially, that could add noise to a clinical trial. Certainly, in terms of a treatment in pre-symptomatic individuals it argues strongly that modulating amyloid at this stage could be dangerous.

Aged *APP*$^{-NL-F}$ mice demonstrated a deficit in OiP memory in the pre-treatment assessment of baseline performance, replicating the result of Experiment 5 in a separate cohort of mice, supporting its reliability. Experiment 4 revealed that object novelty recognition was intact in aged *APP*$^{-NL-F}$ mice, suggesting that the OiP deficit was not due to a failure to identify different objects, but rather impaired spatial processing. A number of studies by the Warburton group have produced a model whereby associative recognition memory depends on interactions within a network consisting of the hippocampus, perirhinal cortex and mPFC (Warburton and Brown 2015). Therefore, the age-related deficit in OiP memory is likely due to Aβ-mediated dysfunction of this network.

Aβ peptides have been implicated in cognitive dysfunction in numerous studies using either direct infusion or transgenic models to investigate their impact (Hsiao et al. 1996, Flood et al. 1994). The Iberian mutation at the C-terminus of the Aβ sequence ensures that Aβ42 is the predominant species in *APP*$^{-NL-F}$ mice (Saito et al. 2014), and this peptide is thought to induce greater neurotoxicity than Aβ40 (Klein, Kowall, and Ferrante 1999). Experiment 13 revealed that 2B3 infusion reduced Aβ42 levels in aged *APP*$^{-NL-F}$ mice by 20%. Considering 2B3 infusion rescued OiP memory alongside reducing Aβ accumulation in these mice, a probable mechanism is rescue of Aβ-mediated hippocampal function within this network. This is a similar result to that observed by the Evans group following 2B3 administration in aged PDAPP mice. Moreover, as the first example of an anti-amyloid treatment delivering benefit to a knock-in AD model, the current study indicates that the effect in PDAPP mice was not an artefact of APP overexpression.

The lack of studies investigating the alleviation of pathology in *APP*$^{-NL-F}$ mice may have been caused by the absence of a robust cognitive phenotype currently in the literature. The observation of a significant decline in OiP performance by aged *APP*$^{-NL-F}$ mice in Chapter 4 permitted the current experiment. Izumi *et al* (2018) reported that administration of SAK3, a T-type voltage-gated calcium channel enhancer, to *APP*$^{-NL-F}$ mice between 9 and 12 months of age impeded the development of object recognition and step-through passive avoidance deficits that were present in 12-month-old, vehicle-treated, *APP*$^{-NL-F}$ mice. Although SAK3 treatment also inhibited plaque deposition in these mice, it is not specifically targetted to amyloid, and chronic prophylactic treatment is a different strategy compared to the attempted reversal of the deficit observed in the pre-surgery condition of the current experiment. The Izumi group also reported no impact of treatment in WT mice, while the current chapter presents the first significant effect of selective BACE1-APP cleavage inhibition in aged WT mice. Although 2B3-treated WT mice did not discriminate objects that had switched positions in experiment 2, no group differences were significant. This was likely due to lack of statistical power. The current experiment used a larger group size and reveled a significant detrimental effect of 2B3 in WT mice on memory.

Western blot analyses of the impact of 2B3 in WT mice revealed a reduction in NR2B phosphorylation that was consistent between Experiments 2 and 13. Phosphorylation of the tyrosine 1472 residue inhibits internalisation of NR2B-containing NMDA receptors from the synaptic membrane (Snyder et al. 2005). Therefore, the results suggest that 2B3-mediated reduction of Aβ led to a decreased amount of NR2B-containing

NMDA complexes in the synaptic membrane in WT mice. Hippocampal NMDA receptors are critical to OiP associative memory, as shown by Barker & Warburton (2015) who observed impaired performance in rats following infusion of AP5, an NMDA-antagonist into the hippocampus. Furthermore, the Evans group demonstrated that the specific NR2B inhibitor/antagonist Ro25-6981 induced an OiP memory deficit, while also observing that reduced phosphorylation of NR2B in aged PDAPP mice was also correlated with OiP impairment. However, while aged *APP*$^{-NL-F}$ mice exhibited a similar cognitive deficit as PDAPP mice, the current experiment did not show changes in expression or phosphorylation of NMDA receptors. It may be that the reduced amyloid burden exhibited by knock-in mice resulted in more subtle changes in NMDA dynamics which were not discernible by the same methods. On the other hand, there may be distinct mechanisms leading to disruption of visuospatial memory in the PDAPP and *APP*$^{-NL-F}$ mice.

One putative mechanism underpinning disrupted OiP memory that was consistent across the two genotypes in this experiment is the increased phosphorylation of GluA1 subunits of AMPA receptors, which was observed in both the 2B3-treated WT and control-treated *APP*$^{-NL-F}$ mice. The fact that dephosphorylation of GluA1 correlated with the two genotype/treatment groups that demonstrated the ability to discriminate novel object-place associations increases the reliability that this represents a mechanism underpinning cognitive performance. Dephosphorylation of the serine 845 residue on the GluA1 subunit leads to endocytosis of AMPA receptors and is associated with long term depression (LTD), a synaptic plasticity mechanism involved in memory formation (He et al. 2009). In fact, while it is speculative to associate the changes in AMPA receptor dynamics observed in the current experiment to specific synaptic mechanisms in the absence of *in vivo* electrophysiological recordings; LTD has previously been associated with visual recognition memory. Blocking endocytosis of AMPA receptors in the perirhinal cortex resulted in impairment of object recognition performance and LTD expression in the perirhinal cortex during slice electrophysiology (Griffiths et al. 2008). There is also evidence specifically relating to the hippocampus and OiP performance. Ro25-6981, which inhibits NR2B activation and OiP memory (as mentioned above), has also been shown to impair the induction of LTD. Meanwhile, Goh and Manahan-Vaughan (2013) suggested LTD as the major cellular substrate underpinning visuospatial learning, as they measured the enabling of LTD in the hippocampus of mice during the learning of a novel spatial arrangement of objects. They also reported that this effect was dependent on the activation of NMDA and mGluR5 receptors (Goh and Manahan-Vaughan 2013). There is evidence

of Aβ peptides enhancing LTD in the dentate gyrus through the activation of mGluR5 (Chen et al. 2013). Meanwhile, Aβ is able to bind to α7-NAChR with high affinity and this has been linked to the recycling of synaptic NMDA (Wang et al. 2000, Snyder et al. 2005). In fact, the interaction of Aβ with α7-NAChR is intriguing due to the concentration-dependent switch from a facilitatory to inhibitory effect which mirrors its impact on cognition (Mura et al. 2012). Further discussion of this receptor is presented in section 8.3.

While the observed impact of 2B3 on NR2B phosphorylation and cognition in WT mice in the current experiment was consistent with that observed in Experiment 2; the significant increase in GluA1-Ser845 phosphorylation is the opposite effect to the decrease previously reported. The explanation for this change is not clear. It is unlikely to be related to genetic variability, as although the subjects of the current experiment were bred from an *APP*$^{NL-F}$ colony, this model uses a C57Bl/6l background strain and so genetic differences with the C57Bl/6l mice used in Experiment 2 will be minimal. However, Experiment 2 involved 5-month-old mice while the current experiment used 17-month-old mice, and this age difference may be responsible for the observed change in effect on glutamate receptor dynamics. While there are no direct studies on AMPA receptor trafficking in models of normal ageing, analysis of synaptic plasticity mechanisms in the hippocampus may provide the best available evidence of age-related changes in the function of this receptor. During normal ageing, there is a decrease in basal synaptic transmission and LTP is impaired, requiring stronger stimulation for induction (Barnes, Rao, and Houston 2000), while, LTD is facilitated (Norris, Korol, and Foster 1996). It is possible that the age-dependent change in synaptic plasticity mechanisms reported in these studies may explain the inconsistency in 2B3 impact on GluA1 phosphorylation observed in 5- and 17-month old mice.

While this is the first example of an anti-amyloid therapy demonstrating cognitive benefit in a non-transgenic APP model, many studies using a variety of strategies have been effective in APP overexpressors. The first of such studies involved anti-Aβ antibodies which alleviated amyloid burden in 16-month-old PDAPP mice (Bard et al. 2000). Another study observed a rapid 1000-fold increase in plasma Aβ along with its clearance from the brain following peripheral injection of the m266 mAb (DeMattos et al. 2001). Administration of this antibody was also associated with a rapid rescue of object recognition and spatial working memory deficits (Dodart et al. 2002). Kotilinek et al (2002) published similar results with BAM-10, an Aβ N-terminal region-targeted antibody, which reduced both soluble and insoluble Aβ levels and improved cognitive function in Tg2576

mice. Passive anti-Aβ immunotherapy has also demonstrated the potential to influence other symptoms of amyloid pathology in multiple FAD models, such as synaptic plasticity impairment, dendritic spine loss and reactive gliosis (Chauhan and Siegel 2002, 2003, Klyubin et al. 2005, Spires-Jones et al. 2009). Antibody treatment also reduced the rate of early aggregation of hyperphosphorylated tau in 12-month-old 3xTg-AD mice (Oddo et al. 2004). However, this effect did not extend to more advanced stages of NFT pathology in older mice; further hinting at a therapeutic window that is restricted to early stage AD. Inhibition of β-secretase has also shown benefit in reducing the pathology and cognitive impairments of transgenic mice (Asai et al. 2006, Hussain et al. 2007). However, due to concerns regarding potential off-target effects caused by inhibition of other BACE1-dependent pathways, the alternative strategy of using anti-APP antibodies targeting the β-secretase cleavage site to sterically inhibit metabolism has been developed. While administration of 2B3 reduced amyloid burden and cognitive deficits in aged PDAPP mice, another antibody with a similar mechanism, BBS1 ameliorated amyloid pathology and a recognition memory deficit in the Tg2576 and 3xTg transgenic mouse models (Rakover, Arbel, and Solomon 2007, Rabinovich-Nikitin et al. 2012).

Collectively, these studies provide clear evidence of the beneficial effect of reducing amyloid in transgenic mice which overexpress mutant hAPP. However, as the current experiments used a knock-in mouse line that does not suffer from the artefacts of overexpression, they provide increased clinical relevance and suggests that this therapeutic strategy may be beneficial even in the absence of overexpression. While anti-amyloid therapies have failed to translate to the clinic, this may be due to a lack of understanding of what stage of AD is actually recapitulated in these mouse models. Considering the lack of neurodegeneration and NFT pathology in APP mutant mice, they most likely represent preclinical AD, and so suggest that anti-amyloid treatments would be most effective during this stage of the human disease. However, while the evidence of cognitive benefit in $APP^{-}{}^{NL\text{-}F}$ mice promotes early intervention strategies, the fact that Aβ reduction was detrimental to mice which lack amyloid accumulation supports the argument that the synaptic impairment associated with small molecule BACE inhibitors was caused by the impact on APP cleavage and not alternative substrates (Filser *et al*, 2016). Therefore, while selective amyloid inhibition represents a promising strategy to treat patients who have developed amyloid pathology, it may be detrimental to those who do not. This may offer an explanation for the failure of so many clinical trials, and greater understanding of the precise therapeutic window for anti-amyloid treatments is required.

Chapter 8 –

Thesis Discussion

8.1 Thesis Overview

The major aims of this thesis were to examine the role of Aβ in normal neuronal function and the effect of age-related accumulation of Aβ in a knock-in mouse model of amyloid pathology. In order to investigate the first aim, an anti-APP antibody, 2B3, was administered to young WT mice to selectively inhibit production of mouse Aβ and assess the effect on cognitive function and biochemical markers of synaptic activity. The second aim involved the breeding and aging of a cohort of APP^{-NL-F} mice that express a mutated human Aβ sequence under the control of the endogenous murine APP promoter. These mice were tested on a battery of behavioral tasks in order to characterize any cognitive decline and were subsequently used to examine age- and amyloid-related changes in synaptic proteins. The final study assessed the effect of 2B3 in both WT and APP^{-NL-F} mice and showed a dissociable effect of the antibody on cognition in WT and knock-in mice, disrupting memory in the former and improving memory in the latter.

8.2 Summary of Findings

While the widely accepted amyloid cascade hypothesis presents accumulation of Aβ as the central event in the pathogenesis of AD, understanding of the peptide's normal physiological function (if any) is limited. This latter point is important because reduction of endogenous amyloid production (by anti-amyloid therapies) remains a potential strategy for treatment of those at risk of dementia or displaying early stage biomarkers of disease onset. However, the consequences of inhibiting/reducing amyloid production at the pre-symptomatic stage for mental health remains unclear. The administration of BACE inhibitors in both clinical and preclinical studies has resulted in greater cognitive decline and synaptic impairment in patients or WT mice (Filser et al. 2015, Egan et al. 2019). However, because alternative substrates of BACE may be affected, it is not possible to conclude that the detrimental effects of inhibitors were specific to the impact on APP cleavage and Aβ production.

Chapter 3 evaluated the hypothesis that selectively inhibiting BACE cleavage of APP would lead to a memory impairment in healthy WT mice. The 2B3 antibody reduced Aβ production in primary cortical cultures derived from PDAPP mice, as well as *in vivo* by icv infusion into aged mice of the same strain (Evans et al. 2019, Thomas, Liddell, and Kidd 2011). The results of Experiment 2 revealed that icv infusion of 2B3 by osmotic minipump over 14 days significantly reduced Aβ40 in the hippocampus, but not the cortex

of 5-month-old WT mice. The WT mice given 2B3 lost their ability to detect changes in object-place associations, which had been evident prior to the drug treatment. Western blot analysis in Experiment 2 revealed significant reductions in hippocampal NMDA and AMPA receptor phosphorylation in the 2B3-infused mice, indicating a putative synaptic mechanism linking the reduction in Aβ to cognitive dysfunction. Previous research has indeed linked these two glutamate receptors to OiP memory (Barker and Warburton 2015). These experiments demonstrated a normal physiological role of Aβ in synaptic function and memory in WT mice (Morley et al. 2010, Puzzo et al. 2011).

While many therapeutic interventions for AD have demonstrated promising results in preclinical models, they have all failed to translate into a clinical benefit for patients. One explanation that has gained traction is that the overexpression of mutant human APP in first generation models can induce phenotypic artefacts that are independent of the activity of the protein of interest (Sasaguri et al. 2017). In fact, some estimates suggest that approximately 60% of the phenotypes reported in transgenic AD models are artefacts of gene overexpression. APP presents particular issues due to the existence of multiple fragments, all of which are overexpressed. Therefore, the second main aim of this thesis was to characterise the phenotype of a recently developed knock-in model, the *APP*$^{-NL-F}$, which expresses murine APP with a humanised Aβ sequence containing two FAD mutations, all under the endogenous mouse promoter. Given the extensive evidence that hippocampal dependent memory is particularly susceptible to Aβ accumulation, the cognitive changes in *APP*$^{-NL-F}$ mice were assessed on the OiP task, which is exceptionally sensitive to disruption of activity within the hippocampus – perirhinal cortex – mPFC network (Barker and Warburton 2011, Webster et al. 2014). OiP assessment was counterbalanced with object novelty recognition as a control task to confirm the capability of recognizing four individual objects. These tests revealed that while *APP*$^{-NL-F}$ performance was intact at 8 months of age, a deficit in OiP memory developed by 17 months of age. This specificity of the deficit in associative object-in-place memory was then assessed further. In contrast to associative recognition memory, object novelty and object location memory were intact in the *APP*$^{-NL-F}$ mice. However, one other attribute of object exposure, that of the temporal order in which objects had been encountered, was also impaired in *APP*$^{-NL-F}$ compared to WT littermates. It is argued that the two tasks sensitive to *APP*$^{-NL-F}$ mice mutation required integration of multiple object features, and these associative recognition tasks involve a network comprising the hippocampus, perirhinal cortex and mPFC. The specific deficit in associative tasks suggests that the Aβ

accumulation induced dysfunction at the network level rather than in a selective brain region, as disconnection analysis has demonstrated that communication within the aforementioned network is vital to associative recognition memory performance (Barker and Warburton 2011). In Chapter 5, the *APP*$^{NL-F}$ mice were assessed on a foraging-based working memory task to examine the generality of their cognitive deficit. They demonstrated an age-related decline in performance of the spatial but not the non-spatial version of the task, the former is sensitive to hippocampal lesion (Evans et al. 2018). This pattern clearly established the sensitivity of aged *APP*$^{NL-F}$ to encoding spatial and temporal features of objects and reward locations. In summary, the data presented in Chapters 4 and 5 indicate that the more refined mechanism of Aβ accumulation exhibited by the knock-in *APP*$^{NL-F}$ model represents a suitable system in which to assess the mechanisms of early AD pathogenesis and the consequences of disrupting amyloid production.

The above pattern of results suggest that the benefical effects of disrupting Aβ production relies on the level of amyloid pathology. This observation could provide a possible explanation for the failure of anti-amyloid therapies in clinical trials. Patients who did not have widespread amyloid pathology might have been adversely affteced by Aβ-related therapies. Therefore, experiments within Chapter 7 tested the hypothesis that reduction of Aβ in mice would lead to differential effects on cognition depending on the presence or absence of amyloid pathology. Infusion of 2B3 into aged *APP*$^{NL-F}$ mice rescued memory dysfunction in the knock-in mice but impaired the healthy WT control mice. This dissociation of effect has not been reported previously, as most studies only examined the impact of treating mice with widespread amyloid pathology. The results of this experiment are important for our understanding of the normal role of amyloid in the brain and have wider implications for therapeutic stratgeies targetting this peptide in psychologically normal individuals at risk of developing AD.

8.3 Does Aβ Play a Role in Neuronal Function?

Experiments within Chapters 3 and 7 of this thesis have demonstrated that specific inhibition of BACE-dependent APP cleavage resulted in significant reduction of the Aβ concentration in the hippocampus, along with deficits in the ability to discriminate objects that had changed spatial location. These results were consistent with previous studies that used alternative methods to inhibit Aβ function and observed cognitive impairment. Filser

et al demonstrated that administration of BACE inhibitors to young WT mice resulted in deficits in Y-maze spontaneous alternation (Filser et al. 2015). However, the potential impact of BACE inhibitors on alternative BACE substrates challenges the conclusion that this effect was due specifically to reduction in Aβ concentration. Nevertheless, three groups have observed similar detrimental effects following either icv or intrahippocampal infusion of anti-Aβ antibodies (Garcia-Osta and Alberini 2009, Morley et al. 2010, Puzzo et al. 2011). Furthermore, they also demonstrated that the detrimental effect of Aβ-antibodies was rescued by concurrent application of exogenous Aβ42 peptide. The Puzzo group injected the anti-Aβ antibodies into the hippocampi of 3-4-month-old mice 15-minutes prior to assessment in either the MWM or fear conditioning paradigms. They observed impairment of spatial reference memory and contextual (but not cue-related) freezing response. In a related study, Puzzo et also showed that application of picomolar concentrations of exogenous Aβ actually enhanced memory performance in the same tests, further indicating that endogenous concentrations of the peptide are involved in normal neuronal function (Puzzo et al. 2008).

Interestingly, the inhibitory effect of anti-Aβ antibodies only occurred when they were injected prior to, not immediately following training, addition of picomolar Aβ enhanced memory performance when injected at either time point. This suggests that, while the peptide is required for encoding mechanisms, a small excess is also able to enhance retention. Puzzo *et al* (2011) also showed that production of the peptide was increased in the hippocampus 1-minute following a foot shock associated with a context. The effect was not found following foot shock alone, suggesting that the increase was correlated with associative learning. Aβ has previously been shown to be specifically upregulated by excitatory synaptic activity (Cirrito et al. 2005, Kamenetz et al. 2003). The effect of both picomolar Aβ and antibodies binding to the peptide on memory performance has been replicated *in vitro*, as the treatments either enhanced or impaired LTP in hippocampal slices, respectively (Puzzo et al. 2008, Puzzo et al. 2011, Morley et al. 2010). It is possible that Aβ is secreted during processes involved in synaptic plasticity, and that 2B3 inhibits this activity-driven release, impairing synaptic function and memory formation.

The molecular mechanism through which physiological Aβ functions in synaptic plasticity and memory performance remains unclear. The studies by the Puzzo, Morley and Garcia-Osta groups have cited the α7-NAChR as a putative mediator of Aβ's effect on synaptic plasticity. More specifically, Morley and Garcia-Osta showed that administration

of Aβ increased acetylcholine (ACh) production, while α7-NAChR antagonists exhibited the same pattern of effects as anti-Aβ antibodies (Morley and Farr 2012, Garcia-Osta and Alberini 2009). The Puzzo group reported that the facilitatory effect of Aβ on LTP induction was blocked by co-administration of α7-NAChR antagonists and it was also abolished in hippocampal slices taken from mice with genetic deletion of the receptor (Puzzo et al. 2008). Furthermore, anti-Aβ antibodies showed no effect on LTP or memory in these knock-out mice (Puzzo et al. 2011). Aβ binds the α7-NAChR with high affinity, and is known to activate it (Wang et al. 2000, Dineley, Bell, et al. 2002). At endogenous concentrations, the interaction of Aβ with the α7-NAChR is facilitatory and potentiates the release of glutamate (Mura et al. 2012, Hascup and Hascup 2016). Importantly in the context of the current studies, activation of this receptor has been linked to enhancement of NMDA receptor activity in the hippocampus (Bali, Nagy, and Hernádi 2017, Cheng and Yakel 2015, Dougherty, Wu, and Nichols 2003). Therefore, while expression of α7-NAChRs was unchanged following 2B3 administration in Experiments 2 and 13, it is tempting to speculate that depletion of Aβ in the hippocampus by 2B3 reduced the endogenous Aβ-mediated activation of the receptor and led to the subsequent decrease in NR2B phosphorylation. In turn, this reduction in phosphorylation has been linked to increased internalisation of NR2B-containing NMDA complexes from the synaptic membrane and impaired OiP performance (Evans et al. 2019).

An alternative possible mechanism is that 2B3 infusion causes dysfunction within the hippocampus – mPFC – perirhinal cortex network (Barker and Warburton 2011). Nicotinic ACh receptors are responsible for gating synaptic plasticity at hippocampal – prefrontal synapses and α7NAChR activation in the mPFC is critical to the encoding of a spatial array of objects in the OiP task (Sabec et al. 2018). Reduction of the Aβ concentration by 2B3 may reduce the activation of this receptor, thereby impairing performance. However, analysis of Aβ levels in the mPFC following icv infusion of 2B3 would be required to evaluate whether this putative mechanism is viable, particularly as there is no direct evidence of the role of hippocampal α7-NAChRs in OiP memory performance. In summary, although Aβ clearly performs an important role in neuronal function, further investigation is required to elucidate the specific synaptic processes in which it is involved.

8.4 How Does the role of Aβ Change in AD?

Despite evidence of a neurotrophic function of Aβ emerging soon after the peptide was first identified (Yankner, Duffy, and Kirschner 1990), research has mainly focussed on its neurotoxicity due to its role in the pathogenesis of AD. A vast number of studies have shown that application or overexpression of the peptide induces synaptic and memory impairment. However, a large body of evidence now indicates that the switch in role of the peptide from physiological to pathogenic is dependent on its concentration and aggregation state. Studies by the Puzzo group mentioned above showed that Aβ exerts a hormetic effect on synaptic (and cognitive) function. Hormesis describes a biphasic dose-response interaction where the effect reverses with increasing concentration of a chemical (Kendig, Le, and Belcher 2010). Specifically, Aβ demonstrated a facilitatory effect on hippocampal LTP at concentrations ranging between 20 picomolar and 2 nanomolar, above which it exerted an inhibitory effect (Puzzo, Privitera, and Palmeri 2012). The effect was replicated *in vivo*, as 200 pM Aβ enhanced reference memory in the MWM, while 20 μM impaired it. The endogenous concentration of the peptide in the healthy brain is estimated to be in the picomolar range, which may explain the concentrations at which a facilitatory effect was observed (Puzzo et al. 2008, Schmidt, Nixon, and Mathews 2005). This feature of Aβ seems to be consistent with the experiments in this thesis (summarised by figure 8.1), as reduction of endogenous Aβ levels was detrimental to cognition, while accumulation of mutant human Aβ in *APP*$^{NL\text{-}F}$ mice also resulted in an age-related decline in associative memory tests. The specific mechanisms underpinning how increasing concentration and aggregation state of the peptide inhibit neuronal function are not yet fully elucidated, but may involve interaction with α7-NAChRs, in an opposite way to that described above.

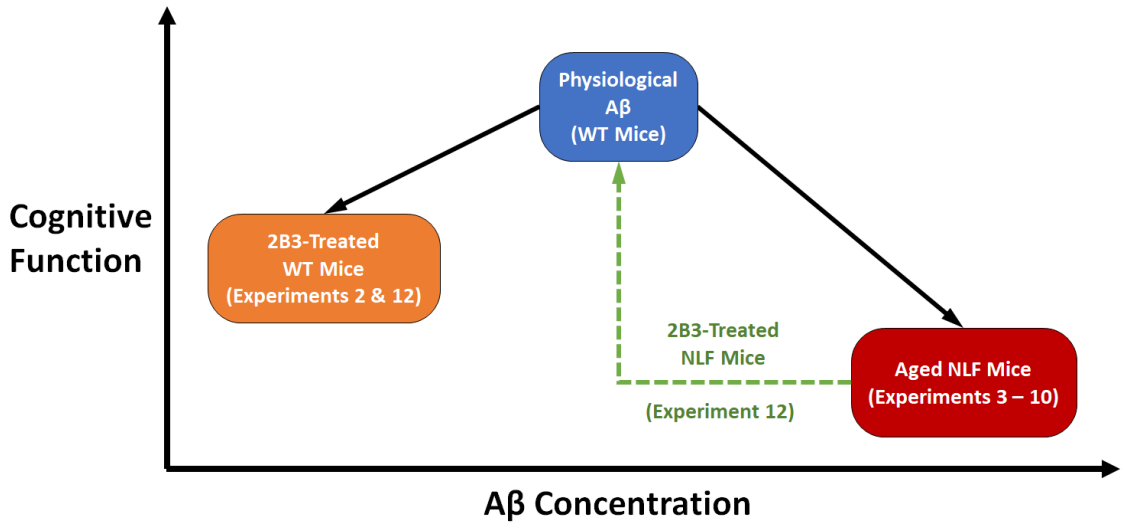


Figure 8.1: Diagram summarising how the results presented in this thesis demonstrate the hormetic effect of Aβ on cognition. The normal physiological concentration of Aβ is represented by the blue box and is present in healthy WT mice. Infusion of 2B3 reduces the concentration below this level by selectively inhibiting BACE-dependent APP cleavage, resulting in cognitive impairment, as observed in experiments 2 and 12. Increased concentration of the peptide following age-related accumulation in *APP*$^{NL-F}$ mice also results in a cognitive deficit. However, reduction of Aβ concentration in these mice rescued cognitive performance, further indicating that the role of this peptide is highly concentration-dependent.

The proposal that α7-NAChRs mediate at least some of the physiological effects of Aβ is intriguing, as the hormetic effect of the peptide on synaptic plasticity and memory enhancement is mirrored by its effect on α7-NAChR activity. At low endogenous concentrations, Aβ activates the receptor and potentiates neurotransmitter release; however, following increased concentration or oligomerisation of the peptide, its effect becomes antagonistic (Mura et al. 2012, Sadigh-Eteghada et al. 2014, Hascup and Hascup 2016, Lazarevic et al. 2017). Aβ aggregates over time and with increasing concentration (Bharadwaj et al. 2009). Thus, it is possible that the reversal in α7-NAChR activity with higher Aβ concentrations may be due to the oligomerisation state of the peptide. Considering the specific nature of protein-protein interactions, oligomerisation will likely change the binding properties. For example, Mura and colleagues reported Aβ binding to α7-NAChR and α4β2-NAChR receptors at high Aβ concentrations but only to α7-NAChR when Aβ levels were low (Mura et al. 2012). It is tempting to speculate that the detrimental effect of the peptide on cognition detected in the current study following its age-related accumulation in *APP*$^{NL-F}$ mice is linked to a change in interaction with α7-NAChRs. Specific activation of α7-NAChRs rescued both memory performance and synaptic plasticity following administration of nanomolar Aβ into the brains of WT mice in a dose-dependent manner (Chen et al. 2010, Sadigh-Eteghad et al. 2015). In fact, following the continued use of anticholinesterase inhibitors as a symptomatic treatment for AD patients, nicotinic agonists have been in clinical trials but none have been approved (Florian et al. 2016). Meanwhile, another study reported that Aβ interaction with α7-NAChRs increased the endocytosis of NR2B-NMDA receptors in hippocampal cultures, a mechanism previously associated with disruption of associative memory in aged PDAPP mice (Snyder et al. 2005, Evans et al. 2019). Furthermore, considering the involvement of mPFC α7-NAChR activation in associative memory encoding described above, the antagonistic effect of Aβ accumulation on this receptor may have contributed to the deficits observed

in Chapter 4. Histochemical analysis to confirm accumulation of the peptide in this brain region would be useful to provide evidence for this hypothesis.

In addition to mediation of the synaptic functions of Aβ, the α7-NAChR has been associated with aspects of amyloid-dependent neurotoxicity, as excessive glutamate release following excessive stimulation of this receptor may contribute to excitotoxicity (Rudy et al. 2015). Meanwhile, following the observation that Aβ colocalises with the receptor within neurons of AD brains, studies have shown that the interaction between the peptide and receptor can result in endocytosis of the complex leading to intracellular accumulation of Aβ (Nagele et al. 2002). This in turn can lead to cell lysis and plaque formation (D'Andrea et al. 2001). α7-NAChRs have been shown to desensitize quickly in the presence of Aβ (Dineley, Xia, et al. 2002). Furthermore, loss of the physiological Aβ-α7-NAChR interaction may drive the observed upregulation of the receptor in the brains of AD patients and aged Tg2576 mice in a compensatory manner (Chu et al. 2005, Dineley et al. 2001). In Experiment 11 of this thesis, there was a significant effect of group when analysing expression of α7-NAChRs. However, although there was a numerical increase in *APP-NL-F* brains, this did not reach significance during *post hoc* analysis.

However, evidence concerning the overall impact of α7-NAChRs in AD pathogenesis is controversial. One study showed that genetic deletion of the receptor exacerbated cognitive impairments and neuropathology in a transgenic APP mouse model (Hernandez et al. 2010), while another showed a protective effect (Dziewczapolski et al. 2009). The difference in age of the mice assessed in the two studies may explain the divergent results and offer an insight to the role of the receptor. α7-NAChR-KO was detrimental to 5-month-old APP transgenic mice, but advantageous to animals over 13-months of age, as MWM performance, LTP induction and synaptic marker density was preserved compared to APP transgenic expressing the receptor. This reversal of effect seems to agree with the general influence of α7-NAChRs, which seem to facilitate both the physiological role of Aβ in young, healthy systems but also the pathogenesis associated with accumulation of the peptide. Further analysis of the effect of age on APP mice with α7-NAChR-KO would be useful to resolve the role of the receptor, particularly considering the transgenic model used was inconsistent between the aforementioned studies.

In reality, the detrimental effects of amyloid accumulation most likely involves a variety of mechanisms as the peptide interacts with many different receptors. This section

has focused on the α7-NAChR due to the evidence supporting its role in facilitating both physiological and pathological aspects of Aβ function, which appears to mirror the concentration-dependent switch in the peptide's influence on synaptic and memory function. The results of Chapters 4-6 demonstrated an age-related decline in cognitive function in *APP*$^{-NL-F}$ mice that was associated with Aβ accumulation. Although this study did not observe significant differences in baseline expression of synaptic markers or receptors between aged *APP*$^{-NL-F}$ and WT mice, this may indicate the lower severity of pathology in the knock-in model compared to transgenics. Importantly, the Aβ accumulation in these mice did not depend on overexpression of mutant hAPP, indicating that this more subtle pathology was sufficient to affect cognition.

8.5 Are Knock-In Models More Reliable than Transgenic Mouse Models?

A major aim of the research conducted within this thesis was to assess how cognitive function changed with age in a knock-in mouse model of amyloid pathology. Knock-in models have been described as more physiologically relevant, compared to first-generation, transgenic models of AD, however a robust cognitive phenotype has not yet been established. The lack of overexpression provides a distinct benefit in assessing the pathological processes responsible for changes in cognition, and has already been useful in exposing results in studies of transgenic AD models that may be artefacts of APP overexpression. The only age-related cognitive effects published prior to the current study were deficits in spontaneous alternation and reversal learning measured in the Y-maze and MWM at 18 and 15 months of age respectively. The observation of a significant age-dependent deficit in visuospatial memory in two different cohorts of *APP*$^{-NL-F}$ mice in this thesis nevertheless raises the potential for further investigation of how natural Aβ accumulation leads to memory impairment in the context of an aged brain. The fact that cognitive changes are observable in the absence of overexpression removes the possibility that cognitive changes were artefacts of overexpression and provides further evidence in support of the amyloid cascade hypothesis. However, results of chapter 7 demonstrated that inhibition of Aβ production rescued memory deficits even with the subtle pathology exhibited by aged *APP*$^{-NL-F}$ mice, reinforcing the idea that anti-amyloid strategies may be beneficial during a precise, preclinical stage of AD.

The current study has also elucidated some potential artefacts of APP overexpression reported in studies using transgenic AD mouse models. The foraging task used during experiments presented in Chapter 5 replicated the methods used by Evans and colleagues (2018), who assessed PDAPP mice. However, while PDAPP mice exhibited both age-dependent and age-independent changes in foraging strategy, these were not observed in the *APP*$^{-NL-F}$ mice of the current study, suggesting that these behaviours were artefacts of APP overexpression in the transgenic model. This was particularly important for the reliability of the age-dependent decline in performance observed in the *APP*$^{-NL-F}$ mice in Chapter 5, as the results were not confounded by these artefacts. Furthermore, the current study did not observe significant differences in anxiety-related behavior by *APP*$^{-NL-F}$ mice (Experiment 3). This was consistent with previous groups, although none aged the mice beyond 12 months of age. Examination of *APP*$^{-NL-G-F}$ mice has consistently returned an anxiolytic phenotype (Latif-Hernandez *et al* (2017); Sakakibara *et al*, Pervoloraki *et al* (2018)). However, reports of anxiety phenotypes in AD models are controversial as different studies have observed either anxiogenic or anxiolytic behavior (for a review see Lalonde *et al*, 2012). Therefore, further examination of anxiety-related behavior in aged *APP*$^{-NL-F}$ mice would increase the reliability of this result. However, if the lack of anxiety phenotype is valid, it increases the reliability of using this model for investigating the impact of Aβ accumulation on behaviour, as results will not be confounded by altered emotionality, which is possible in alternative models.

The validity of modelling a human disease within another species will always be questioned. For example, clinical assessment of AD patients requires verbal communication of episodic memory which is not possible when analysing mice. Therefore, due to the differences in tests used, the memory systems being examined in each species may diverge substantially. Furthermore, mice do not naturally develop dementia, and genetic models rarely display all of the hallmarks of AD (such as tau pathology and cell loss), indicating a fundamental physiological difference between the species. However, this is not currently understood. Elucidation of the mechanisms protecting mice from the progression of NFTs and brain atrophy despite widespread amyloid pathology may enable the development of treatments for this later stage of the condition. The 3xTg model, which overexpresses mutant human tau protein in addition to APP and PS1, exhibits a comprehensive range of human AD pathology and may be crucial for this. In addition, novel human MAPT knock-in mice have recently been developed that express human tau protein (Saito et al. 2019). These mice will be useful for understanding the progression of

tau pathology in AD, particularly when they are crossed with the APP knock-in mice. However, the fact that the mutations in the MAPT gene are not associated with human AD means that, although these mice may recapitulate a greater breadth of the human AD-like pathology, their physiological relevance diminishes potentially along with their predictive validity in terms of treatment outcomes. This trade-off between the simulation of pathology versus the extent of artificial manipulation is a commonly acknowledged issue with animal models. For example, APP^{-NL-F} mice express two FAD mutations in their APP sequence. This combination does not occur naturally, but is required because knock-in of the Swedish mutation alone did not result in AD pathogenesis. Furthermore, FAD-linked mutations are not relevant for late onset AD, but are a necessary compromise. The complex combination of genetic and environmental factors that contribute to late onset AD renders exact modelling extremely challenging. Expression of genetic variants associated with the condition (such as the ApoE4 allele) in mice does not reproduce many features of AD but has developed our understanding of pathogenic mechanisms.

The crucial question that must be addressed is: what stage of human pathology do knock-in mouse models of AD represent? Despite demonstrating Aβ accumulation, plaque formation and, in some cases, reactive gliosis and hyperphosphorylation of tau; knock-in (nor transgenic) models do not develop NFTs or neurodegeneration. This suggests that they imitate a preclinical stage of AD, in which underlying amyloid pathology is present in the absence of NFTs, neurodegeneration and detectable cognitive symptoms. This may explain why so many anti-amyloid therapies reversed cognitive deficits in mouse studies but not clinical trials. Brain pathology that has progressed beyond amyloid accumulation may no longer be sensitive to treatments related to this peptide. It is possible that the current models expressing FAD mutations are only relevant for the <5% of AD patients that develop this early onset form, and clinical trials involving these patients are in development (www.clinicaltrials.gov). Alternatively, enhanced diagnostic protocols that can quickly, cheaply and reliably detect increasing amyloid concentrations or other brain changes may allow clinicians to pinpoint the exact timepoint at which pathology mimics that seen in preclinical models and is therefore potentially sensitive to treatment.

8.6 Are Anti-Amyloid Therapies Still Viable?

The widely held conclusion following the failure of so many anti-amyloid therapies in clinical trials is that, while these strategies are efficacious in preclinical models of AD, their administration provides no beneficial effect in patients with late-stage AD pathology such as NFTs and neurodegeneration. Therefore, trials have aimed to recruit patients at earlier stages of the disease, prior to the end of the putative therapeutic window. However, the results presented in Chapters 3 and 7 of this thesis indicate that reducing Aβ below physiological levels may be detrimental. This raises the concern that administration of anti-amyloid drugs to patients who do not have widespread amyloid pathology may be detrimental, further narrowing the period in which these drugs may be beneficial. However, although this information presents further complications, Chapter 7 also revealed that selective inhibition of Aβ production rescued the memory impairment in a knock-in model. This is the first evidence of this strategy demonstrating beneficial effects in a non-transgenic model, reinforcing the amyloid cascade hypothesis. Therefore, the data presented in this thesis indicate that, while the restricted therapeutic window must be acknowledged, targeting amyloid is still a viable therapeutic strategy.

The fact that treatment of AD may only be possible within a specific stage of pathology necessitates the development of quick and reliable diagnostic tests. While PET scans remain the gold standard, they are not feasible for widespread use due to their expense and low throughput. Recently, multiple studies have published blood tests that can predict AD pathology with strong reliability. A 2017 study reported that measurement of BACE1 activity in the plasma was able to discriminate individuals exhibiting normal cognition, MCI or AD with 88% specificity (Shen et al. 2018). Intriguingly, follow up tests revealed that MCI patients who converted to AD had shown significantly greater BACE1 activity in the original test than those who did not convert, suggesting that this assay may also predict progression from prodromal to probable AD dementia. Another promising test identified novel serum microRNA markers that enabled discrimination between MCI / AD and vascular dementia (Dong et al. 2015). Furthermore, analysis of the plasma Aβ42/Aβ40 ratio by immunoprecipitation and liquid-chromatography mass spectrometry has been able to predict current and developing amyloid pathology in multiple different studies, with a success rate up to 94% when comparing to the current gold standard: PET neuroimaging (Schindler et al. 2019, Nakamura et al. 2018, Perez-Grijalba et al. 2019, Ashton et al. 2019). These non-invasive diagnostic tests will reduce cost and increase the scalability of such

diagnostics. Their introduction should enable identification of more patients in the prodromal stages of AD with the potential for more successful clinical trials.

Is There Existing Hope for Amyloid Therapies?

Some of the lessons learned from clinical studies using anti-amyloid treatments have been applied to trials of the passive immunotherapy BAN2401 (Biogen / Eisai). This antibody is targeted to Aβ protofibrils, theoretically only binding intermediate peptide aggregates and therefore does not impair the physiological synaptic function of Aβ monomers. Following a favourable safety profile reported in a phase I trial (Logovinsky et al. 2016), a phase II trial involving 856 participants was designed. Importantly, this trial only recruited patients with significant amyloid pathology observed during PET scans, thereby decreasing the likelihood of detrimental effects caused by the anti-amyloid treatment. Furthermore, the trial used an innovative Bayesian "adaptive randomisation" enrolment design in which, new patients were allocated into one of five different dose regimes that had been deemed most likely to be effective by interim analyses (Satlin et al. 2016). The 18-month treatment phase results were presented at the 2018 Alzheimer's Association International Conference (AAIC) and, strikingly, represented the largest ever trial reporting a significant beneficial effect on cognitive decline. While brain amyloid was reduced by up to 93% in the maximum dose group (10 mg/kg biweekly), cognitive decline was slowed by 47% and 30% as measured on the ADAS-Cog and ADCOMS, respectively. However, following a ruling by the European Medicines Agency (EMA) one year into the trial, APOE ε4 carriers were no longer permitted to be allocated the highest dose of BAN2401, due to Amyloid-related imaging abnormalities (ARIA). This is a phenomenon whereby MRI signals detect vasogenic oedema or microhaemorrhages, typically within the first three months of Aβ immunotherapy (Penninkilampi, Brothers, and Eslick 2017). It is thought to be linked to the increased inflammatory reaction following clearance of perivascular Aβ and increased BBB permeability, both of which increases with APOE ε4 status (Sperling et al. 2012). The effect of the EMA's ruling was that allocation was no longer randomised. However, despite this confound, the potential of BAN2401 is promising for patients and researchers. Moreover, the rationale behind the treatment and trial strategy seems to be consistent with the data within this thesis, as the antibody targets aggregated, not monomeric Aβ, while only patients with Aβ pathology (as diagnosed by PET scans) were included in the trial. Results following the continuation of Ban2401 into larger scale trials may potentially validate the amyloid cascade hypothesis.

8.7 Future Directions

While the experiments within Chapters 4 and 5 demonstrate that *APP*$^{-NL-F}$ mice have developed a deficit in associative recognition memory and spatial working memory by 16-17 months of age, more frequent testing would elucidate the precise age-point at which performance becomes impaired. Correlation with ELISA analysis of Aβ accumulation at all of these age-points would provide information regarding the exact concentration at which the peptide starts to impair cognitive processes. Furthermore, the impact of 2B3 on young *APP*$^{-NL-F}$ mice was not assessed in the current study. Hypothetically, young *APP*$^{-NL-F}$ mice would respond to 2B3 infusion in a similar way to young WT mice, as they do not exhibit amyloid pathology at this age. Investigating the effect of 2B3 at multiple age-points in the *APP*$^{-NL-F}$ mice could pinpoint the amyloid burden required to switch the impact of Aβ inhibition from being detrimental to restorative. When combined with the ELISA analysis described above, this experiment may elucidate vital information regarding the influence of Aβ accumulation and the therapeutic window of anti-amyloid treatment.

Furthermore, repeating the experiments in Chapter 7 using peripheral administration would increase the clinical relevance of the study and permit an increased duration of 2B3 administration, as the minipump-dependent infusion techniques have time limits imposed by the size of the capsule. The increased duration would enable use of the alternative cognitive tests described above and investigation into whether 2B3 rescues the SWM deficit reported in Chapter 5. Finally, intraperitoneal administration of 2B3 would also permit analysis of long-term 2B3 treatment in order to examine any chronic effects of BACE-dependent cleavage of APP, as well as whether the impact is reversible after treatment has stopped.

Following evidence of altered glutamate receptor phosphorylation in 2B3-treated WT animals, it would be worthwhile to investigate the effect of reduced Aβ on synaptic function by using techniques such as slice electrophysiology or *in vivo* 2-photon imaging. Previous studies have used BACE inhibitors, anti-Aβ antibodies or APP-siRNA to show impairment of synaptic plasticity (Morley et al. 2010, Puzzo et al. 2011, Filser et al. 2015), and it would be interesting to see the effect of selective inhibition of APP-BACE1 cleavage by 2B3. Furthermore, considering the involvement of hippocampal glutamate receptor

activation in OiP memory, *in vivo* electrophysiological measurements during the test could elucidate the plasticity mechanisms involved in recognition of object-place associations.

Considering the evidence of the putative influence of the α7-NAChR in facilitating both the physiological and pathogenic effects of Aβ, further investigation of the role of this receptor would be informative. Previous studies have suggested specific agonism of this receptor rescued the cognitive deficit associated with either Aβ infusion or APP overexpression (Chen et al. 2010, Sadigh-Eteghad et al. 2015, Medeiros et al. 2014). Therefore, it would be interesting to assess whether α7-NAChR agonism rescues the cognitive deficits associated with the infusion of 2B3 in WT mice, as well as in aged APP^{-NL-F} mice. However, it must be acknowledged that these drugs are nootropic and so appropriate controls would be required to assess whether any cognitive enhancement was a specific response to Aβ manipulation (Thomsen et al. 2010). Section 8.4 describes two studies that reported opposite effects of α7-NAChR deletion in APP transgenic mice, depending on the age at which testing occurred. However, the models examined were not consistent. Crossing KO APP^{-NL-F} with α7-NAChR-KO mice would permit longitudinal analysis of the impact of the receptor at multiple stages of amyloid accumulation.

The plethora of behavioural tasks available for cognitive testing, permits the use of alternative tests which would help refine the understanding of the exact memory processes that are impaired in aged APP^{-NL-F} mice. The deficits in recognition of object-in-place and object-time associations represent some of the core elements that ultimately contribute to episodic memory, which is severely impaired in human AD patients (Bellassen et al. 2012). However, the combination of these aspects into a single task permits examination of a rodent's ability to form an integrated memory of "what happened", "where" and "when", in paradigms of object recognition or fear conditioning (Good, Hale, and Staal 2007, Iordanova, Good, and Honey 2008). These tasks are comparable to tests that are diagnostic of the human condition and have been previously used to demonstrate episodic-like memory deficits in transgenic mouse models. Repeating these methods with the knock-in APP^{-NL-F} mice would support the view that Aβ pathology is sufficient to drive changes in episodic-like memory.

8.8 Thesis Summary and Conclusions

This thesis provides evidence of the role of Aβ in cognition in both normal physiological and abnormal, pathological states. The age-related deficit in associative recognition memory reported in the *APP*$^{-NL-F}$ model is a novel phenotype and, along with the Aβ accumulation in absence of APP overexpression, validates this knock-in strain as a physiological representation of AD pathology. The robust OiP deficit measured across multiple cohorts can be used by future studies aiming to assess novel treatments for AD. Meanwhile, the restoration of memory performance by selectively inhibiting BACE1 cleavage of APP provides further evidence supporting the amyloid cascade hypothesis. This is the first report of an anti-amyloid treatment showing a beneficial effect in the absence of APP overexpression, which reinforces confidence in the potential for that strategy to yield clinical success.

However, the data presented in this thesis also demonstrated the first evidence that administration of anti-amyloid therapies induces dissociable effects depending on the existence of amyloid pathology. Cognition was impaired in both young and aged WT mice following inhibition of BACE1-dependent APP cleavage. This highlights the fact that Aβ plays an important and dynamic role in normal cognition and highlights an issue for amyloid-based prevention strategies in human clinical trials. Therefore, the results of this thesis will be important in determining how we treat AD in the future.

References

Abushouk, A. I., A. Elmaraezy, A. Aglan, R. Salama, S. Fouda, R. Fouda, and A. M. AlSafadi. 2017. "Bapineuzumab for mild to moderate Alzheimer's disease: a meta-analysis of randomized controlled trials." In *BMC Neurol*.

Adolfsson, O., M. Pihlgren, N. Toni, Y. Varisco, A. L. Buccarello, K. Antoniello, S. Lohmann, K. Piorkowska, V. Gafner, J. K. Atwal, J. Maloney, M. Chen, A. Gogineni, R. M. Weimer, D. L. Mortensen, M. Friesenhahn, C. Ho, R. Paul, A. Pfeifer, A. Muhs, and R. J. Watts. 2012. "An effector-reduced anti-beta-amyloid (Abeta) antibody with unique abeta binding properties promotes neuroprotection and glial engulfment of Abeta." *J Neurosci* 32 (28):9677-89. doi: 10.1523/jneurosci.4742-11.2012.

Aggleton, J. P. 1985. "One-Trial Object Recognition by Rats." *The Quartely Journal of Experimental Psychology* 37 (4):279-294. doi: 10.1080_14640748508401171.

Aggleton, J. P., P. R. Hunt, and J. N. Rawlins. 1986. "The effects of hippocampal lesions upon spatial and non-spatial tests of working memory." *Behav Brain Res* 19 (2):133-46.

Aguilar-Valles, A., E. Sanchez, P. de Gortari, I. Balderas, V. Ramirez-Amaya, F. Bermudez-Rattoni, and P. Joseph-Bravo. 2005. "Analysis of the stress response in rats trained in the water-maze: differential expression of corticotropin-releasing hormone, CRH-R1, glucocorticoid receptors and brain-derived neurotrophic factor in limbic regions." *Neuroendocrinology* 82 (5-6):306-19. doi: 10.1159/000093129.

Albert, M. S., S. T. DeKosky, D. Dickson, B. Dubois, H. H. Feldman, N. C. Fox, A. Gamst, D. M. Holtzman, W. J. Jagust, R. C. Petersen, P. J. Snyder, M. C. Carrillo, B. Thies, and C. H. Phelps. 2011. "The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease." *Alzheimers Dement* 7 (3):270-9. doi: 10.1016/j.jalz.2011.03.008.

Anderson, J. J., G. Holtz, P. P. Baskin, R. Wang, L. Mazzarelli, S. L. Wagner, and F. Menzaghi. 1999. "Reduced cerebrospinal fluid levels of alpha-secretase-cleaved amyloid precursor protein in aged rats: correlation with spatial memory deficits." *Neuroscience* 93 (4):1409-20. doi: 10.1016/s0306-4522(99)00244-4.

Antunes, M., and G. Biala. 2012. "The novel object recognition memory: neurobiology, test procedure, and its modifications." In *Cogn Process*, 93-110.

Arriagada, P. V., J. H. Growdon, E. T. Hedley-Whyte, and B. T. Hyman. 1992. "Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease." *Neurology* 42 (3 Pt 1):631-9. doi: 10.1212/wnl.42.3.631.

Asai, M., C. Hattori, N. Iwata, T. C. Saido, N. Sasagawa, B. Szabo, Y. Hashimoto, K. Maruyama, S. Tanuma, Y. Kiso, and S. Ishiura. 2006. "The novel beta-secretase inhibitor KMI-429 reduces amyloid beta peptide production in amyloid precursor protein transgenic and wild-type mice." *J Neurochem* 96 (2):533-40. doi: 10.1111/j.1471-4159.2005.03576.x.

Asai, M., C. Hattori, B. Szabo, N. Sasagawa, K. Maruyama, S. Tanuma, and S. Ishiura. 2003. "Putative function of ADAM9, ADAM10, and ADAM17 as APP alpha-secretase." *Biochem Biophys Res Commun* 301 (1):231-5. doi: 10.1016/s0006-291x(02)02999-6.

Ashton, N. J., A. J. Nevado-Holgado, I. S. Barber, S. Lynham, V. Gupta, P. Chatterjee, K. Goozee, E. Hone, S. Pedrini, K. Blennow, M. Scholl, H. Zetterberg, K. A.

Ellis, A. I. Bush, C. C. Rowe, V. L. Villemagne, D. Ames, C. L. Masters, D. Aarsland, J. Powell, S. Lovestone, R. Martins, and A. Hye. 2019. "A plasma protein classifier for predicting amyloid burden for preclinical Alzheimer's disease." *Sci Adv* 5 (2):eaau7220. doi: 10.1126/sciadv.aau7220.

Axmacher, N., M. M. Henseler, O. Jensen, I. Weinreich, C. E. Elger, and J. Fell. 2010. "Cross-frequency coupling supports multi-item working memory in the human hippocampus." In *Proc Natl Acad Sci U S A*, 3228-33.

Aydin, D., M. A. Filippov, J. A. Tschape, N. Gretz, M. Prinz, R. Eils, B. Brors, and U. C. Muller. 2011. "Comparative transcriptome profiling of amyloid precursor protein family members in the adult cortex." *BMC Genomics* 12:160. doi: 10.1186/1471-2164-12-160.

Bachevalier, J., and S. Nemanic. 2008. "Memory for spatial location and object-place associations are differently processed by the hippocampal formation, parahippocampal areas TH/TF and p... - PubMed - NCBI." *Hippocampus* 18 (1):64-80. doi: 10.1002/hipo.20369.

Bacskai, B. J., S. T. Kajdasz, M. E. McLellan, D. Games, P. Seubert, D. Schenk, and B. T. Hyman. 2002. "Non-Fc-mediated mechanisms are involved in clearance of amyloid-beta in vivo by immunotherapy." *J Neurosci* 22 (18):7873-8.

Baker, K. B., and J. J. Kim. 2002. "Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats." *Learn Mem* 9 (2):58-65. doi: 10.1101/lm.46102.

Bali, Z. K., L. V. Nagy, and I. Hernádi. 2017. "Alpha7 Nicotinic Acetylcholine Receptors Play a Predominant Role in the Cholinergic Potentiation of N-Methyl-D-Aspartate Evoked Firing Responses of Hippocampal CA1 Pyramidal Cells." *Front Cell Neurosci* 11. doi: 10.3389/fncel.2017.00271.

Banks, W. A., B. Terrell, S. A. Farr, S. M. Robinson, N. Nonaka, and J. E. Morley. 2002. "Passage of amyloid beta protein antibody across the blood-brain barrier in a mouse model of Alzheimer's disease." *Peptides* 23 (12):2223-6.

Bannerman, D. M., B. K. Yee, M. Lemaire, L. Wilbrecht, L. Jarrard, S. D. Iversen, J. N. Rawlins, and M. A. Good. 2001. "The role of the entorhinal cortex in two forms of spatial learning and memory." *Exp Brain Res* 141 (3):281-303. doi: 10.1007/s002210100868.

Bao, F., L. Wicklund, P. N. Lacor, W. L. Klein, A. Nordberg, and A. Marutle. 2012. "Different beta-amyloid oligomer assemblies in Alzheimer brains correlate with age of disease onset and impaired cholinergic activity." *Neurobiol Aging* 33 (4):825.e1-13. doi: 10.1016/j.neurobiolaging.2011.05.003.

Baranger, K., Y. Marchalant, A. E. Bonnet, N. Crouzin, A. Carrete, J. M. Paumier, N. A. Py, A. Bernard, C. Bauer, E. Charrat, K. Moschke, M. Seiki, M. Vignes, S. F. Lichtenthaler, F. Checler, M. Khrestchatisky, and S. Rivera. 2016. "MT5-MMP is a new pro-amyloidogenic proteinase that promotes amyloid pathology and cognitive decline in a transgenic mouse model of Alzheimer's disease." *Cell Mol Life Sci* 73 (1):217-36. doi: 10.1007/s00018-015-1992-1.

Bard, F., C. Cannon, R. Barbour, R. L. Burke, D. Games, H. Grajeda, T. Guido, K. Hu, J. Huang, K. Johnson-Wood, K. Khan, D. Kholodenko, M. Lee, I. Lieberburg, R. Motter, M. Nguyen, F. Soriano, N. Vasquez, K. Weiss, B. Welch, P. Seubert, D. Schenk, and T. Yednock. 2000. "Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease." *Nat Med* 6 (8):916-9. doi: 10.1038/78682.

Barker, G. R., P. J. Banks, H. Scott, G. S. Ralph, K. A. Mitrophanous, L. F. Wong, Z. I. Bashir, J. B. Uney, and E. C. Warburton. 2017. "Separate elements of episodic

memory subserved by distinct hippocampal-prefrontal connections." *Nat Neurosci* 20 (2):242-250. doi: 10.1038/nn.4472.

Barker, G. R., F. Bird, V. Alexander, and E. C. Warburton. 2007. "Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex." *J Neurosci* 27 (11):2948-57. doi: 10.1523/jneurosci.5289-06.2007.

Barker, G. R. I., and E. C. Warburton. 2015. "Object-in-Place Associative Recognition Memory Depends on Glutamate Receptor Neurotransmission Within Two Defined Hippocampal-Cortical Circuits: A Critical Role for AMPA and NMDA Receptors in the Hippocampus, Perirhinal, and Prefrontal Cortices." *Cereb Cortex* 25 (2):472-81. doi: 10.1093/cercor/bht245.

Barker, G.R.I., and E.C. Warburton. 2011. "When Is the Hippocampus Involved in Recognition Memory?" *J Neuroscience* 31 (29):10721-10731. doi: 10.1523/JNEUROSCI.6413-10.2011.

Barnes, C. A. 1979. "Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat." *J Comp Physiol Psychol* 93 (1):74-104.

Barnes, C. A., and B. L. McNaughton. 1985. "An age comparison of the rates of acquisition and forgetting of spatial information in relation to long-term enhancement of hippocampal synapses." *Behav Neurosci* 99 (6):1040-8.

Barnes, C. A., G. Rao, and F. P. Houston. 2000. "LTP induction threshold change in old rats at the perforant path--granule cell synapse." *Neurobiol Aging* 21 (5):613-20. doi: 10.1016/s0197-4580(00)00163-9.

Bateman, R. J., E. R. Siemers, K. G. Mawuenyega, G. Wen, K. R. Browning, W. C. Sigurdson, K. E. Yarasheski, S. W. Friedrich, R. B. Demattos, P. C. May, S. M. Paul, and D. M. Holtzman. 2009. "A gamma-secretase inhibitor decreases amyloid-beta production in the central nervous system." *Ann Neurol* 66 (1):48-54. doi: 10.1002/ana.21623.

Bateman, R. J., C. Xiong, T. L. Benzinger, A. M. Fagan, A. Goate, N. C. Fox, D. S. Marcus, N. J. Cairns, X. Xie, T. M. Blazey, D. M. Holtzman, A. Santacruz, V. Buckles, A. Oliver, K. Moulder, P. S. Aisen, B. Ghetti, W. E. Klunk, E. McDade, R. N. Martins, C. L. Masters, R. Mayeux, J. M. Ringman, M. N. Rossor, P. R. Schofield, R. A. Sperling, S. Salloway, and J. C. Morris. 2012. "Clinical and biomarker changes in dominantly inherited Alzheimer's disease." *N Engl J Med* 367 (9):795-804. doi: 10.1056/NEJMoa1202753.

Baudier, J., and R. D. Cole. 1987. "Phosphorylation of tau proteins to a state like that in Alzheimer's brain is catalyzed by a calcium/calmodulin-dependent kinase and modulated by phospholipids." *J Biol Chem* 262 (36):17577-83.

Bayer, T. A., R. Cappai, C. L. Masters, K. Beyreuther, and G. Multhaup. 1999. "It all sticks together--the APP-related family of proteins and Alzheimer's disease." *Mol Psychiatry* 4 (6):524-8.

Bayer, T. A., K. Paliga, S. Weggen, O. D. Wiestler, K. Beyreuther, and G. Multhaup. 1997. "Amyloid precursor-like protein 1 accumulates in neuritic plaques in Alzheimer's disease." *Acta Neuropathol* 94 (6):519-24.

Bellassen, V., K. Igloi, L. C. de Souza, B. Dubois, and L. Rondi-Reig. 2012. "Temporal order memory assessed during spatiotemporal navigation as a behavioral cognitive marker for differential Alzheimer's disease diagnosis." *J Neurosci* 32 (6):1942-52. doi: 10.1523/jneurosci.4556-11.2012.

Benilova, I., E. Karran, and B. De Strooper. 2012. "The toxic A beta oligomer and Alzheimer's disease: an emperor in need of clothes." *Nature Neuroscience* 15 (3):349-357. doi: 10.1038/nn.3028.

Berger-Sweeney, J., D. L. McPhie, J. A. Arters, J. Greenan, M. L. Oster-Granite, and R. L. Neve. 1999. "Impairments in learning and memory accompanied by neurodegeneration in mice transgenic for the carboxyl-terminus of the amyloid precursor protein." *Brain Res Mol Brain Res* 66 (1-2):150-62.

Bertoglio, L. J., and A. P. Carobrez. 2000. "Previous maze experience required to increase open arms avoidance in rats submitted to the elevated plus-maze model of anxiety." *Behav Brain Res* 108 (2):197-203.

Bettens, K., K. Sleegers, and C. Van Broeckhoven. 2013. "Genetic insights in Alzheimer's disease." *Lancet Neurol* 12 (1):92-104. doi: 10.1016/s1474-4422(12)70259-4.

Bharadwaj, P. R., A. K. Dubey, C. L. Masters, R. N. Martins, and I. G. Macreadie. 2009. "Aβ aggregation and possible implications in Alzheimer's disease pathogenesis." *J Cell Mol Med* 13 (3):412-21. doi: 10.1111/j.1582-4934.2009.00609.x.

Bianchi, S. L., T. Tran, C. Liu, S. Lin, Y. Li, J. M. Keller, R. G. Eckenhoff, and M. F. Eckenhoff. 2008. "Brain and behavior changes in 12-month-old Tg2576 and nontransgenic mice exposed to anesthetics." *Neurobiol Aging* 29 (7):1002-10. doi: 10.1016/j.neurobiolaging.2007.02.009.

Billings, L. M., S. Oddo, K. N. Green, J. L. McGaugh, and F. M. LaFerla. 2005. "Intraneuronal Abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice." *Neuron* 45 (5):675-88. doi: 10.1016/j.neuron.2005.01.040.

Birks, J. 2006. "Cholinesterase inhibitors for Alzheimer's disease." *Cochrane Database Syst Rev* (1):Cd005593. doi: 10.1002/14651858.cd005593.

Bjerknes, T. L., N. C. Dagslott, E. I. Moser, and M. B. Moser. 2018. "Path integration in place cells of developing rats." *Proc Natl Acad Sci U S A* 115 (7):E1637-e1646. doi: 10.1073/pnas.1719054115.

Blume, T., S. Filser, A. Jaworska, J. F. Blain, G. Koenig, K. Moschke, S. F. Lichtenthaler, and J. Herms. 2018. "BACE1 Inhibitor MK-8931 Alters Formation but Not Stability of Dendritic Spines." *Front Aging Neurosci* 10:229. doi: 10.3389/fnagi.2018.00229.

Bohrmann, B., K. Baumann, J. Benz, F. Gerber, W. Huber, F. Knoflach, J. Messer, K. Oroszlan, R. Rauchenberger, W. F. Richter, C. Rothe, M. Urban, M. Bardroff, M. Winter, C. Nordstedt, and H. Loetscher. 2012. "Gantenerumab: a novel human anti-Abeta antibody demonstrates sustained cerebral amyloid-beta binding and elicits cell-mediated removal of human amyloid-beta." *J Alzheimers Dis* 28 (1):49-69. doi: 10.3233/jad-2011-110977.

Bonardi, C., M. C. Pardon, and P. Armstrong. 2016. "Deficits in object-in-place but not relative recency performance in the APPswe/PS1dE9 mouse model of Alzheimer's disease: Implications for object recognition." *Behav Brain Res* 313:71-81. doi: 10.1016/j.bbr.2016.07.008.

Bondi, M. W., A. J. Jak, L. Delano-Wood, M. W. Jacobson, D. C. Delis, and D. P. Salmon. 2008. "Neuropsychological Contributions to the Early Identification of Alzheimer's Disease." *Neuropsychol Rev* 18 (1):73-90. doi: 10.1007/s11065-008-9054-1.

Borchelt, D. R., T. Ratovitski, J. van Lare, M. K. Lee, V. Gonzales, N. A. Jenkins, N. G. Copeland, D. L. Price, and S. S. Sisodia. 1997. "Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins." *Neuron* 19 (4):939-45.

Braak, H., and E. Braak. 1991. "NEUROPATHOLOGICAL STAGING OF ALZHEIMER-RELATED CHANGES." *Acta Neuropathologica* 82 (4):239-259.

Breitner, J. C., B. A. Gau, K. A. Welsh, B. L. Plassman, W. M. McDonald, M. J. Helms, and J. C. Anthony. 1994. "Inverse association of anti-inflammatory treatments and Alzheimer's disease: initial results of a co-twin control study." *Neurology* 44 (2):227-32. doi: 10.1212/wnl.44.2.227.

Briz, V., and M. Baudry. 2017. "Calpains: Master Regulators of Synaptic Plasticity." *Neuroscientist* 23 (3):221-231. doi: 10.1177/1073858416649178.

Broadbent, N. J., S. Gaskin, L. R. Squire, and R. E. Clark. 2010. "Object recognition memory and the rodent hippocampus." *Learn Mem* 17 (1):5-11. doi: 10.1101/lm.1650110.

Brodaty, H., D. Pond, N. M. Kemp, G. Luscombe, L. Harding, K. Berman, and F. A. Huppert. 2002. "The GPCOG: a new screening test for dementia designed for general practice." *J Am Geriatr Soc* 50 (3):530-4. doi: 10.1046/j.1532-5415.2002.50122.x.

Brody, D. L., S. Magnoni, K. E. Schwetye, M. L. Spinner, T. J. Esparza, N. Stocchetti, G. J. Zipfel, and D. M. Holtzman. 2008. "Amyloid-beta dynamics correlate with neurological status in the injured human brain." *Science* 321 (5893):1221-4. doi: 10.1126/science.1161591.

Brothers, H. M., M. L. Gosztyla, and S. R. Robinson. 2018. "The Physiological Roles of Amyloid-β Peptide Hint at New Ways to Treat Alzheimer's Disease." *Front Aging Neurosci* 10 (118). doi: 10.3389/fnagi.2018.00118.

Bu, X. L., Y. Xiang, W. S. Jin, J. Wang, L. L. Shen, Z. L. Huang, K. Zhang, Y. H. Liu, F. Zeng, J. H. Liu, H. L. Sun, Z. Q. Zhuang, S. H. Chen, X. Q. Yao, B. Giunta, Y. C. Shan, J. Tan, X. W. Chen, Z. F. Dong, H. D. Zhou, X. F. Zhou, W. Song, and Y. J. Wang. 2018. "Blood-derived amyloid-beta protein induces Alzheimer's disease pathologies." *Mol Psychiatry* 23 (9):1948-1956. doi: 10.1038/mp.2017.204.

Bussey, T. J., J. L. Muir, and J. P. Aggleton. 1999. "Functionally dissociating aspects of event memory: the effects of combined perirhinal and postrhinal cortex lesions on object and place memory in the rat." *J Neurosci* 19 (1):495-502.

Cai, H., Y. Wang, D. McCarthy, H. Wen, D. R. Borchelt, D. L. Price, and P. C. Wong. 2001. "BACE1 is the major beta-secretase for generation of Abeta peptides by neurons." *Nat Neurosci* 4 (3):233-4. doi: 10.1038/85064.

Campion, D., C. Dumanchin, D. Hannequin, B. Dubois, S. Belliard, M. Puel, C. Thomas-Anterion, A. Michon, C. Martin, F. Charbonnier, G. Raux, A. Camuzat, C. Penet, V. Mesnage, M. Martinez, F. Clerget-Darpoux, A. Brice, and T. Frebourg. 1999. "Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum." *Am J Hum Genet* 65 (3):664-70.

Cao, X., and T. C. Sudhof. 2001. "A transcriptionally [correction of transcriptively] active complex of APP with Fe65 and histone acetyltransferase Tip60." *Science* 293 (5527):115-20. doi: 10.1126/science.1058783.

Carmona, S., K. Zahs, E. Wu, K. Dakin, J. Bras, and R. Guerreiro. 2018. "The role of TREM2 in Alzheimer's disease and other neurodegenerative disorders." *Lancet Neurol* 17 (8):721-730. doi: 10.1016/s1474-4422(18)30232-1.

Carnevale, D., M. Perrotta, G. Lembo, and B. Trimarco. 2016. "Pathophysiological Links Among Hypertension and Alzheimer's Disease." *High Blood Press Cardiovasc Prev* 23 (1):3-7. doi: 10.1007/s40292-015-0108-1.

Carobrez, A. P., and L. J. Bertoglio. 2005. "Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on." *Neurosci Biobehav Rev* 29 (8):1193-205. doi: 10.1016/j.neubiorev.2005.04.017.

Cataldo, A. M., J. L. Barnett, C. Pieroni, and R. A. Nixon. 1997. "Increased neuronal endocytosis and protease delivery to early endosomes in sporadic Alzheimer's

disease: Neuropathologic evidence for a mechanism of increased beta-amyloidogenesis." *Journal of Neuroscience* 17 (16):6142-6151.
Chang, E. H., M. J. Savage, D. G. Flood, J. M. Thomas, R. B. Levy, V. Mahadomrongkul, T. Shirao, C. Aoki, and P. T. Huerta. 2006. "AMPA receptor downscaling at the onset of Alzheimer's disease pathology in double knockin mice." *Proc Natl Acad Sci U S A* 103 (9):3410-5. doi: 10.1073/pnas.0507313103.
Chang, K. A., and Y. H. Suh. 2005. "Pathophysiological roles of amyloidogenic carboxy-terminal fragments of the beta-amyloid precursor protein in Alzheimer's disease." *J Pharmacol Sci* 97 (4):461-71.
Chang, K. A., and Y. H. Suh. 2010. "Possible roles of amyloid intracellular domain of amyloid precursor protein." *BMB Rep* 43 (10):656-63. doi: 10.5483/BMBRep.2010.43.10.656.
Chartier-Harlin, M. C., F. Crawford, H. Houlden, A. Warren, D. Hughes, L. Fidani, A. Goate, M. Rossor, P. Roques, J. Hardy, and et al. 1991. "Early-onset Alzheimer's disease caused by mutations at codon 717 of the beta-amyloid precursor protein gene." *Nature* 353 (6347):844-6. doi: 10.1038/353844a0.
Chaudhury, S., K. J. Brookes, T. Patel, A. Fallows, T. Guetta-Baranes, J. C. Turton, R. Guerreiro, J. Bras, J. Hardy, P. T. Francis, R. Croucher, C. Holmes, K. Morgan, and A. J. Thomas. 2019. "Alzheimer's disease polygenic risk score as a predictor of conversion from mild-cognitive impairment." *Transl Psychiatry* 9 (1):154. doi: 10.1038/s41398-019-0485-7.
Chauhan, N. B., and G. J. Siegel. 2002. "Reversal of amyloid beta toxicity in Alzheimer's disease model Tg2576 by intraventricular antiamyloid beta antibody." *J Neurosci Res* 69 (1):10-23. doi: 10.1002/jnr.10286.
Chauhan, N. B., and G. J. Siegel. 2003. "Intracerebroventricular passive immunization with anti-Abeta antibody in Tg2576." *J Neurosci Res* 74 (1):142-7. doi: 10.1002/jnr.10721.
Chen, G., K. S. Chen, J. Knox, J. Inglis, A. Bernard, S. J. Martin, A. Justice, L. McConlogue, D. Games, S. B. Freedman, and R. G. Morris. 2000. "A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease." *Nature* 408 (6815):975-9. doi: 10.1038/35050103.
Chen, L., H. Wang, Z. Zhang, Z. Li, D. He, and M. Sokabe. 2010. "DMXB (GTS-21) ameliorates the cognitive deficits in beta amyloid(25-35(-)) injected mice through preventing the dysfunction of alpha7 nicotinic receptor." *J Neurosci Res* 88 (8):1784-94. doi: 10.1002/jnr.22345.
Chen, X. H., V. E. Johnson, K. Uryu, J. Q. Trojanowski, and D. H. Smith. 2009. "A lack of amyloid beta plaques despite persistent accumulation of amyloid beta in axons of long-term survivors of traumatic brain injury." *Brain Pathol* 19 (2):214-23. doi: 10.1111/j.1750-3639.2008.00176.x.
Chen, X., R. Lin, L. Chang, S. Xu, X. Wei, J. Zhang, C. Wang, R. Anwyl, and Q. Wang. 2013. "Enhancement of long-term depression by soluble amyloid beta protein in rat hippocampus is mediated by metabotropic glutamate receptor and involves activation of p38MAPK, STEP and caspase-3." *Neuroscience* 253:435-43. doi: 10.1016/j.neuroscience.2013.08.054.
Cheng, G., Z. Yu, D. Zhou, and M. P. Mattson. 2002. "Phosphatidylinositol-3-kinase-Akt kinase and p42/p44 mitogen-activated protein kinases mediate neurotrophic and excitoprotective actions of a secreted form of amyloid precursor protein." *Exp Neurol* 175 (2):407-14. doi: 10.1006/exnr.2002.7920.
Cheng, I. H., J. J. Palop, L. A. Esposito, N. Bien-Ly, F. Yan, and L. Mucke. 2004. "Aggressive amyloidosis in mice expressing human amyloid peptides with the Arctic mutation." *Nat Med* 10 (11):1190-2. doi: 10.1038/nm1123.

Cheng, Q., and J. L. Yakel. 2015. "The effect of α7 nicotinic receptor activation on glutamatergic transmission in the hippocampus." *Biochem Pharmacol* 97 (4):439-44. doi: 10.1016/j.bcp.2015.07.015.

Cheung, H. N., C. Dunbar, G. M. Morotz, W. H. Cheng, H. Y. Chan, C. C. Miller, and K. F. Lau. 2014. "FE65 interacts with ADP-ribosylation factor 6 to promote neurite outgrowth." *Faseb j* 28 (1):337-49. doi: 10.1096/fj.13-232694.

Chiba, A. A., R. P. Kesner, and A. M. Reynolds. 1994. "Memory for spatial location as a function of temporal lag in rats: role of hippocampus and medial prefrontal cortex." *Behav Neural Biol* 61 (2):123-31.

Chiba, K., M. Araseki, K. Nozawa, K. Furukori, Y. Araki, T. Matsushima, T. Nakaya, S. Hata, Y. Saito, S. Uchida, Y. Okada, A. C. Nairn, R. J. Davis, T. Yamamoto, M. Kinjo, H. Taru, and T. Suzuki. 2014. "Quantitative analysis of APP axonal transport in neurons: role of JIP1 in enhanced APP anterograde transport." *Mol Biol Cell* 25 (22):3569-80. doi: 10.1091/mbc.E14-06-1111.

Chu, L. W., E. S. Ma, K. K. Lam, M. F. Chan, and D. H. Lee. 2005. "Increased alpha 7 nicotinic acetylcholine receptor protein levels in Alzheimer's disease patients." *Dement Geriatr Cogn Disord* 19 (2-3):106-12. doi: 10.1159/000082661.

Chui, D. H., H. Tanahashi, K. Ozawa, S. Ikeda, F. Checler, O. Ueda, H. Suzuki, W. Araki, H. Inoue, K. Shirotani, K. Takahashi, F. Gallyas, and T. Tabira. 1999. "Transgenic mice with Alzheimer presenilin 1 mutations show accelerated neurodegeneration without amyloid plaque formation." *Nat Med* 5 (5):560-4. doi: 10.1038/8438.

Chung, J. A., and J. L. Cummings. 2000. "Neurobehavioral and neuropsychiatric symptoms in Alzheimer's disease: characteristics and treatment." *Neurol Clin* 18 (4):829-46.

Chételat, G., V. L. Villemagne, N. Villain, G. Jones, K. A. Ellis, D. Ames, R. N. Martins, C. L. Masters, and C. C. Rowe. 2012. "Accelerated cortical atrophy in cognitively normal elderly with high beta-amyloid deposition." *Neurology* 78 (7):477-84. doi: 10.1212/WNL.0b013e318246d67a.

Cirrito, J. R., K. A. Yamada, M. B. Finn, R. S. Sloviter, K. R. Bales, P. C. May, D. D. Schoepp, S. M. Paul, S. Mennerick, and D. M. Holtzman. 2005. "Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo." *Neuron* 48 (6):913-22. doi: 10.1016/j.neuron.2005.10.028.

Citron, M., T. Oltersdorf, C. Haass, L. McConlogue, A. Y. Hung, P. Seubert, C. Vigo-Pelfrey, I. Lieberburg, and D. J. Selkoe. 1992. "Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production." *Nature* 360 (6405):672-4. doi: 10.1038/360672a0.

Citron, M., D. Westaway, W. Xia, G. Carlson, T. Diehl, G. Levesque, K. Johnson-Wood, M. Lee, P. Seubert, A. Davis, D. Kholodenko, R. Motter, R. Sherrington, B. Perry, H. Yao, R. Strome, I. Lieberburg, J. Rommens, S. Kim, D. Schenk, P. Fraser, P. St George Hyslop, and D. J. Selkoe. 1997. "Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice." *Nat Med* 3 (1):67-72.

Claasen, A. M., D. Guevremont, S. E. Mason-Parker, K. Bourne, W. P. Tate, W. C. Abraham, and J. M. Williams. 2009. "Secreted amyloid precursor protein-alpha upregulates synaptic protein synthesis by a protein kinase G-dependent mechanism." *Neurosci Lett* 460 (1):92-6. doi: 10.1016/j.neulet.2009.05.040.

Clark, C. M., M. J. Pontecorvo, T. G. Beach, B. J. Bedell, R. E. Coleman, P. M. Doraiswamy, A. S. Fleisher, E. M. Reiman, M. N. Sabbagh, C. H. Sadowsky, J. A. Schneider, A. Arora, A. P. Carpenter, M. L. Flitter, A. D. Joshi, M. J. Krautkramer, M. Lu, M. A. Mintun, and D. M. Skovronsky. 2012. "Cerebral PET

with florbetapir compared with neuropathology at autopsy for detection of neuritic amyloid-beta plaques: a prospective cohort study." *Lancet Neurol* 11 (8):669-78. doi: 10.1016/s1474-4422(12)70142-4.
Clark, R. E., S. M. Zola, and L. R. Squire. 2000. "Impaired recognition memory in rats after damage to the hippocampus." *J Neurosci* 20 (23):8853-60.
Cleary, J. P., D. M. Walsh, J. J. Hofmeister, G. M. Shankar, M. A. Kuskowski, D. J. Selkoe, and K. H. Ashe. 2005. "Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function." *Nat Neurosci* 8 (1):79-84. doi: 10.1038/nn1372.
Coburger, I., S. Hoefgen, and M. E. Than. 2014. "The structural biology of the amyloid precursor protein APP - a complex puzzle reveals its multi-domain architecture." *Biol Chem* 395 (5):485-98. doi: 10.1515/hsz-2013-0280.
Cockrell, J. R., and M. F. Folstein. 1988. "Mini-Mental State Examination (MMSE)." *Psychopharmacol Bull* 24 (4):689-92.
Collett, T. S., and P. Graham. 2004. "Animal navigation: path integration, visual landmarks and cognitive maps." *Curr Biol* 14 (12):R475-7. doi: 10.1016/j.cub.2004.06.013.
Cooper, P. R., G. J. Ciambrone, C. M. Kliwinski, E. Maze, L. Johnson, Q. Li, Y. Feng, and P. J. Hornby. 2013. "Efflux of monoclonal antibodies from rat brain by neonatal Fc receptor, FcRn." *Brain Res* 1534:13-21. doi: 10.1016/j.brainres.2013.08.035.
Corder, E. H., A. M. Saunders, N. J. Risch, W. J. Strittmatter, D. E. Schmechel, P. C. Gaskell, Jr., J. B. Rimmler, P. A. Locke, P. M. Conneally, K. E. Schmader, and et al. 1994. "Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease." *Nat Genet* 7 (2):180-4. doi: 10.1038/ng0694-180.
Coric, V., C. H. van Dyck, S. Salloway, N. Andreasen, M. Brody, R. W. Richter, H. Soininen, S. Thein, T. Shiovitz, G. Pilcher, S. Colby, L. Rollin, R. Dockens, C. Pachai, E. Portelius, U. Andreasson, K. Blennow, H. Soares, C. Albright, H. H. Feldman, and R. M. Berman. 2012. "Safety and Tolerability of the gamma-Secretase Inhibitor Avagacestat in a Phase 2 Study of Mild to Moderate Alzheimer Disease." *Archives of Neurology* 69 (11):1430-1440. doi: 10.1001/archneurol.2012.2194.
Corrigan, F., R. Vink, P. C. Blumbergs, C. L. Masters, R. Cappai, and C. van den Heuvel. 2012. "sAPPalpha rescues deficits in amyloid precursor protein knockout mice following focal traumatic brain injury." *J Neurochem* 122 (1):208-20. doi: 10.1111/j.1471-4159.2012.07761.x.
Cousins, S. L., W. Dai, and F. A. Stephenson. 2015. "APLP1 and APLP2, members of the APP family of proteins, behave similarly to APP in that they associate with NMDA receptors and enhance NMDA receptor surface expression." *J Neurochem* 133 (6):879-85. doi: 10.1111/jnc.13063.
Craig, D., A. Mirakhur, D. J. Hart, S. P. McIlroy, and A. P. Passmore. 2005. "A cross-sectional study of neuropsychiatric symptoms in 435 patients with Alzheimer's disease." *Am J Geriatr Psychiatry* 13 (6):460-8. doi: 10.1176/appi.ajgp.13.6.460.
Crump, C. J., D. S. Johnson, and Y. M. Li. 2013. "Development and mechanism of gamma-secretase modulators for Alzheimer's disease." *Biochemistry* 52 (19):3197-216. doi: 10.1021/bi400377p.
Cullen, K. M., Z. Kocsi, and J. Stone. 2006. "Microvascular pathology in the aging human brain: evidence that senile plaques are sites of microhaemorrhages." *Neurobiol Aging* 27 (12):1786-96.

Cummings, J. L., T. Morstorf, and K. Zhong. 2014. "Alzheimer's disease drug-development pipeline: few candidates, frequent failures." *Alzheimers Research & Therapy* 6 (4). doi: 10.1186/alzrt269.

Cummings, J., G. Lee, A. Ritter, and K. Zhong. 2018. "Alzheimer's disease drug development pipeline: 2018." In *Alzheimers Dement (N Y)*, 195-214.

D'Andrea, M. R., R. G. Nagele, H. Y. Wang, P. A. Peterson, and D. H. Lee. 2001. "Evidence that neurones accumulating amyloid can undergo lysis to form amyloid plaques in Alzheimer's disease." *Histopathology* 38 (2):120-34. doi: 10.1046/j.1365-2559.2001.01082.x.

Danysz, W., and C. G. Parsons. 2003. "The NMDA receptor antagonist memantine as a symptomatological and neuroprotective treatment for Alzheimer's disease: preclinical evidence." *Int J Geriatr Psychiatry* 18 (Suppl 1):S23-32. doi: 10.1002/gps.938.

Das, P., V. Howard, N. Loosbrock, D. Dickson, M. P. Murphy, and T. E. Golde. 2003. "Amyloid-beta immunization effectively reduces amyloid deposition in FcRgamma-/- knock-out mice." *J Neurosci* 23 (24):8532-8.

Das, P., M. P. Murphy, L. H. Younkin, S. G. Younkin, and T. E. Golde. 2001. "Reduced effectiveness of Abeta1-42 immunization in APP transgenic mice with significant amyloid deposition." *Neurobiol Aging* 22 (5):721-7.

Daumas, S., J. Sandin, K. S. Chen, D. Kobayashi, J. Tulloch, S. J. Martin, D. Games, and R. G. Morris. 2008. "Faster forgetting contributes to impaired spatial memory in the PDAPP mouse: deficit in memory retrieval associated with increased sensitivity to interference?" *Learn Mem* 15 (9):625-32. doi: 10.1101/lm.990208.

Davies, P., and A. J. Maloney. 1976. "Selective loss of central cholinergic neurons in Alzheimer's disease." In *Lancet*, 1403. England.

Dawson, G. R., G. R. Seabrook, H. Zheng, D. W. Smith, S. Graham, G. O'Dowd, B. J. Bowery, S. Boyce, M. E. Trumbauer, H. Y. Chen, L. H. Van der Ploeg, and D. J. Sirinathsinghji. 1999. "Age-related cognitive deficits, impaired long-term potentiation and reduction in synaptic marker density in mice lacking the beta-amyloid precursor protein." *Neuroscience* 90 (1):1-13.

de Bruin, J. P., F. Sanchez-Santed, R. P. Heinsbroek, A. Donker, and P. Postmes. 1994. "A behavioural analysis of rats with damage to the medial prefrontal cortex using the Morris water maze: evidence for behavioural flexibility, but not for impaired spatial navigation." *Brain Res* 652 (2):323-33. doi: 10.1016/0006-8993(94)90243-7.

De Strooper, B., W. Annaert, P. Cupers, P. Saftig, K. Craessaerts, J. S. Mumm, E. H. Schroeter, V. Schrijvers, M. S. Wolfe, W. J. Ray, A. Goate, and R. Kopan. 1999. "A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain." *Nature* 398 (6727):518-522. doi: 10.1038/19083.

Deacon, R. M., E. Koros, K. D. Bornemann, and J. N. Rawlins. 2009. "Aged Tg2576 mice are impaired on social memory and open field habituation tests." *Behav Brain Res* 197 (2):466-8. doi: 10.1016/j.bbr.2008.09.042.

Dean, R. L., 3rd, J. Scozzafava, J. A. Goas, B. Regan, B. Beer, and R. T. Bartus. 1981. "Age-related differences in behavior across the life span of the C57BL/6J mouse." *Exp Aging Res* 7 (4):427-51. doi: 10.1080/03610738108259823.

Deane, R., A. Sagare, K. Hamm, M. Parisi, B. LaRue, H. Guo, Z. Wu, D. M. Holtzman, and B. V. Zlokovic. 2005. "IgG-assisted age-dependent clearance of Alzheimer's amyloid beta peptide by the blood-brain barrier neonatal Fc receptor." *J Neurosci* 25 (50):11495-503. doi: 10.1523/jneurosci.3697-05.2005.

DeCoteau, W. E., and R. P. Kesner. 2000. "A double dissociation between the rat hippocampus and medial caudoputamen in processing two forms of knowledge." *Behav Neurosci* 114 (6):1096-108.

Delabar, J. M., D. Goldgaber, Y. Lamour, A. Nicole, J. L. Huret, J. de Grouchy, P. Brown, D. C. Gajdusek, and P. M. Sinet. 1987. "Beta amyloid gene duplication in Alzheimer's disease and karyotypically normal Down syndrome." *Science* 235 (4794):1390-2. doi: 10.1126/science.2950593.

DeMattos, R. B., K. R. Bales, D. J. Cummins, J. C. Dodart, S. M. Paul, and D. M. Holtzman. 2001. "Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease." *Proc Natl Acad Sci U S A* 98 (15):8850-5. doi: 10.1073/pnas.151261398.

Devi, L., B. M. Prabhu, D. F. Galati, N. G. Avadhani, and H. K. Anandatheerthavarada. 2006. "Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction." *J Neurosci* 26 (35):9057-68. doi: 10.1523/jneurosci.1469-06.2006.

Dewachter, I., R. K. Filipkowski, C. Priller, L. Ris, J. Neyton, S. Croes, D. Terwel, M. Gysemans, H. Devijver, P. Borghgraef, E. Godaux, L. Kaczmarek, J. Herms, and F. Van Leuven. 2009. "Deregulation of NMDA-receptor function and down-stream signaling in APP[V717I] transgenic mice." *Neurobiol Aging* 30 (2):241-56. doi: 10.1016/j.neurobiolaging.2007.06.011.

Dineley, K. T., K. A. Bell, D. Bui, and J. D. Sweatt. 2002. "beta -Amyloid peptide activates alpha 7 nicotinic acetylcholine receptors expressed in Xenopus oocytes." *J Biol Chem* 277 (28):25056-61. doi: 10.1074/jbc.M200066200.

Dineley, K. T., M. Westerman, D. Bui, K. Bell, K. H. Ashe, and J. D. Sweatt. 2001. "Beta-amyloid activates the mitogen-activated protein kinase cascade via hippocampal alpha7 nicotinic acetylcholine receptors: In vitro and in vivo mechanisms related to Alzheimer's disease." *J Neurosci* 21 (12):4125-33.

Dineley, K. T., X. Xia, D. Bui, J. D. Sweatt, and H. Zheng. 2002. "Accelerated plaque accumulation, associative learning deficits, and up-regulation of alpha 7 nicotinic receptor protein in transgenic mice co-expressing mutant human presenilin 1 and amyloid precursor proteins." *J Biol Chem* 277 (25):22768-80. doi: 10.1074/jbc.M200164200.

Dodart, J. C., K. R. Bales, K. S. Gannon, S. J. Greene, R. B. DeMattos, C. Mathis, C. A. DeLong, S. Wu, X. Wu, D. M. Holtzman, and S. M. Paul. 2002. "Immunization reverses memory deficits without reducing brain Abeta burden in Alzheimer's disease model." *Nat Neurosci* 5 (5):452-7. doi: 10.1038/nn842.

Dodart, J. C., C. Mathis, K. R. Bales, S. M. Paul, and A. Ungerer. 1999. "Early regional cerebral glucose hypometabolism in transgenic mice overexpressing the V717F beta-amyloid precursor protein." *Neurosci Lett* 277 (1):49-52. doi: 10.1016/s0304-3940(99)00847-2.

Dodart, J. C., H. Meziane, C. Mathis, K. R. Bales, S. M. Paul, and A. Ungerer. 1999. "Behavioral disturbances in transgenic mice overexpressing the V717F beta-amyloid precursor protein." *Behav Neurosci* 113 (5):982-90.

Dominguez, D., J. Tournoy, D. Hartmann, T. Huth, K. Cryns, S. Deforce, L. Serneels, I. E. Camacho, E. Marjaux, K. Craessaerts, A. J. M. Roebroek, M. Schwake, R. D'Hooge, P. Bach, U. Kalinke, D. Moechars, C. Alzheimer, K. Reiss, P. Saftig, and B. De Strooper. 2005. "Phenotypic and biochemical analyses of BACE1- and BACE2-deficient mice." *Journal of Biological Chemistry* 280 (35):30797-30806. doi: 10.1074/jbc.M505249200.

Dong, H., J. Li, L. Huang, X. Chen, D. Li, T. Wang, C. Hu, J. Xu, C. Zhang, K. Zen, S. Xiao, Q. Yan, C. Wang, and C. Y. Zhang. 2015. "Serum MicroRNA Profiles Serve as Novel Biomarkers for the Diagnosis of Alzheimer's Disease." *Dis Markers* 2015:625659. doi: 10.1155/2015/625659.

Donohue, M. C., R. A. Sperling, D. P. Salmon, D. M. Rentz, R. Raman, R. G. Thomas, M. Weiner, and P. S. Aisen. 2014. "The preclinical Alzheimer cognitive composite: measuring amyloid-related decline." *JAMA Neurol* 71 (8):961-70. doi: 10.1001/jamaneurol.2014.803.

Doody, R. S., R. Raman, M. Farlow, T. Iwatsubo, B. Vellas, S. Joffe, K. Kieburtz, F. He, X. Y. Sun, R. G. Thomas, P. S. Aisen, E. Siemers, G. Sethuraman, and R. Mohs. 2013. "A Phase 3 Trial of Semagacestat for Treatment of Alzheimer's Disease." *New England Journal of Medicine* 369 (4):341-350. doi: 10.1056/NEJMoa1210951.

Dougherty, J. J., J. Wu, and R. A. Nichols. 2003. "Beta-amyloid regulation of presynaptic nicotinic receptors in rat hippocampus and neocortex." *J Neurosci* 23 (17):6740-7.

Dovey, H. F., V. John, J. P. Anderson, L. Z. Chen, P. D. Andrieu, L. Y. Fang, S. B. Freedman, B. Folmer, E. Goldbach, E. J. Holsztynska, K. L. Hu, K. L. Johnson-Wood, S. L. Kennedy, D. Kholedenko, J. E. Knops, L. H. Latimer, M. Lee, Z. Liao, I. M. Lieberburg, R. N. Motter, L. C. Mutter, J. Nietz, K. P. Quinn, K. L. Sacchi, P. A. Seubert, G. M. Shopp, E. D. Thorsett, J. S. Tung, J. Wu, S. Yang, C. T. Yin, D. B. Schenk, P. C. May, L. D. Altstiel, M. H. Bender, L. N. Boggs, T. C. Britton, J. C. Clemens, D. L. Czilli, D. K. Dieckman-McGinty, J. J. Droste, K. S. Fuson, B. D. Gitter, P. A. Hyslop, E. M. Johnstone, W. Y. Li, S. P. Little, T. E. Mabry, F. D. Miller, B. Ni, J. S. Nissen, W. J. Porter, B. D. Potts, J. K. Reel, D. Stephenson, Y. Su, L. A. Shipley, C. A. Whitesitt, T. Yin, and J. E. Audia. 2001. "Functional gamma-secretase inhibitors reduce beta-amyloid peptide levels in brain." *Journal of Neurochemistry* 76 (1):173-181. doi: 10.1046/j.1471-4159.2001.00012.x.

Du, Y., R. Dodel, H. Hampel, K. Buerger, S. Lin, B. Eastwood, K. Bales, F. Gao, H. J. Moeller, W. Oertel, M. Farlow, and S. Paul. 2001. "Reduced levels of amyloid beta-peptide antibody in Alzheimer disease." *Neurology* 57 (5):801-5. doi: 10.1212/wnl.57.5.801.

Dudchenko, P. A. 2004. "An overview of the tasks used to test working memory in rodents." *Neurosci Biobehav Rev* 28 (7):699-709. doi: 10.1016/j.neubiorev.2004.09.002.

Dziewczapolski, G., C. M. Glogowski, E. Masliah, and S. F. Heinemann. 2009. "Deletion of the alpha 7 nicotinic acetylcholine receptor gene improves cognitive deficits and synaptic pathology in a mouse model of Alzheimer's disease." *J Neurosci* 29 (27):8805-15. doi: 10.1523/jneurosci.6159-08.2009.

Egan, M. F., J. Kost, P. N. Tariot, P. S. Aisen, J. L. Cummings, B. Vellas, C. Sur, Y. Mukai, T. Voss, C. Furtek, E. Mahoney, L. Harper Mozley, R. Vandenberghe, Y. Mo, and D. Michelson. 2018. "Randomized Trial of Verubecestat for Mild-to-Moderate Alzheimer's Disease." *N Engl J Med* 378 (18):1691-1703. doi: 10.1056/NEJMoa1706441.

Egan, M. F., J. Kost, T. Voss, Y. Mukai, P. S. Aisen, J. L. Cummings, P. N. Tariot, B. Vellas, C. H. van Dyck, M. Boada, Y. Zhang, W. Li, C. Furtek, E. Mahoney, L. Harper Mozley, Y. Mo, C. Sur, and D. Michelson. 2019. "Randomized Trial of Verubecestat for Prodromal Alzheimer's Disease." *N Engl J Med* 380 (15):1408-1420. doi: 10.1056/NEJMoa1812840.

Emes, R. D., and S. G. Grant. 2012. "Evolution of synapse complexity and diversity." *Annu Rev Neurosci* 35:111-31. doi: 10.1146/annurev-neuro-062111-150433.
Ennaceur, A., and J. Delacour. 1988. "A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data." *Behav Brain Res* 31 (1):47-59.
Ennaceur, A., N. Neave, and J. P. Aggleton. 1996. "Neurotoxic lesions of the perirhinal cortex do not mimic the behavioural effects of fornix transection in the rat." *Behav Brain Res* 80 (1-2):9-25.
Esparza, T. J., H. Zhao, J. R. Cirrito, N. J. Cairns, R. J. Bateman, D. M. Holtzman, and D. L. Brody. 2013. "Amyloid-beta oligomerization in Alzheimer dementia versus high-pathology controls." *Ann Neurol* 73 (1):104-19. doi: 10.1002/ana.23748.
Evans, C. E., R. S. Thomas, T. J. Freeman, M. Hvoslef-Eide, M. A. Good, and E. J. Kidd. 2019. "Selective reduction of APP-BACE1 activity improves memory via NMDA-NR2B receptor-mediated mechanisms in aged PDAPP mice." *Neurobiol Aging* 75:136-149. doi: 10.1016/j.neurobiolaging.2018.11.011.
Evans, C., M. Hvoslef-Eide, R. Thomas, E. Kidd, and M. A. Good. 2018. "A rapidly acquired foraging-based working memory task, sensitive to hippocampal lesions, reveals age-dependent and age-independent behavioural changes in a mouse model of amyloid pathology." *Neurobiol Learn Mem* 149:46-57. doi: 10.1016/j.nlm.2018.02.004.
Fanutza, T., D. Del Prete, M. J. Ford, P. E. Castillo, and L. D'Adamio. 2015. "APP and APLP2 interact with the synaptic release machinery and facilitate transmitter release at hippocampal synapses." *Elife* 4:e09743. doi: 10.7554/eLife.09743.
Farrer, L. A., L. A. Cupples, J. L. Haines, B. Hyman, W. A. Kukull, R. Mayeux, R. H. Myers, M. A. Pericak-Vance, N. Risch, and C. M. van Duijn. 1997. "Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium." *Jama* 278 (16):1349-56.
Fazeli, M. S., K. Breen, M. L. Errington, and T. V. Bliss. 1994. "Increase in extracellular NCAM and amyloid precursor protein following induction of long-term potentiation in the dentate gyrus of anaesthetized rats." *Neurosci Lett* 169 (1-2):77-80. doi: 10.1016/0304-3940(94)90360-3.
Ferrara, M., E. Langiano, T. Di Brango, E. De Vito, L. Di Cioccio, and C. Bauco. 2008. "Prevalence of stress, anxiety and depression in with Alzheimer caregivers." In *Health Qual Life Outcomes*, 93.
Filser, S., S. V. Ovsepian, M. Masana, L. Blazquez-Llorca, A. Brandt Elvang, C. Volbracht, M. B. Muller, C. K. Jung, and J. Herms. 2015. "Pharmacological inhibition of BACE1 impairs synaptic plasticity and cognitive functions." *Biol Psychiatry* 77 (8):729-39. doi: 10.1016/j.biopsych.2014.10.013.
Fish, J. 2019. "Kreutzer, J.S; DeLuca, J; Caplan, B. Alzheimer's Disease Cooperative Study ADL Scale." In *Encyclopedia of Clinical Neuropsychology*. New York: SpringerLink.
Fleisher, A. S., R. Raman, E. R. Siemers, L. Becerra, C. M. Clark, R. A. Dean, M. R. Farlow, J. E. Galvin, E. R. Peskind, J. F. Quinn, A. Sherzai, B. B. Sowell, P. S. Aisen, and L. J. Thal. 2008. "Phase 2 safety trial targeting amyloid beta production with a gamma-secretase inhibitor in Alzheimer disease." *Arch Neurol* 65 (8):1031-8. doi: 10.1001/archneur.65.8.1031.
Flood, D. G., A. G. Reaume, K. S. Dorfman, Y. G. Lin, D. M. Lang, S. P. Trusko, M. J. Savage, W. G. Annaert, B. De Strooper, R. Siman, and R. W. Scott. 2002. "FAD mutant PS-1 gene-targeted mice: increased A beta 42 and A beta deposition without APP overproduction." *Neurobiol Aging* 23 (3):335-48.

Flood, J.F, E Roberts, M. A Sherman, B. E Kaplan, and J. E Morley. 1994. "Topography of a binding site for small amnestic peptides deduced from structure-activity studies: relation to amnestic effect of amyloid beta protein."

Florian, H., A. Meier, S. Gauthier, S. Lipschitz, Y. Lin, Q. Tang, A. A. Othman, W. Z. Robieson, and L. M. Gault. 2016. "Efficacy and Safety of ABT-126 in Subjects with Mild-to-Moderate Alzheimer's Disease on Stable Doses of Acetylcholinesterase Inhibitors: A Randomized, Double-Blind, Placebo-Controlled Study." *J Alzheimers Dis* 51 (4):1237-47. doi: 10.3233/jad-150978.

Folstein, M. F., S. E. Folstein, and P. R. McHugh. 1975. ""Mini-mental state". A practical method for grading the cognitive state of patients for the clinician." *J Psychiatr Res* 12 (3):189-98.

Forstl, H., and A. Kurz. 1999. "Clinical features of Alzheimer's disease." *Eur Arch Psychiatry Clin Neurosci* 249 (6):288-90.

Fortin, N. J., K. L. Agster, and H. B. Eichenbaum. 2002. "Critical role of the hippocampus in memory for sequences of events." *Nat Neurosci* 5 (5):458-62. doi: 10.1038/nn834.

Forwood, S. E., B. D. Winters, and T. J. Bussey. 2005. "Hippocampal lesions that abolish spatial maze performance spare object recognition memory at delays of up to 48 hours." *Hippocampus* 15 (3):347-55. doi: 10.1002/hipo.20059.

Francis, D. D., M. D. Zaharia, N. Shanks, and H. Anisman. 1995. "Stress-induced disturbances in Morris water-maze performance: interstrain variability." *Physiol Behav* 58 (1):57-65.

Francis, R., G. McGrath, J. H. Zhang, D. A. Ruddy, M. Sym, J. Apfeld, M. Nicoll, M. Maxwell, B. Hai, M. C. Ellis, A. L. Parks, W. Xu, J. H. Li, M. Gurney, R. L. Myers, C. S. Himes, R. Hiebsch, C. Ruble, J. S. Nye, and D. Curtis. 2002. "aph-1 and pen-2 are required for notch pathway signaling, gamma-secretase cleavage of beta APP, and presenilin protein accumulation." *Developmental Cell* 3 (1):85-97. doi: 10.1016/s1534-5807(02)00189-2.

Fray, J, T.W Robbins, and B.J Sahakian. 1996. "Neuorpsychiatyric applications of CANTAB." *International Journal of Geriatric Psychiatry*. doi: 10.1002/(SICI)1099-1166(199604)11:4<329::AID-GPS453>3.0.CO;2-6.

Fukumori, A., M. Okochi, S. Tagami, J. Jiang, N. Itoh, T. Nakayama, K. Yanagida, Y. Ishizuka-Katsura, T. Morihara, K. Kamino, T. Tanaka, T. Kudo, H. Tanii, A. Ikuta, C. Haass, and M. Takeda. 2006. "Presenilin-dependent gamma-secretase on plasma membrane and endosomes is functionally distinct." *Biochemistry* 45 (15):4907-14. doi: 10.1021/bi052412w.

Fukumoto, H., H. Takahashi, N. Tarui, J. Matsui, T. Tomita, M. Hirode, M. Sagayama, R. Maeda, M. Kawamoto, K. Hirai, J. Terauchi, Y. Sakura, M. Kakihana, K. Kato, T. Iwatsubo, and M. Miyamoto. 2010. "A noncompetitive BACE1 inhibitor TAK-070 ameliorates Abeta pathology and behavioral deficits in a mouse model of Alzheimer's disease." *J Neurosci* 30 (33):11157-66. doi: 10.1523/jneurosci.2884-10.2010.

Furukawa, K., S. W. Barger, E. M. Blalock, and M. P. Mattson. 1996. "Activation of K+ channels and suppression of neuronal activity by secreted beta-amyloid-precursor protein." *Nature* 379 (6560):74-8. doi: 10.1038/379074a0.

Games, D., D. Adams, R. Alessandrini, R. Barbour, P. Berthelette, C. Blackwell, T. Carr, J. Clemens, T. Donaldson, F. Gillespie, and et al. 1995. "Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein." *Nature* 373 (6514):523-7. doi: 10.1038/373523a0.

Garcia-Alloza, M., E. M. Robbins, S. X. Zhang-Nunes, S. M. Purcell, R. A. Betensky, S. Raju, C. Prada, S. M. Greenberg, B. J. Bacskai, and M. P. Frosch. 2006.

"Characterization of amyloid deposition in the APPswe/PS1dE9 mouse model of Alzheimer disease." *Neurobiol Dis* 24 (3):516-24. doi: 10.1016/j.nbd.2006.08.017.

Garcia-Osta, A., and C. M. Alberini. 2009. "Amyloid beta mediates memory formation." *Learn Mem* 16 (4):267-72. doi: 10.1101/lm.1310209.

Garwood, C. J., A. M. Pooler, J. Atherton, D. P. Hanger, and W. Noble. 2011. "Astrocytes are important mediators of Abeta-induced neurotoxicity and tau phosphorylation in primary culture." *Cell Death Dis* 2:e167. doi: 10.1038/cddis.2011.50.

Gatz, M., C. A. Reynolds, L. Fratiglioni, B. Johansson, J. A. Mortimer, S. Berg, A. Fiske, and N. L. Pedersen. 2006. "Role of genes and environments for explaining Alzheimer disease." *Arch Gen Psychiatry* 63 (2):168-74. doi: 10.1001/archpsyc.63.2.168.

Gauthier, S., H. H. Feldman, L. S. Schneider, G. K. Wilcock, G. B. Frisoni, J. H. Hardlund, H. J. Moebius, P. Bentham, K. A. Kook, D. J. Wischik, B. O. Schelter, C. S. Davis, R. T. Staff, L. Bracoud, K. Shamsi, J. M. Storey, C. R. Harrington, and C. M. Wischik. 2016. "Efficacy and safety of tau-aggregation inhibitor therapy in patients with mild or moderate Alzheimer's disease: a randomised, controlled, double-blind, parallel-arm, phase 3 trial." *Lancet* 388 (10062):2873-2884. doi: 10.1016/s0140-6736(16)31275-2.

Ghosal, K., D. L. Vogt, M. Liang, Y. Shen, B. T. Lamb, and S. W. Pimplikar. 2009. "Alzheimer's disease-like pathological features in transgenic mice expressing the APP intracellular domain." *Proc Natl Acad Sci U S A* 106 (43):18367-72. doi: 10.1073/pnas.0907652106.

Ghosh, A. K., M. Brindisi, and J. Tang. 2012. "Developing β-secretase inhibitors for treatment of Alzheimer's disease." *J Neurochem* 120 (Suppl 1):71-83. doi: 10.1111/j.1471-4159.2011.07476.x.

Giliberto, L., C. d'Abramo, C. M. Acker, P. Davies, and L. D'Adamio. 2010. "Transgenic expression of the amyloid-beta precursor protein-intracellular domain does not induce Alzheimer's Disease-like traits in vivo." *PLoS One* 5 (7):e11609. doi: 10.1371/journal.pone.0011609.

Gilman, S., M. Koller, R. S. Black, L. Jenkins, S. G. Griffith, N. C. Fox, L. Eisner, L. Kirby, M. B. Rovira, F. Forette, and J. M. Orgogozo. 2005. "Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial." *Neurology* 64 (9):1553-62. doi: 10.1212/01.wnl.0000159740.16984.3c.

Glenner, G. G., and C. W. Wong. 1984. "Alzheimers-Disease and Downs-Syndrome - sharing of a unique cerebrovascular amyloid fibril protein." *Biochemical and Biophysical Research Communications* 122 (3):1131-1135. doi: 10.1016/0006-291x(84)91209-9.

Goate, A., M. C. Chartier-Harlin, M. Mullan, J. Brown, F. Crawford, L. Fidani, L. Giuffra, A. Haynes, N. Irving, L. James, and et al. 1991. "Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease." *Nature* 349 (6311):704-6. doi: 10.1038/349704a0.

Goh, J. J., and D. Manahan-Vaughan. 2013. "Spatial Object Recognition Enables Endogenous LTD that Curtails LTP in the Mouse Hippocampus." *Cereb Cortex* 23 (5):1118-25. doi: 10.1093/cercor/bhs089.

Goh, L. K., W. S. Lim, S. Teo, A. Vijayaraghavan, M. Chan, L. Tay, T. P. Ng, C. H. Tan, T. S. Lee, and M. S. Chong. 2015. "TOMM40 alterations in Alzheimer's disease over a 2-year follow-up period." *J Alzheimers Dis* 44 (1):57-61. doi: 10.3233/jad-141590.

Goldgaber, D., H. W. Harris, T. Hla, T. Maciag, R. J. Donnelly, J. S. Jacobsen, M. P. Vitek, and D. C. Gajdusek. 1989. "Interleukin 1 regulates synthesis of amyloid beta-protein precursor mRNA in human endothelial cells." *Proc Natl Acad Sci U S A* 86 (19):7606-10. doi: 10.1073/pnas.86.19.7606.

Goldgaber, D., M. I. Lerman, W. O. McBride, U. Saffiotti, and D. C. Gajdusek. 1987. "Isolation, characterization, and chromosomal localization of human brain cDNA clones coding for the precursor of the amyloid of brain in Alzheimer's disease, Down's syndrome and aging." *J Neural Transm Suppl* 24:23-8.

Gomez-Isla, T., J. L. Price, D. W. McKeel, Jr., J. C. Morris, J. H. Growdon, and B. T. Hyman. 1996. "Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease." *J Neurosci* 16 (14):4491-500.

Good, M. A., P. Barnes, V. Staal, A. McGregor, and R. C. Honey. 2007. "Context- but not familiarity-dependent forms of object recognition are impaired following excitotoxic hippocampal lesions in rats." *Behav Neurosci* 121 (1):218-23. doi: 10.1037/0735-7044.121.1.218.

Good, M. A., and G. Hale. 2007. "The "Swedish" mutation of the amyloid precursor protein (APPswe) dissociates components of object-location memory in aged Tg2576 mice." *Behav Neurosci* 121 (6):1180-91. doi: 10.1037/0735-7044.121.6.1180.

Good, M. A., G. Hale, and V. Staal. 2007. "Impaired "episodic-like" object memory in adult APPswe transgenic mice." *Behav Neurosci* 121 (2):443-8. doi: 10.1037/0735-7044.121.2.443.

Graham, D. I., S. M. Gentleman, A. Lynch, and G. W. Roberts. 1995. "Distribution of beta-amyloid protein in the brain following severe head injury." *Neuropathol Appl Neurobiol* 21 (1):27-34.

Graham, M. E. 2017. "From wandering to wayfaring: Reconsidering movement in people with dementia in long-term care." *Dementia (London)* 16 (6):732-749. doi: 10.1177/1471301215614572.

Green, K. N., L. M. Billings, B. Roozendaal, J. L. McGaugh, and F. M. LaFerla. 2006. "Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease." *J Neurosci* 26 (35):9047-56. doi: 10.1523/jneurosci.2797-06.2006.

Griffiths, S., H. Scott, C. Glover, A. Bienemann, M. T. Ghorbel, J. Uney, M. W. Brown, E. C. Warburton, and Z. I. Bashir. 2008. "Expression of long-term depression underlies visual recognition memory." *Neuron* 58 (2):186-94. doi: 10.1016/j.neuron.2008.02.022.

Guerreiro, R., A. Wojtas, J. Bras, M. Carrasquillo, E. Rogaeva, E. Majounie, C. Cruchaga, C. Sassi, J. S. Kauwe, S. Younkin, L. Hazrati, J. Collinge, J. Pocock, T. Lashley, J. Williams, J. C. Lambert, P. Amouyel, A. Goate, R. Rademakers, K. Morgan, J. Powell, P. St George-Hyslop, A. Singleton, and J. Hardy. 2013. "TREM2 variants in Alzheimer's disease." *N Engl J Med* 368 (2):117-27. doi: 10.1056/NEJMoa1211851.

Haass, C. 2004. "Take five - BACE and the gamma-secretase quartet conduct Alzheimer's amyloid beta-peptide generation." *Embo Journal* 23 (3):483-488. doi: 10.1038/sj.emboj.7600061.

Haass, C., A. Y. Hung, M. G. Schlossmacher, D. B. Teplow, and D. J. Selkoe. 1993. "beta-Amyloid peptide and a 3-kDa fragment are derived by distinct cellular mechanisms." *J Biol Chem* 268 (5):3021-4.

Haass, C., E. H. Koo, A. Mellon, A. Y. Hung, and D. J. Selkoe. 1992. "Targeting of cell-surface beta-amyloid precursor protein to lysosomes: alternative processing into amyloid-bearing fragments." *Nature* 357 (6378):500-3. doi: 10.1038/357500a0.

Haass, C., M. G. Schlossmacher, A. Y. Hung, C. Vigo-Pelfrey, A. Mellon, B. L. Ostaszewski, I. Lieberburg, E. H. Koo, D. Schenk, D. B. Teplow, and et al. 1992. "Amyloid beta-peptide is produced by cultured cells during normal metabolism." *Nature* 359 (6393):322-5. doi: 10.1038/359322a0.

Haass, C., and D. J. Selkoe. 2007. "Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide." *Nat Rev Mol Cell Biol* 8 (2):101-12. doi: 10.1038/nrm2101.

Haass, C., and H. Steiner. 2002. "Alzheimer disease gamma-secretase: a complex story of GxGD-type presenilin proteases." *Trends in Cell Biology* 12 (12):556-562. doi: 10.1016/s0962-8924(02)02394-2.

Hale, G., and M. Good. 2005. "Impaired visuospatial recognition memory but normal object novelty detection and relative familiarity judgments in adult mice expressing the APPswe Alzheimer's disease mutation." *Behav Neurosci* 119 (4):884-91. doi: 10.1037/0735-7044.119.4.884.

Hall, JL, and RF Berman. 1995. "Juvenile experience alters strategies used to solve the radial arm maze in rats. - PsycNET." *Psychobiology* 23 (3):195-198. doi: TBD.

Hamaguchi, T., I. Tsutsui-Kimura, M. Mimura, T. Saito, T. C. Saido, and K. F. Tanaka. 2019. "App(NL-G-F/NL-G-F) mice overall do not show impaired motivation, but cored amyloid plaques in the striatum are inversely correlated with motivation." *Neurochem Int* 129:104470. doi: 10.1016/j.neuint.2019.104470.

Hannesson, D. K., G. Vacca, J. G. Howland, and A. G. Phillips. 2004. "Medial prefrontal cortex is involved in spatial temporal order memory but not spatial recognition memory in tests relying on spontaneous exploration in rats." *Behav Brain Res* 153 (1):273-85. doi: 10.1016/j.bbr.2003.12.004.

Hardy, J. A., and G. A. Higgins. 1992. "Alzheimers-Disease - The Amyloid Cascade Hypothesis." *Science* 256 (5054):184-185. doi: 10.1126/science.1566067.

Harkany, T., I. Abraham, W. Timmerman, G. Laskay, B. Toth, M. Sasvari, C. Konya, J. B. Sebens, J. Korf, C. Nyakas, M. Zarandi, K. Soos, B. Penke, and P. G. Luiten. 2000. "beta-amyloid neurotoxicity is mediated by a glutamate-triggered excitotoxic cascade in rat nucleus basalis." *Eur J Neurosci* 12 (8):2735-45.

Harold, D., R. Abraham, P. Hollingworth, R. Sims, A. Gerrish, M. Hamshere, J. Singh Pahwa, V. Moskvina, K. Dowzell, A. Williams, N. Jones, C. Thomas, A. Stretton, A. Morgan, S. Lovestone, J. Powell, P. Proitsi, M. K. Lupton, C. Brayne, D. C. Rubinsztein, M. Gill, B. Lawlor, A. Lynch, K. Morgan, K. Brown, P. Passmore, D. Craig, B. McGuinness, S. Todd, C. Holmes, D. Mann, A. D. Smith, S. Love, P. G. Kehoe, J. Hardy, S. Mead, N. Fox, M. Rossor, J. Collinge, W. Maier, F. Jessen, B. Schürmann, H. van den Bussche, I. Heuser, J. Kornhuber, J. Wiltfang, M. Dichgans, L. Frölich, H. Hampel, M. Hüll, D. Rujescu, A. Goate, J. S. Kauwe, C. Cruchaga, P. Nowotny, J. C. Morris, K. Mayo, K. Sleegers, K. Bettens, S. Engelborghs, P. De Deyn, C. van Broeckhoven, G. Livingston, N. J. Bass, H. Gurling, A. McQuillin, R. Gwilliam, P. Deloukas, A. Al-Chalabi, C. E. Shaw, M. Tsolaki, A. Singleton, R. Guerreiro, T. W. Mühleisen, M. M. Nöthen, S. Moebus, K. H. Jöckel, N. Klopp, H. E. Wichmann, M. M. Carrasquillo, V. S. Pankratz, S. G. Younkin, P. Holmans, M. O'Donovan, M. J. Owen, and J. Williams. 2009. "Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease, and shows evidence for additional susceptibility genes." *Nat Genet* 41 (10):1088-93. doi: 10.1038/ng.440.

Harris, J. A., N. Devidze, L. Verret, K. Ho, B. Halabisky, M. T. Thwin, D. Kim, P. Hamto, I. Lo, G. Q. Yu, J. J. Palop, E. Masliah, and L. Mucke. 2010. "Transsynaptic progression of amyloid-β-induced neuronal dysfunction within the

entorhinal-hippocampal network." *Neuron* 68 (3):428-41. doi: 10.1016/j.neuron.2010.10.020.

Harrison, S. M., A. J. Harper, J. Hawkins, G. Duddy, E. Grau, P. L. Pugh, P. H. Winter, C. S. Shilliam, Z. A. Hughes, L. A. Dawson, M. I. Gonzalez, N. Upton, M. N. Pangalos, and C. Dingwall. 2003. "BACE1 (beta-secretase) transgenic neurochernical deficits ind knockout mice: identification of and behavioral changes." *Molecular and Cellular Neuroscience* 24 (3):646-655. doi: 10.1016/s1044-7431(03)00227-4.

Hartman, R. E., Y. Izumi, K. R. Bales, S. M. Paul, D. F. Wozniak, and D. M. Holtzman. 2005. "Treatment with an amyloid-beta antibody ameliorates plaque load, learning deficits, and hippocampal long-term potentiation in a mouse model of Alzheimer's disease." *J Neurosci* 25 (26):6213-20. doi: 10.1523/jneurosci.0664-05.2005.

Hascup, K. N., and E. R. Hascup. 2016. "Soluble Amyloid-beta42 Stimulates Glutamate Release through Activation of the alpha7 Nicotinic Acetylcholine Receptor." *J Alzheimers Dis* 53 (1):337-47. doi: 10.3233/jad-160041.

He, K., L. Song, L. W. Cummings, J. Goldman, R. L. Huganir, and H. K. Lee. 2009. "Stabilization of Ca2+-permeable AMPA receptors at perisynaptic sites by GluR1-S845 phosphorylation." *Proc Natl Acad Sci U S A* 106 (47):20033-8. doi: 10.1073/pnas.0910338106.

Head, E., V. Pop, V. Vasilevko, M. Hill, T. Saing, F. Sarsoza, M. Nistor, L. A. Christie, S. Milton, C. Glabe, E. Barrett, and D. Cribbs. 2008. "A two-year study with fibrillar beta-amyloid (Abeta) immunization in aged canines: effects on cognitive function and brain Abeta." *J Neurosci* 28 (14):3555-66. doi: 10.1523/jneurosci.0208-08.2008.

Hefti, F., W. F. Goure, J. Jerecic, K. S. Iverson, P. A. Walicke, and G. A. Krafft. 2013. "The case for soluble Abeta oligomers as a drug target in Alzheimer's disease." *Trends Pharmacol Sci* 34 (5):261-6. doi: 10.1016/j.tips.2013.03.002.

Heneka, M. T., M. K. O'Banion, D. Terwel, and M. P. Kummer. 2010. "Neuroinflammatory processes in Alzheimer's disease." *J Neural Transm (Vienna)* 117 (8):919-47. doi: 10.1007/s00702-010-0438-z.

Herms, J., B. Anliker, S. Heber, S. Ring, M. Fuhrmann, H. Kretzschmar, S. Sisodia, and U. Muller. 2004. "Cortical dysplasia resembling human type 2 lissencephaly in mice lacking all three APP family members." *Embo j* 23 (20):4106-15. doi: 10.1038/sj.emboj.7600390.

Hernandez, C. M., R. Kayed, H. Zheng, J. D. Sweatt, and K. T. Dineley. 2010. "Loss of alpha7 nicotinic receptors enhances beta-amyloid oligomer accumulation, exacerbating early-stage cognitive decline and septohippocampal pathology in a mouse model of Alzheimer's disease." *J Neurosci* 30 (7):2442-53. doi: 10.1523/jneurosci.5038-09.2010.

Hick, M., U. Herrmann, S. W. Weyer, J. P. Mallm, J. A. Tschape, M. Borgers, M. Mercken, F. C. Roth, A. Draguhn, L. Slomianka, D. P. Wolfer, M. Korte, and U. C. Muller. 2015. "Acute function of secreted amyloid precursor protein fragment APPsalpha in synaptic plasticity." *Acta Neuropathol* 129 (1):21-37. doi: 10.1007/s00401-014-1368-x.

Hickman, S. E., E. K. Allison, and J. El Khoury. 2008. "Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice." *J Neurosci* 28 (33):8354-60. doi: 10.1523/jneurosci.0616-08.2008.

Higuchi, M., N. Iwata, Y. Matsuba, J. Takano, T. Suemoto, J. Maeda, B. Ji, M. Ono, M. Staufenbiel, T. Suhara, and T. C. Saido. 2012. "Mechanistic involvement of the

calpain-calpastatin system in Alzheimer neuropathology." *Faseb j* 26 (3):1204-17. doi: 10.1096/fj.11-187740.

Hitt, B. D., T. C. Jaramillo, D. M. Chetkovich, and R. Vassar. 2010. "BACE1(-/-) mice exhibit seizure activity that does not correlate with sodium channel level or axonal localization." *Molecular Neurodegeneration* 5. doi: 10.1186/1750-1326-5-31.

Hitt, B., S. M. Riordan, L. Kukreja, W. A. Eimer, T. W. Rajapaksha, and R. Vassar. 2012. "beta-Site Amyloid Precursor Protein (APP)-cleaving Enzyme 1 (BACE1)-deficient Mice Exhibit a Close Homolog of L1 (CHL1) Loss-of-function Phenotype Involving Axon Guidance Defects." *Journal of Biological Chemistry* 287 (46):38408-38425. doi: 10.1074/jbc.M112.415505.

Hodges, J. R., D. P. Salmon, and N. Butters. 1990. "Differential impairment of semantic and episodic memory in Alzheimer's and Huntington's diseases: a controlled prospective study." *J Neurol Neurosurg Psychiatry* 53 (12):1089-95.

Holmes, C., D. Boche, D. Wilkinson, G. Yadegarfar, V. Hopkins, A. Bayer, R. W. Jones, R. Bullock, S. Love, J. W. Neal, E. Zotova, and J. A. Nicoll. 2008. "Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial." *Lancet* 372 (9634):216-23. doi: 10.1016/s0140-6736(08)61075-2.

Honey, R. C., V. J. Marshall, A. Mcgregor, J. Futter, and M. Good. 2007. "Revisiting places passed: Sensitization of exploratory activity in rats with hippocampal lesions:." *Quarterly Journal of Experimental Psychology* 60 (5):625-634. doi: 10.1080_17470210601155252.

Hong, S., B. L. Ostaszewski, T. Yang, T. T. O'Malley, M. Jin, K. Yanagisawa, S. Li, T. Bartels, and D. J. Selkoe. 2014. "Soluble Abeta oligomers are rapidly sequestered from brain ISF in vivo and bind GM1 ganglioside on cellular membranes." *Neuron* 82 (2):308-19. doi: 10.1016/j.neuron.2014.02.027.

Honig, L. S., B. Vellas, M. Woodward, M. Boada, R. Bullock, M. Borrie, K. Hager, N. Andreasen, E. Scarpini, H. Liu-Seifert, M. Case, R. A. Dean, A. Hake, K. Sundell, V. Poole Hoffmann, C. Carlson, R. Khanna, M. Mintun, R. DeMattos, K. J. Selzler, and E. Siemers. 2018. "Trial of Solanezumab for Mild Dementia Due to Alzheimer's Disease." *N Engl J Med* 378 (4):321-330. doi: 10.1056/NEJMoa1705971.

Honig, W.K. 1978. "Studies of Working Memory in the Pigeon." In *Cognitive Processes in Animal Behavior*, edited by Lawrence Erlbaum. Hillsdale.

Hort, J., J. Laczo, M. Vyhnalek, M. Bojar, J. Bures, and K. Vlcek. 2007. "Spatial navigation deficit in amnestic mild cognitive impairment." *Proc Natl Acad Sci U S A* 104 (10):4042-7. doi: 10.1073/pnas.0611314104.

Hsiao, K., P. Chapman, S. Nilsen, C. Eckman, Y. Harigaya, S. Younkin, F. Yang, and G. Cole. 1996. "Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice." *Science* 274 (5284):99-102.

Hsieh, H., J. Boehm, C. Sato, T. Iwatsubo, T. Tomita, S. Sisodia, and R. Malinow. 2006. "AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss." *Neuron* 52 (5):831-43. doi: 10.1016/j.neuron.2006.10.035.

Hu, X. Y., W. X. He, X. Y. Luo, K. E. Tsubota, and R. Q. Yan. 2013. "BACE1 Regulates Hippocampal Astrogenesis via the Jagged1-Notch Pathway." *Cell Reports* 4 (1):40-49. doi: 10.1016/j.celrep.2013.06.005.

Hu, X. Y., C. W. Hicks, W. X. He, P. Wong, W. B. Macklin, B. D. Trapp, and R. Q. Yan. 2006. "Bace1 modulates myelination in the central and peripheral nervous system." *Nature Neuroscience* 9 (12):1520-1525. doi: 10.1038/nn1797.

Huber, G., J. R. Martin, J. Loffler, and J. L. Moreau. 1993. "Involvement of amyloid precursor protein in memory formation in the rat: an indirect antibody approach." *Brain Res* 603 (2):348-52. doi: 10.1016/0006-8993(93)91261-p.

Huitron-Resendiz, S., M. Sanchez-Alavez, R. Gallegos, G. Berg, E. Crawford, J. L. Giacchino, D. Games, S. J. Henriksen, and J. R. Criado. 2002. "Age-independent and age-related deficits in visuospatial learning, sleep-wake states, thermoregulation and motor activity in PDAPP mice." *Brain Res* 928 (1-2):126-37.

Hull, M., C. Sadowsky, H. Arai, G. Le Prince Leterme, A. Holstein, K. Booth, Y. Peng, T. Yoshiyama, H. Suzuki, N. Ketter, E. Liu, and J. M. Ryan. 2017. "Long-Term Extensions of Randomized Vaccination Trials of ACC-001 and QS-21 in Mild to Moderate Alzheimer's Disease." *Curr Alzheimer Res* 14 (7):696-708. doi: 10.2174/1567205014666170117101537.

Hung, V. K., P. K. Yeung, A. K. Lai, M. C. Ho, A. C. Lo, K. C. Chan, E. X. Wu, S. S. Chung, C. W. Cheung, and S. K. Chung. 2015. "Selective astrocytic endothelin-1 overexpression contributes to dementia associated with ischemic stroke by exaggerating astrocyte-derived amyloid secretion." *J Cereb Blood Flow Metab* 35 (10):1687-96. doi: 10.1038/jcbfm.2015.109.

Huse, J. T., D. S. Pijak, G. J. Leslie, V. M. Lee, and R. W. Doms. 2000. "Maturation and endosomal targeting of beta-site amyloid precursor protein-cleaving enzyme. The Alzheimer's disease beta-secretase." *J Biol Chem* 275 (43):33729-37. doi: 10.1074/jbc.M004175200.

Hussain, I., J. Hawkins, D. Harrison, C. Hille, G. Wayne, L. Cutler, T. Buck, D. Walter, E. Demont, C. Howes, A. Naylor, P. Jeffrey, M. I. Gonzalez, C. Dingwall, A. Michel, S. Redshaw, and J. B. Davis. 2007. "Oral administration of a potent and selective non-peptidic BACE-1 inhibitor decreases beta-cleavage of amyloid precursor protein and amyloid-beta production in vivo." *J Neurochem* 100 (3):802-9. doi: 10.1111/j.1471-4159.2006.04260.x.

Hussain, I., D. Powell, D. R. Howlett, D. G. Tew, T. D. Week, C. Chapman, I. S. Gloger, K. E. Murphy, C. D. Southan, D. M. Ryan, T. S. Smith, D. L. Simmons, F. S. Walsh, C. Dingwall, and G. Christie. 1999. "Identification of a novel aspartic protease (Asp 2) as beta-secretase." *Molecular and Cellular Neuroscience* 14 (6):419-427. doi: 10.1006/mcne.1999.0811.

Iaccarino, H. F., A. C. Singer, A. J. Martorell, A. Rudenko, F. Gao, T. Z. Gillingham, H. Mathys, J. Seo, O. Kritskiy, F. Abdurrob, C. Adaikkan, R. G. Canter, R. Rueda, E. N. Brown, E. S. Boyden, and L. H. Tsai. 2016. "Gamma frequency entrainment attenuates amyloid load and modifies microglia." *Nature* 540 (7632):230-235. doi: 10.1038/nature20587.

Igarashi, K. M., L. Lu, L. L. Colgin, M. B. Moser, and E. I. Moser. 2014. "Coordination of entorhinal-hippocampal ensemble activity during associative learning." *Nature* 510 (7503):143-7. doi: 10.1038/nature13162.

Imbimbo, B., B. Hutter-Paier, G. Villetti, F. Facchinetti, V. Cenacchi, R. Volta, A. Lanzillotta, M. Pizzi, and M. Windisch. 2009. "CHF5074, a novel γ-secretase modulator, attenuates brain β-amyloid pathology and learning deficit in a mouse model of Alzheimer's disease." *Br J Pharmacol* 156 (6):982-93. doi: 10.1111/j.1476-5381.2008.00097.x.

Iordanova, M. D., M. A. Good, and R. C. Honey. 2008. "Configural learning without reinforcement: integrated memories for correlates of what, where, and when." *Q J Exp Psychol (Hove)* 61 (12):1785-92. doi: 10.1080/17470210802194324.

Irizarry, M. C., M. McNamara, K. Fedorchak, K. Hsiao, and B. T. Hyman. 1997. "APPSw transgenic mice develop age-related A beta deposits and neuropil

abnormalities, but no neuronal loss in CA1." *J Neuropathol Exp Neurol* 56 (9):965-73. doi: 10.1097/00005072-199709000-00002.

Ittner, L. M., Y. D. Ke, F. Delerue, M. A. Bi, A. Gladbach, J. van Eersel, H. Wolfing, B. C. Chieng, M. J. Christie, I. A. Napier, A. Eckert, M. Staufenbiel, E. Hardeman, and J. Gotz. 2010. "Dendritic Function of Tau Mediates Amyloid-beta Toxicity in Alzheimer's Disease Mouse Models." *Cell* 142 (3):387-397. doi: 10.1016/j.cell.2010.06.036.

Izumi, H., Y. Shinoda, T. Saito, T. C. Saido, K. Sato, Y. Yabuki, Y. Matsumoto, Y. Kanemitsu, Y. Tomioka, N. Abolhassani, Y. Nakabeppu, and K. Fukunaga. 2018. "The Disease-modifying Drug Candidate, SAK3 Improves Cognitive Impairment and Inhibits Amyloid beta Deposition in App Knock-in Mice." *Neuroscience* 377:87-97. doi: 10.1016/j.neuroscience.2018.02.031.

Jack, C. R., Jr., D. S. Knopman, W. J. Jagust, R. C. Petersen, M. W. Weiner, P. S. Aisen, L. M. Shaw, P. Vemuri, H. J. Wiste, S. D. Weigand, T. G. Lesnick, V. S. Pankratz, M. C. Donohue, and J. Q. Trojanowski. 2013. "Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers." *Lancet Neurol* 12 (2):207-16. doi: 10.1016/s1474-4422(12)70291-0.

Jack, C. R., Jr., V. J. Lowe, S. D. Weigand, H. J. Wiste, M. L. Senjem, D. S. Knopman, M. M. Shiung, J. L. Gunter, B. F. Boeve, B. J. Kemp, M. Weiner, and R. C. Petersen. 2009. "Serial PIB and MRI in normal, mild cognitive impairment and Alzheimer's disease: implications for sequence of pathological events in Alzheimer's disease." *Brain* 132 (Pt 5):1355-65. doi: 10.1093/brain/awp062.

Jacobsen, J. S., C. C. Wu, J. M. Redwine, T. A. Comery, R. Arias, M. Bowlby, R. Martone, J. H. Morrison, M. N. Pangalos, P. H. Reinhart, and F. E. Bloom. 2006. "Early-onset behavioral and synaptic deficits in a mouse model of Alzheimer's disease." *Proc Natl Acad Sci U S A* 103 (13):5161-6. doi: 10.1073/pnas.0600948103.

Jarrard, LE. 1983. "Selective hippocampal lesions and behavior: Effects of kainic acid lesions on performance of place and cue tasks." *Behavioural Neuroscience* 97 (6):873-889. doi: 10.1037/0735-7044.97.6.873.

Jarrett, J. T., E. P. Berger, and P. T. Lansbury, Jr. 1993. "The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease." *Biochemistry* 32 (18):4693-7.

Jellinger, K. A. 1995. "Alzheimer's changes in non-demented and demented patients." *Acta Neuropathol* 89 (1):112-3.

Jeong, Y. H., C. H. Park, J. Yoo, K. Y. Shin, S. M. Ahn, H. S. Kim, S. H. Lee, P. C. Emson, and Y. H. Suh. 2006. "Chronic stress accelerates learning and memory impairments and increases amyloid deposition in APPV717I-CT100 transgenic mice, an Alzheimer's disease model." *Faseb j* 20 (6):729-31. doi: 10.1096/fj.05-4265fje.

Jin, M., N. Shepardson, T. Yang, G. Chen, D. Walsh, and D. J. Selkoe. 2011. "Soluble amyloid beta-protein dimers isolated from Alzheimer cortex directly induce Tau hyperphosphorylation and neuritic degeneration." *Proc Natl Acad Sci U S A* 108 (14):5819-24. doi: 10.1073/pnas.1017033108.

Jonsson, T., J. K. Atwal, S. Steinberg, J. Snaedal, P. V. Jonsson, S. Bjornsson, H. Stefansson, P. Sulem, D. Gudbjartsson, J. Maloney, K. Hoyte, A. Gustafson, Y. Liu, Y. Lu, T. Bhangale, R. R. Graham, J. Huttenlocher, G. Bjornsdottir, O. A. Andreassen, E. G. Jonsson, A. Palotie, T. W. Behrens, O. T. Magnusson, A. Kong, U. Thorsteinsdottir, R. J. Watts, and K. Stefansson. 2012. "A mutation in

APP protects against Alzheimer's disease and age-related cognitive decline." *Nature* 488 (7409):96-9. doi: 10.1038/nature11283.

Joyashiki, Eri, Yuji Matsuya, and Chihiro Tohda. 2011. "Sominone Improves Memory Impairments and Increases Axonal Density in Alzheimer's Disease Model Mice, 5XFAD." *International Journal of Neuroscience* 121 (4):181-190. doi: 10.3109/00207454.2010.541571.

Kamenetz, F., T. Tomita, H. Hsieh, G. Seabrook, D. Borchelt, T. Iwatsubo, S. Sisodia, and R. Malinow. 2003. "APP processing and synaptic function." *Neuron* 37 (6):925-37.

Kaur, U., P. Banerjee, A. Bir, M. Sinha, A. Biswas, and S. Chakrabarti. 2015. "Reactive oxygen species, redox signaling and neuroinflammation in Alzheimer's disease: the NF-kappaB connection." *Curr Top Med Chem* 15 (5):446-57.

Kendig, E. L., H. H. Le, and S. M. Belcher. 2010. "Defining hormesis: evaluation of a complex concentration response phenomenon." *Int J Toxicol* 29 (3):235-46. doi: 10.1177/1091581810363012.

Kennedy, M. E., A. W. Stamford, X. Chen, K. Cox, J. N. Cumming, M. F. Dockendorf, M. Egan, L. Ereshefsky, R. A. Hodgson, L. A. Hyde, S. Jhee, H. J. Kleijn, R. Kuvelkar, W. Li, B. A. Mattson, H. Mei, J. Palcza, J. D. Scott, M. Tanen, M. D. Troyer, J. L. Tseng, J. A. Stone, E. M. Parker, and M. S. Forman. 2016. "The BACE1 inhibitor verubecestat (MK-8931) reduces CNS beta-amyloid in animal models and in Alzheimer's disease patients." *Science Translational Medicine* 8 (363):14. doi: 10.1126/scitranslmed.aad9704.

Kim, D. Y., M. T. Gersbacher, P. Inquimbert, and D. M. Kovacs. 2011. "Reduced Sodium Channel Na(v)1.1 Levels in BACE1-null Mice." *Journal of Biological Chemistry* 286 (10):8106-8116. doi: 10.1074/jbc.M110.134692.

Kim, J. J., and D. M. Diamond. 2002. "The stressed hippocampus, synaptic plasticity and lost memories." *Nat Rev Neurosci* 3 (6):453-62. doi: 10.1038/nrn849.

Kimura, R., L. Devi, and M. Ohno. 2010. "Partial reduction of BACE1 improves synaptic plasticity, recent and remote memories in Alzheimer’s disease transgenic mice." *J Neurochem* 113 (1):248-61. doi: 10.1111/j.1471-4159.2010.06608.x.

Kimura, T., D. Shuto, Y. Hamada, N. Igawa, S. Kasai, P. Liu, K. Hidaka, T. Hamada, Y. Hayashi, and Y. Kiso. 2005. "Design and synthesis of highly active Alzheimer's beta-secretase (BACE1) inhibitors, KMI-420 and KMI-429, with enhanced chemical stability." *Bioorg Med Chem Lett* 15 (1):211-5. doi: 10.1016/j.bmcl.2004.09.090.

Kinoshita, A., H. Fukumoto, T. Shah, C. M. Whelan, M. C. Irizarry, and B. T. Hyman. 2003. "Demonstration by FRET of BACE interaction with the amyloid precursor protein at the cell surface and in early endosomes." *J Cell Sci* 116 (Pt 16):3339-46. doi: 10.1242/jcs.00643.

Kitazawa, M., T. R. Yamasaki, and F. M. LaFerla. 2004. "Microglia as a potential bridge between the amyloid beta-peptide and tau." *Ann N Y Acad Sci* 1035:85-103. doi: 10.1196/annals.1332.006.

Klein, A. M., N. W. Kowall, and R. J. Ferrante. 1999. "Neurotoxicity and oxidative damage of beta amyloid 1-42 versus beta amyloid 1-40 in the mouse cerebral cortex." *Ann N Y Acad Sci* 893:314-20. doi: 10.1111/j.1749-6632.1999.tb07845.x.

Klevanski, M., U. Herrmann, S. W. Weyer, R. Fol, N. Cartier, D. P. Wolfer, J. H. Caldwell, M. Korte, and U. C. Muller. 2015. "The APP Intracellular Domain Is Required for Normal Synaptic Morphology, Synaptic Plasticity, and Hippocampus-Dependent Behavior." *J Neurosci* 35 (49):16018-33. doi: 10.1523/jneurosci.2009-15.2015.

Klyubin, I., D. M. Walsh, C. A. Lemere, W. K. Cullen, G. M. Shankar, V. Betts, E. T. Spooner, L. Jiang, R. Anwyl, D. J. Selkoe, and M. J. Rowan. 2005. "Amyloid beta protein immunotherapy neutralizes Abeta oligomers that disrupt synaptic plasticity in vivo." *Nat Med* 11 (5):556-61. doi: 10.1038/nm1234.

Kobayashi, D. T., and K. S. Chen. 2005. "Behavioral phenotypes of amyloid-based genetically modified mouse models of Alzheimer's disease." *Genes Brain Behav* 4 (3):173-96. doi: 10.1111/j.1601-183X.2005.00124.x.

Kobayashi, D., M. Zeller, T. Cole, M. Buttini, L. McConlogue, S. Sinha, S. Freedman, R. G. Morris, and K. S. Chen. 2008. "BACE1 gene deletion: impact on behavioral function in a model of Alzheimer's disease." *Neurobiol Aging* 29 (6):861-73. doi: 10.1016/j.neurobiolaging.2007.01.002.

Koffie, R. M., M. Meyer-Luehmann, T. Hashimoto, K. W. Adams, M. L. Mielke, M. Garcia-Alloza, K. D. Micheva, S. J. Smith, M. L. Kim, V. M. Lee, B. T. Hyman, and T. L. Spires-Jones. 2009. "Oligomeric amyloid beta associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques." *Proc Natl Acad Sci U S A* 106 (10):4012-7. doi: 10.1073/pnas.0811698106.

Koh, J. Y., L. L. Yang, and C. W. Cotman. 1990. "Beta-amyloid protein increases the vulnerability of cultured cortical neurons to excitotoxic damage." *Brain Res* 533 (2):315-20. doi: 10.1016/0006-8993(90)91355-k.

Kohli, B. M., D. Pflieger, L. N. Mueller, G. Carbonetti, R. Aebersold, R. M. Nitsch, and U. Konietzko. 2012. "Interactome of the amyloid precursor protein APP in brain reveals a protein network involved in synaptic vesicle turnover and a close association with Synaptotagmin-1." *J Proteome Res* 11 (8):4075-90. doi: 10.1021/pr300123g.

Koike, M. A., A. J. Lin, J. Pham, E. Nguyen, J. J. Yeh, R. Rahimian, B. J. Tromberg, B. Choi, K. N. Green, and F. M. LaFerla. 2012. "APP knockout mice experience acute mortality as the result of ischemia." *PLoS One* 7 (8):e42665. doi: 10.1371/journal.pone.0042665.

Kojro, E., and F. Fahrenholz. 2005. "The non-amyloidogenic pathway: structure and function of alpha-secretases." *Subcell Biochem* 38:105-27.

Kok, E., S. Haikonen, T. Luoto, H. Huhtala, S. Goebeler, H. Haapasalo, and P. J. Karhunen. 2009. "Apolipoprotein E-dependent accumulation of Alzheimer disease-related lesions begins in middle age." *Ann Neurol* 65 (6):650-7. doi: 10.1002/ana.21696.

Koo, E. H., S. S. Sisodia, D. R. Archer, L. J. Martin, A. Weidemann, K. Beyreuther, P. Fischer, C. L. Masters, and D. L. Price. 1990. "Precursor of amyloid protein in Alzheimer disease undergoes fast anterograde axonal transport." *Proc Natl Acad Sci U S A* 87 (4):1561-5. doi: 10.1073/pnas.87.4.1561.

Koppensteiner, P., F. Trinchese, M. R. Fa, D. Puzzo, W. Gulisano, S. Yan, A. Poussin, S. Liu, I. Orozco, E. Dale, A. F. Teich, A. Palmeri, I. Ninan, S. Boehm, and O. Arancio. 2016. "Time-dependent reversal of synaptic plasticity induced by physiological concentrations of oligomeric A beta 42: an early index of Alzheimer's disease." *Scientific Reports* 6. doi: 10.1038/srep32553.

Kotilinek, L. A., B. Bacskai, M. Westerman, T. Kawarabayashi, L. Younkin, B. T. Hyman, S. Younkin, and K. H. Ashe. 2002. "Reversible memory loss in a mouse transgenic model of Alzheimer's disease." *J Neurosci* 22 (15):6331-5. doi: 20026675.

Kounnas, M. Z., A. M. Danks, S. Cheng, C. Tyree, E. Ackerman, X. Zhang, K. Ahn, P. Nguyen, D. Comer, L. Mao, C. Yu, D. Pleynet, P. J. Digregorio, G. Velicelebi, K. A. Stauderman, W. T. Comer, W. C. Mobley, Y. M. Li, S. S. Sisodia, R. E. Tanzi,

and S. L. Wagner. 2010. "Modulation of gamma-secretase reduces beta-amyloid deposition in a transgenic mouse model of Alzheimer's disease." *Neuron* 67 (5):769-80. doi: 10.1016/j.neuron.2010.08.018.

Kumaran, D., and E. A. Maguire. 2006. "The dynamics of hippocampal activation during encoding of overlapping sequences." *Neuron* 49 (4):617-29. doi: 10.1016/j.neuron.2005.12.024.

Kuro-o, M., Y. Matsumura, H. Aizawa, H. Kawaguchi, T. Suga, T. Utsugi, Y. Ohyama, M. Kurabayashi, T. Kaname, E. Kume, H. Iwasaki, A. Iida, T. Shiraki-Iida, S. Nishikawa, R. Nagai, and Y. I. Nabeshima. 1997. "Mutation of the mouse klotho gene leads to a syndrome resembling ageing." *Nature* 390 (6655):45-51. doi: 10.1038/36285.

Laczo, J., R. Andel, M. Vyhnalek, K. Vlcek, H. Magerova, A. Varjassyova, M. Tolar, and J. Hort. 2010. "Human analogue of the morris water maze for testing subjects at risk of Alzheimer's disease." *Neurodegener Dis* 7 (1-3):148-52. doi: 10.1159/000289226.

LaFerla, F. M., and K. N. Green. 2012. "Animal Models of Alzheimer Disease." *Cold Spring Harb Perspect Med* 2 (11). doi: 10.1101/cshperspect.a006320.

Laird, F. M., H. Cai, A. V. Savonenko, M. H. Farah, K. He, T. Melnikova, H. Wen, H. C. Chiang, G. Xu, V. E. Koliatsos, D. R. Borchelt, D. L. Price, H. K. Lee, and P. C. Wong. 2005. "BACE1, a major determinant of selective vulnerability of the brain to amyloid-beta amyloidogenesis, is essential for cognitive, emotional, and synaptic functions." *J Neurosci* 25 (50):11693-709. doi: 10.1523/jneurosci.2766-05.2005.

Lalonde, R., K. Fukuchi, and C. Strazielle. 2012. "APP transgenic mice for modelling behavioural and psychological symptoms of dementia (BPSD)." *Neurosci Biobehav Rev* 36 (5):1357-75. doi: 10.1016/j.neubiorev.2012.02.011.

Langston, R. F., and E. R. Wood. 2010. "Associative recognition and the hippocampus: differential effects of hippocampal lesions on object-place, object-context and object-place-context memory." *Hippocampus* 20 (10):1139-53. doi: 10.1002/hipo.20714.

Lashley, K. S. 1917. "The Effects of Strychnine and Caffeine Upon the Rate of Learning." *Psychobiology* 1 (2):141. doi: 10.1037/h0075094.

Lassek, M., J. Weingarten, A. Acker-Palmer, S. M. Bajjalieh, U. Muller, and W. Volknandt. 2014. "Amyloid precursor protein knockout diminishes synaptic vesicle proteins at the presynaptic active zone in mouse brain." *Curr Alzheimer Res* 11 (10):971-80.

Latif-Hernandez, A., D. Shah, T. Ahmed, A. C. Lo, Z. Callaerts-Vegh, A. Van der Linden, D. Balschun, and R. D'Hooge. 2016. "Quinolinic acid injection in mouse medial prefrontal cortex affects reversal learning abilities, cortical connectivity and hippocampal synaptic plasticity." *Sci Rep* 6:36489. doi: 10.1038/srep36489.

Latif-Hernandez, A., D. Shah, K. Craessaerts, T. Saido, T. Saito, B. De Strooper, A. Van der Linden, and R. D'Hooge. 2017. "Subtle behavioral changes and increased prefrontal-hippocampal network synchronicity in APP(NL-G-F) mice before prominent plaque deposition." *Behav Brain Res* 364:431-441. doi: 10.1016/j.bbr.2017.11.017.

Latif-Hernandez, A., D. Shah, K. Craessaerts, T. Saido, T. Saito, B. De Strooper, A. Van der Linden, and R. D'Hooge. 2019. "Subtle behavioral changes and increased prefrontal-hippocampal network synchronicity in APP(NL-G-F) mice before prominent plaque deposition." *Behav Brain Res* 364:431-441. doi: 10.1016/j.bbr.2017.11.017.

Lawrence, J. L., M. Tong, N. Alfulaij, T. Sherrin, M. Contarino, M. M. White, F. P. Bellinger, C. Todorovic, and R. A. Nichols. 2014. "Regulation of presynaptic Ca2+, synaptic plasticity and contextual fear conditioning by a N-terminal beta-amyloid fragment." *J Neurosci* 34 (43):14210-8. doi: 10.1523/jneurosci.0326-14.2014.

Lazarevic, V., S. Fienko, M. Andres-Alonso, D. Anni, D. Ivanova, C. Montenegro-Venegas, E. D. Gundelfinger, M. A. Cousin, and A. Fejtova. 2017. "Physiological Concentrations of Amyloid Beta Regulate Recycling of Synaptic Vesicles via Alpha7 Acetylcholine Receptor and CDK5/Calcineurin Signaling." *Front Mol Neurosci* 10:221. doi: 10.3389/fnmol.2017.00221.

Lee, H. K., K. Takamiya, J. S. Han, H. Man, C. H. Kim, G. Rumbaugh, S. Yu, L. Ding, C. He, R. S. Petralia, R. J. Wenthold, M. Gallagher, and R. L. Huganir. 2003. "Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory." *Cell* 112 (5):631-43. doi: 10.1016/s0092-8674(03)00122-3.

Lee, J., C. Retamal, L. Cuitino, A. Caruano-Yzermans, J. E. Shin, P. van Kerkhof, M. P. Marzolo, and G. Bu. 2008. "Adaptor protein sorting nexin 17 regulates amyloid precursor protein trafficking and processing in the early endosomes." *J Biol Chem* 283 (17):11501-8. doi: 10.1074/jbc.M800642200.

Lee, K. J., C. E. Moussa, Y. Lee, Y. Sung, B. W. Howell, R. S. Turner, D. T. Pak, and H. S. Hoe. 2010. "Beta amyloid-independent role of amyloid precursor protein in generation and maintenance of dendritic spines." *Neuroscience* 169 (1):344-56. doi: 10.1016/j.neuroscience.2010.04.078.

Lee, S. A., V. Tucci, V. A. Sovrano, and G. Vallortigara. 2015. "Working memory and reference memory tests of spatial navigation in mice (Mus musculus)." *J Comp Psychol* 129 (2):189-197. doi: 10.1037/a0039129.

Lemere, C. A., E. T. Spooner, J. LaFrancois, B. Malester, C. Mori, J. F. Leverone, Y. Matsuoka, J. W. Taylor, R. B. DeMattos, D. M. Holtzman, J. D. Clements, D. J. Selkoe, and K. E. Duff. 2003. "Evidence for peripheral clearance of cerebral Abeta protein following chronic, active Abeta immunization in PSAPP mice." *Neurobiol Dis* 14 (1):10-8.

Lesne, S., M. T. Koh, L. Kotilinek, R. Kayed, C. G. Glabe, A. Yang, M. Gallagher, and K. H. Ashe. 2006. "A specific amyloid-beta protein assembly in the brain impairs memory." *Nature* 440 (7082):352-7. doi: 10.1038/nature04533.

Lesné, S., C. Ali, C. Gabriel, N. Croci, E. T. MacKenzie, C. G. Glabe, M. Plotkine, C. Marchand-Verrecchia, D. Vivien, and A. Buisson. 2005. "NMDA receptor activation inhibits alpha-secretase and promotes neuronal amyloid-beta production." *J Neurosci* 25 (41):9367-77. doi: 10.1523/jneurosci.0849-05.2005.

Leuner, K., T. Schutt, C. Kurz, S. H. Eckert, C. Schiller, A. Occhipinti, S. Mai, M. Jendrach, G. P. Eckert, S. E. Kruse, R. D. Palmiter, U. Brandt, S. Drose, I. Wittig, M. Willem, C. Haass, A. S. Reichert, and W. E. Muller. 2012. "Mitochondrion-derived reactive oxygen species lead to enhanced amyloid beta formation." *Antioxid Redox Signal* 16 (12):1421-33. doi: 10.1089/ars.2011.4173.

Lewis, J., D. W. Dickson, W. L. Lin, L. Chisholm, A. Corral, G. Jones, S. H. Yen, N. Sahara, L. Skipper, D. Yager, C. Eckman, J. Hardy, M. Hutton, and E. McGowan. 2001. "Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP." *Science* 293 (5534):1487-91. doi: 10.1126/science.1058189.

Lewis, J., E. McGowan, J. Rockwood, H. Melrose, P. Nacharaju, M. Van Slegtenhorst, K. Gwinn-Hardy, M. Paul Murphy, M. Baker, X. Yu, K. Duff, J. Hardy, A. Corral, W. L. Lin, S. H. Yen, D. W. Dickson, P. Davies, and M. Hutton. 2000.

"Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein." *Nat Genet* 25 (4):402-5. doi: 10.1038/78078.

Liao, F., H. Yoon, and J. Kim. 2017. "Apolipoprotein E metabolism and functions in brain and its role in Alzheimer's disease." In *Curr Opin Lipidol*, 60-7.

Lichtenthaler, S. F., R. Wang, H. Grimm, S. N. Uljon, C. L. Masters, and K. Beyreuther. 1999. "Mechanism of the cleavage specificity of Alzheimer's disease gamma-secretase identified by phenylalanine-scanning mutagenesis of the transmembrane domain of the amyloid precursor protein." *Proc Natl Acad Sci U S A* 96 (6):3053-8.

Lim, G. P., F. Yang, T. Chu, E. Gahtan, O. Ubeda, W. Beech, J. B. Overmier, K. Hsiao-Ashec, S. A. Frautschy, and G. M. Cole. 2001. "Ibuprofen effects on Alzheimer pathology and open field activity in APPsw transgenic mice." *Neurobiol Aging* 22 (6):983-91.

Lin, X. L., C. Koelsch, S. L. Wu, D. Downs, A. Dashti, and J. Tang. 2000. "Human aspartic protease memapsin 2 cleaves the beta-secretase site of beta-amyloid precursor protein." *Proceedings of the National Academy of Sciences of the United States of America* 97 (4):1456-1460. doi: 10.1073/pnas.97.4.1456.

Lippa, C. F., A. M. Saunders, T. W. Smith, J. M. Swearer, D. A. Drachman, B. Ghetti, L. Nee, D. Pulaski-Salo, D. Dickson, Y. Robitaille, C. Bergeron, B. Crain, M. D. Benson, M. Farlow, B. T. Hyman, S. P. George-Hyslop, A. D. Roses, and D. A. Pollen. 1996. "Familial and sporadic Alzheimer's disease: neuropathology cannot exclude a final common pathway." *Neurology* 46 (2):406-12. doi: 10.1212/wnl.46.2.406.

Lisman, J. 2005. "The theta/gamma discrete phase code occuring during the hippocampal phase precession may be a more general brain coding scheme." *Hippocampus* 15 (7):913-22. doi: 10.1002/hipo.20121.

Liu, D., and Z. X. Niu. 2009. "The structure, genetic polymorphisms, expression and biological functions of complement receptor type 1 (CR1/CD35)." *Immunopharmacol Immunotoxicol* 31 (4):524-35. doi: 10.3109/08923970902845768.

Liu, S., Y. Liu, W. Hao, L. Wolf, A. J. Kiliaan, B. Penke, C. E. Rube, J. Walter, M. T. Heneka, T. Hartmann, M. D. Menger, and K. Fassbender. 2012. "TLR2 is a primary receptor for Alzheimer's amyloid beta peptide to trigger neuroinflammatory activation." *J Immunol* 188 (3):1098-107. doi: 10.4049/jimmunol.1101121.

Logovinsky, V., A. Satlin, R. Lai, C. Swanson, J. Kaplow, G. Osswald, H. Basun, and L. Lannfelt. 2016. "Safety and tolerability of BAN2401--a clinical study in Alzheimer's disease with a protofibril selective Abeta antibody." *Alzheimers Res Ther* 8 (1):14. doi: 10.1186/s13195-016-0181-2.

Lorent, K., L. Overbergh, D. Moechars, B. De Strooper, F. Van Leuven, and H. Van den Berghe. 1995. "Expression in mouse embryos and in adult mouse brain of three members of the amyloid precursor protein family, of the alpha-2-macroglobulin receptor/low density lipoprotein receptor-related protein and of its ligands apolipoprotein E, lipoprotein lipase, alpha-2-macroglobulin and the 40,000 molecular weight receptor-associated protein." *Neuroscience* 65 (4):1009-25. doi: 10.1016/0306-4522(94)00555-j.

Louvi, A., and S. Artavanis-Tsakonas. 2006. "Notch signalling in vertebrate neural development." *Nat Rev Neurosci* 7 (2):93-102. doi: 10.1038/nrn1847.

Lovestone, S., M. Boada, B. Dubois, M. Hull, J. O. Rinne, H. J. Huppertz, M. Calero, M. V. Andres, B. Gomez-Carrillo, T. Leon, and T. del Ser. 2015. "A phase II trial of

tideglusib in Alzheimer's disease." *J Alzheimers Dis* 45 (1):75-88. doi: 10.3233/jad-141959.

Lovestone, S., C. H. Reynolds, D. Latimer, D. R. Davis, B. H. Anderton, J. M. Gallo, D. Hanger, S. Mulot, B. Marquardt, S. Stabel, and et al. 1994. "Alzheimer's disease-like phosphorylation of the microtubule-associated protein tau by glycogen synthase kinase-3 in transfected mammalian cells." *Curr Biol* 4 (12):1077-86. doi: 10.1016/s0960-9822(00)00246-3.

Lu, W., H. Man, W. Ju, W. S. Trimble, J. F. MacDonald, and Y. T. Wang. 2001. "Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons." *Neuron* 29 (1):243-54. doi: 10.1016/s0896-6273(01)00194-5.

Lueptow, L. M. 2017. "Novel Object Recognition Test for the Investigation of Learning and Memory in Mice." In *J Vis Exp*.

Luo, Y., B. Bolon, S. Kahn, B. D. Bennett, S. Babu-Khan, P. Denis, W. Fan, H. Kha, J. Zhang, Y. Gong, L. Martin, J. C. Louis, Q. Yan, W. G. Richards, M. Citron, and R. Vassar. 2001. "Mice deficient in BACE1, the Alzheimer's beta-secretase, have normal phenotype and abolished beta-amyloid generation." *Nat Neurosci* 4 (3):231-2. doi: 10.1038/85059.

Lupien, S. J., and B. S. McEwen. 1997. "The acute effects of corticosteroids on cognition: integration of animal and human model studies." *Brain Res Brain Res Rev* 24 (1):1-27.

Ma, H. F., S. Lesne, L. Kotilinek, J. V. Steidl-Nichols, M. Sherman, L. Younkin, S. Younkin, C. Forster, N. Sergeant, A. Delacourte, R. Vassar, M. Citron, P. Kofuji, L. M. Boland, and K. H. Ashe. 2007. "Involvement of beta-site APP cleaving enzyme 1 (BACE1) in amyloid precursor protein-mediated enhancement of memory and activity-dependent synaptic plasticity." *Proceedings of the National Academy of Sciences of the United States of America* 104 (19):8167-8172. doi: 10.1073/pnas.0609521104.

Magnoni, S., T. J. Esparza, V. Conte, M. Carbonara, G. Carrabba, D. M. Holtzman, G. J. Zipfel, N. Stocchetti, and D. L. Brody. 2012. "Tau elevations in the brain extracellular space correlate with reduced amyloid-beta levels and predict adverse clinical outcomes after severe traumatic brain injury." *Brain* 135 (Pt 4):1268-80. doi: 10.1093/brain/awr286.

Maier, M., T. J. Seabrook, N. D. Lazo, L. Jiang, P. Das, C. Janus, and C. A. Lemere. 2006. "Short amyloid-beta (Abeta) immunogens reduce cerebral Abeta load and learning deficits in an Alzheimer's disease mouse model in the absence of an Abeta-specific cellular immune response." *J Neurosci* 26 (18):4717-28. doi: 10.1523/jneurosci.0381-06.2006.

Malthankar-Phatak, G. H., Y. G. Lin, N. Giovannone, and R. Siman. 2012. "Amyloid deposition and advanced age fails to induce Alzheimer's type progression in a double knock-in mouse model." *Aging Dis* 3 (2):141-55.

Mannix, R. C., J. Zhang, J. Park, C. Lee, and M. J. Whalen. 2011. "Detrimental effect of genetic inhibition of B-site APP-cleaving enzyme 1 on functional outcome after controlled cortical impact in young adult mice." *J Neurotrauma* 28 (9):1855-61. doi: 10.1089/neu.2011.1759.

Martins, I. C., I. Kuperstein, H. Wilkinson, E. Maes, M. Vanbrabant, W. Jonckheere, P. Van Gelder, D. Hartmann, R. D'Hooge, B. De Strooper, J. Schymkowitz, and F. Rousseau. 2008. "Lipids revert inert Abeta amyloid fibrils to neurotoxic protofibrils that affect learning in mice." *Embo j* 27 (1):224-33. doi: 10.1038/sj.emboj.7601953.

Masuda, A., Y. Kobayashi, N. Kogo, T. Saito, T. C. Saido, and S. Itohara. 2016. "Cognitive deficits in single App knock-in mouse models." *Neurobiol Learn Mem*. doi: 10.1016/j.nlm.2016.07.001.

Matrone, C., S. Luvisetto, L. R. La Rosa, R. Tamayev, A. Pignataro, N. Canu, L. Yang, A. P. Barbagallo, F. Biundo, F. Lombino, H. Zheng, M. Ammassari-Teule, and L. D'Adamio. 2012. "Tyr682 in the Abeta-precursor protein intracellular domain regulates synaptic connectivity, cholinergic function, and cognitive performance." *Aging Cell* 11 (6):1084-93. doi: 10.1111/acel.12009.

Matsuoka, Y., Y. Jouroukhin, A. J. Gray, L. Ma, C. Hirata-Fukae, H. F. Li, L. Feng, L. Lecanu, B. R. Walker, E. Planel, O. Arancio, I. Gozes, and P. S. Aisen. 2008. "A neuronal microtubule-interacting agent, NAPVSIPQ, reduces tau pathology and enhances cognitive function in a mouse model of Alzheimer's disease." *J Pharmacol Exp Ther* 325 (1):146-53. doi: 10.1124/jpet.107.130526.

Matthaei, K. I. 2007. "Genetically manipulated mice: a powerful tool with unsuspected caveats." *J Physiol* 582 (Pt 2):481-8. doi: 10.1113/jphysiol.2007.134908.

Mattson, M. P., B. Cheng, A. R. Culwell, F. S. Esch, I. Lieberburg, and R. E. Rydel. 1993. "Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the beta-amyloid precursor protein." *Neuron* 10 (2):243-54.

Mattson, M. P., B. Cheng, D. Davis, K. Bryant, I. Lieberburg, and R. E. Rydel. 1992. "beta-Amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity." *J Neurosci* 12 (2):376-89.

May, P. C., R. A. Dean, S. L. Lowe, F. Martenyi, S. M. Sheehan, L. N. Boggs, S. A. Monk, B. M. Mathes, D. J. Mergott, B. M. Watson, S. L. Stout, D. E. Timm, E. S. LaBell, C. R. Gonzales, M. Nakano, S. S. Jhee, M. Yen, L. Ereshefsky, T. D. Lindstrom, D. O. Calligaro, P. J. Cocke, D. G. Hall, S. Friedrich, M. Citron, and J. E. Audia. 2011. "Robust Central Reduction of Amyloid-beta in Humans with an Orally Available, Non-Peptidic beta-Secretase Inhibitor." *Journal of Neuroscience* 31 (46):16507-16516. doi: 10.1523/jneurosci.3647-11.2011.

May, P. C., B. A. Willis, S. L. Lowe, R. A. Dean, S. A. Monk, P. J. Cocke, J. E. Audia, L. N. Boggs, A. R. Borders, R. A. Brier, D. O. Calligaro, T. A. Day, L. Ereshefsky, J. A. Erickson, H. Gevorkyan, C. R. Gonzales, D. E. James, S. S. Jhee, S. F. Komjathy, L. L. Li, T. D. Lindstrom, B. M. Mathes, F. Martenyi, S. M. Sheehan, S. L. Stout, D. E. Timm, G. M. Vaught, B. M. Watson, L. L. Winneroski, Z. X. Yang, and D. J. Mergott. 2015. "The Potent BACE1 Inhibitor LY2886721 Elicits Robust Central A beta Pharmacodynamic Responses in Mice, Dogs, and Humans." *Journal of Neuroscience* 35 (3):1199-1210. doi: 10.1523/jneurosci.4129-14.2015.

McConlogue, L., M. Buttini, J. P. Anderson, E. F. Brigham, K. S. Chen, S. B. Freedman, D. Games, K. Johnson-Wood, M. Lee, M. Zeller, W. Liu, R. Motter, and S. Sinha. 2007. "Partial reduction of BACE1 has dramatic effects on Alzheimer plaque and synaptic pathology in APP Transgenic Mice." *J Biol Chem* 282 (36):26326-34. doi: 10.1074/jbc.M611687200.

McGeer, P. L., M. Schulzer, and E. G. McGeer. 1996. "Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies." *Neurology* 47 (2):425-32. doi: 10.1212/wnl.47.2.425.

McKhann, G., D. Drachman, M. Folstein, R. Katzman, D. Price, and E. M. Stadlan. 1984. "Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease." *Neurology* 34 (7):939-44. doi: 10.1212/wnl.34.7.939.

McKhann, G. M., D. S. Knopman, H. Chertkow, B. T. Hyman, C. R. Jack, Jr., C. H. Kawas, W. E. Klunk, W. J. Koroshetz, J. J. Manly, R. Mayeux, R. C. Mohs, J. C. Morris, M. N. Rossor, P. Scheltens, M. C. Carrillo, B. Thies, S. Weintraub, and C. H. Phelps. 2011. "The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease." *Alzheimers Dement* 7 (3):263-9. doi: 10.1016/j.jalz.2011.03.005.

McManus, M. J., M. P. Murphy, and J. L. Franklin. 2011. "The mitochondria-targeted antioxidant MitoQ prevents loss of spatial memory retention and early neuropathology in a transgenic mouse model of Alzheimer's disease." *J Neurosci* 31 (44):15703-15. doi: 10.1523/jneurosci.0552-11.2011.

McNaughton, B. L., F. P. Battaglia, O. Jensen, E. I. Moser, and M. B. Moser. 2006. "Path integration and the neural basis of the 'cognitive map'." *Nat Rev Neurosci* 7 (8):663-78. doi: 10.1038/nrn1932.

Meda, L., M. A. Cassatella, G. I. Szendrei, L. Otvos, Jr., P. Baron, M. Villalba, D. Ferrari, and F. Rossi. 1995. "Activation of microglial cells by beta-amyloid protein and interferon-gamma." *Nature* 374 (6523):647-50. doi: 10.1038/374647a0.

Medeiros, R., N. A. Castello, D. Cheng, M. Kitazawa, D. Baglietto-Vargas, K. N. Green, T. A. Esbenshade, R. S. Bitner, M. W. Decker, and F. M. LaFerla. 2014. "alpha7 Nicotinic receptor agonist enhances cognition in aged 3xTg-AD mice with robust plaques and tangles." *Am J Pathol* 184 (2):520-9. doi: 10.1016/j.ajpath.2013.10.010.

Mehla, J., S. G. Lacoursiere, V. Lapointe, B. L. McNaughton, R. J. Sutherland, R. J. McDonald, and M. H. Mohajerani. 2019. "Age-dependent behavioral and biochemical characterization of single APP knock-in mouse (APP(NL-G-F/NL-G-F)) model of Alzheimer's disease." *Neurobiol Aging* 75:25-37. doi: 10.1016/j.neurobiolaging.2018.10.026.

Melov, S., P. A. Adlard, K. Morten, F. Johnson, T. R. Golden, D. Hinerfeld, B. Schilling, C. Mavros, C. L. Masters, I. Volitakis, Q. X. Li, K. Laughton, A. Hubbard, R. A. Cherny, B. Gibson, and A. I. Bush. 2007. "Mitochondrial Oxidative Stress Causes Hyperphosphorylation of Tau." In *PLoS One*.

Meziane, H., J. C. Dodart, C. Mathis, S. Little, J. Clemens, S. M. Paul, and A. Ungerer. 1998. "Memory-enhancing effects of secreted forms of the beta-amyloid precursor protein in normal and amnestic mice." *Proc Natl Acad Sci U S A* 95 (21):12683-8. doi: 10.1073/pnas.95.21.12683.

Middei, S., S. Daniele, A. Caprioli, O. Ghirardi, and M. Ammassari-Teule. 2006. "Progressive cognitive decline in a transgenic mouse model of Alzheimer's disease overexpressing mutant hAPPswe." *Genes Brain Behav* 5 (3):249-56. doi: 10.1111/j.1601-183X.2005.00160.x.

Mitani, Y., J. Yarimizu, K. Saita, H. Uchino, H. Akashiba, Y. Shitaka, K. Ni, and N. Matsuoka. 2012. "Differential effects between gamma-secretase inhibitors and modulators on cognitive function in amyloid precursor protein-transgenic and nontransgenic mice." *J Neurosci* 32 (6):2037-50. doi: 10.1523/jneurosci.4264-11.2012.

Mitchell, J. B., and J. Laiacona. 1998. "The medial frontal cortex and temporal memory: tests using spontaneous exploratory behaviour in the rat." *Behav Brain Res* 97 (1-2):107-13.

Mokrisova, I., J. Laczo, R. Andel, I. Gazova, M. Vyhnalek, Z. Nedelska, D. Levcik, J. Cerman, K. Vlcek, and J. Hort. 2016. "Real-space path integration is impaired in

Alzheimer's disease and mild cognitive impairment." *Behav Brain Res* 307:150-8. doi: 10.1016/j.bbr.2016.03.052.

Montagne, A., S. R. Barnes, M. D. Sweeney, M. R. Halliday, A. P. Sagare, Z. Zhao, A. W. Toga, R. E. Jacobs, C. Y. Liu, L. Amezcua, M. G. Harrington, H. C. Chui, M. Law, and B. V. Zlokovic. 2015. "Blood-brain barrier breakdown in the aging human hippocampus." *Neuron* 85 (2):296-302. doi: 10.1016/j.neuron.2014.12.032.

Moreno, L., C. Rose, A. Mohanraj, B. Allinquant, J. M. Billard, and P. Dutar. 2015. "sAbetaPPalpha Improves Hippocampal NMDA-Dependent Functional Alterations Linked to Healthy Aging." *J Alzheimers Dis* 48 (4):927-35. doi: 10.3233/jad-150297.

Morgan, D., D. M. Diamond, P. E. Gottschall, K. E. Ugen, C. Dickey, J. Hardy, K. Duff, P. Jantzen, G. DiCarlo, D. Wilcock, K. Connor, J. Hatcher, C. Hope, M. Gordon, and G. W. Arendash. 2000. "A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease." *Nature* 408 (6815):982-5. doi: 10.1038/35050116.

Moriguchi, S., S. Kita, M. Fukaya, M. Osanai, R. Inagaki, Y. Sasaki, H. Izumi, K. Horie, J. Takeda, T. Saito, H. Sakagami, T. C. Saido, T. Iwamoto, and K. Fukunaga. 2018. "Reduced expression of Na(+)/Ca(2+) exchangers is associated with cognitive deficits seen in Alzheimer's disease model mice." *Neuropharmacology* 131:291-303. doi: 10.1016/j.neuropharm.2017.12.037.

Morley, J. E., and S. A. Farr. 2012. "Hormesis and amyloid-beta protein: physiology or pathology?" *J Alzheimers Dis* 29 (3):487-92. doi: 10.3233/jad-2011-111928.

Morley, J. E., S. A. Farr, W. A. Banks, S. N. Johnson, K. A. Yamada, and L. Xu. 2010. "A physiological role for amyloid-beta protein:enhancement of learning and memory." *J Alzheimers Dis* 19 (2):441-9. doi: 10.3233/jad-2009-1230.

Morris, R. G., J. J. Hagan, and J. N. Rawlins. 1986. "Allocentric spatial learning by hippocampectomised rats: a further test of the "spatial mapping" and "working memory" theories of hippocampal function." *Q J Exp Psychol B* 38 (4):365-95.

Morris, R.G. 1981. "Spatial localisation does not require the presence of local cues." *Learning and Motivation* 12:239-260.

Mucke, L., E. Masliah, G. Q. Yu, M. Mallory, E. M. Rockenstein, G. Tatsuno, K. Hu, D. Kholodenko, K. Johnson-Wood, and L. McConlogue. 2000. "High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation." *J Neurosci* 20 (11):4050-8.

Mullan, M., F. Crawford, K. Axelman, H. Houlden, L. Lilius, B. Winblad, and L. Lannfelt. 1992. "A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid." *Nat Genet* 1 (5):345-7. doi: 10.1038/ng0892-345.

Muller, T., H. E. Meyer, R. Egensperger, and K. Marcus. 2008. "The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics-relevance for Alzheimer's disease." *Prog Neurobiol* 85 (4):393-406. doi: 10.1016/j.pneurobio.2008.05.002.

Mumby, D. G., S. Gaskin, M. J. Glenn, T. E. Schramek, and H. Lehmann. 2002. "Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts." *Learn Mem* 9 (2):49-57. doi: 10.1101/lm.41302.

Mumby, D. G., and J. P. Pinel. 1994. "Rhinal cortex lesions and object recognition in rats." *Behav Neurosci* 108 (1):11-8.

Mura, E., S. Zappettini, S. Preda, F. Biundo, C. Lanni, M. Grilli, A. Cavallero, G. Olivero, A. Salamone, S. Govoni, and M. Marchi. 2012. "Dual effect of beta-amyloid on alpha7 and alpha4beta2 nicotinic receptors controlling the release of

glutamate, aspartate and GABA in rat hippocampus." *PLoS One* 7 (1):e29661. doi: 10.1371/journal.pone.0029661.

Murrell, J., M. Farlow, B. Ghetti, and M. D. Benson. 1991. "A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease." *Science* 254 (5028):97-9.

Müller, Ulrike C., Thomas Deller, and Martin Korte. 2017. "Not just amyloid: physiological functions of the amyloid precursor protein family." *Nature Reviews Neuroscience* 18:281-298. doi: doi:10.1038/nrn.2017.29.

Nagele, R. G., M. R. D'Andrea, W. J. Anderson, and H. Y. Wang. 2002. "Intracellular accumulation of beta-amyloid(1-42) in neurons is facilitated by the alpha 7 nicotinic acetylcholine receptor in Alzheimer's disease." *Neuroscience* 110 (2):199-211. doi: 10.1016/s0306-4522(01)00460-2.

Nakamura, A., N. Kaneko, V. L. Villemagne, T. Kato, J. Doecke, V. Dore, C. Fowler, Q. X. Li, R. Martins, C. Rowe, T. Tomita, K. Matsuzaki, K. Ishii, Y. Arahata, S. Iwamoto, K. Ito, K. Tanaka, C. L. Masters, and K. Yanagisawa. 2018. "High performance plasma amyloid-beta biomarkers for Alzheimer's disease." *Nature* 554 (7691):249-254. doi: 10.1038/nature25456.

Nalbantoglu, J., G. Tirado-Santiago, A. Lahsaini, J. Poirier, O. Goncalves, G. Verge, F. Momoli, S. A. Welner, G. Massicotte, J. P. Julien, and M. L. Shapiro. 1997. "Impaired learning and LTP in mice expressing the carboxy terminus of the Alzheimer amyloid precursor protein." *Nature* 387 (6632):500-5. doi: 10.1038/387500a0.

Neumann, U., H. Rueeger, R. Machauer, S. J. Veenstra, R. M. Lueoend, M. Tintelnot-Blomley, G. Laue, K. Beltz, B. Vogg, P. Schmid, W. Frieauff, D. R. Shimshek, M. Staufenbiel, and L. H. Jacobson. 2015. "A novel BACE inhibitor NB-360 shows a superior pharmacological profile and robust reduction of amyloid-beta and neuroinflammation in APP transgenic mice." *Mol Neurodegener* 10:44. doi: 10.1186/s13024-015-0033-8.

Nhan, H. S., K. Chiang, and E. H. Koo. 2015. "The multifaceted nature of amyloid precursor protein and its proteolytic fragments: friends and foes." *Acta Neuropathol* 129 (1):1-19. doi: 10.1007/s00401-014-1347-2.

Nicoll, J. A. R., G. R. Buckland, C. H. Harrison, A. Page, S. Harris, S. Love, J. W. Neal, C. Holmes, and D. Boche. 2019. "Persistent neuropathological effects 14 years following amyloid-beta immunization in Alzheimer's disease." *Brain*. doi: 10.1093/brain/awz142.

Nilsberth, C., A. Westlind-Danielsson, C. B. Eckman, M. M. Condron, K. Axelman, C. Forsell, C. Stenh, J. Luthman, D. B. Teplow, S. G. Younkin, J. Naslund, and L. Lannfelt. 2001. "The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation." *Nat Neurosci* 4 (9):887-93. doi: 10.1038/nn0901-887.

Nitsch, R. M., S. A. Farber, J. H. Growdon, and R. J. Wurtman. 1993. "Release of amyloid beta-protein precursor derivatives by electrical depolarization of rat hippocampal slices." *Proc Natl Acad Sci U S A* 90 (11):5191-3. doi: 10.1073/pnas.90.11.5191.

Norris, C. M., D. L. Korol, and T. C. Foster. 1996. "Increased susceptibility to induction of long-term depression and long-term potentiation reversal during aging." *J Neurosci* 16 (17):5382-92.

O'Keefe, J., N. Burgess, J. G. Donnett, K. J. Jeffery, and E. A. Maguire. 1998. "Place cells, navigational accuracy, and the human hippocampus." *Philos Trans R Soc Lond B Biol Sci* 353 (1373):1333-40. doi: 10.1098/rstb.1998.0287.

Oakley, H., S. L. Cole, S. Logan, E. Maus, P. Shao, J. Craft, A. Guillozet-Bongaarts, M. Ohno, J. Disterhoft, L. Van Eldik, R. Berry, and R. Vassar. 2006. "Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation." *J Neurosci* 26 (40):10129-40. doi: 10.1523/jneurosci.1202-06.2006.

Obregon, D., H. Hou, J. Deng, B. Giunta, J. Tian, D. Darlington, M. Shahaduzzaman, Y. Zhu, T. Mori, M. P. Mattson, and J. Tan. 2012. "Soluble amyloid precursor protein-alpha modulates beta-secretase activity and amyloid-beta generation." *Nat Commun* 3:777. doi: 10.1038/ncomms1781.

Oddo, S., L. Billings, J. P. Kesslak, D. H. Cribbs, and F. M. LaFerla. 2004. "Abeta immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome." *Neuron* 43 (3):321-32. doi: 10.1016/j.neuron.2004.07.003.

Oddo, S., A. Caccamo, J. D. Shepherd, M. P. Murphy, T. E. Golde, R. Kayed, R. Metherate, M. P. Mattson, Y. Akbari, and F. M. LaFerla. 2003. "Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction." *Neuron* 39 (3):409-21.

Oddo, S., V. Vasilevko, A. Caccamo, M. Kitazawa, D. H. Cribbs, and F. M. LaFerla. 2006. "Reduction of soluble Abeta and tau, but not soluble Abeta alone, ameliorates cognitive decline in transgenic mice with plaques and tangles." *J Biol Chem* 281 (51):39413-23. doi: 10.1074/jbc.M608485200.

Ohno, M., S. L. Cole, M. Yasvoina, J. Zhao, M. Citron, R. Berry, J. F. Disterhoft, and R. Vassar. 2007. "BACE1 gene deletion prevents neuron loss and memory deficits in 5XFAD APP/PS1 transgenic mice." *Neurobiol Dis* 26 (1):134-45. doi: 10.1016/j.nbd.2006.12.008.

Ohno, M., E. A. Sametsky, L. H. Younkin, H. Oakley, S. G. Younkin, M. Citron, R. Vassar, and J. F. Disterhoft. 2004. "BACE1 deficiency rescues memory deficits and cholinergic dysfunction in a mouse model of Alzheimer's disease." *Neuron* 41 (1):27-33.

Olmos-Alonso, A., S. T. Schetters, S. Sri, K. Askew, R. Mancuso, M. Vargas-Caballero, C. Holscher, V. H. Perry, and D. Gomez-Nicola. 2016. "Pharmacological targeting of CSF1R inhibits microglial proliferation and prevents the progression of Alzheimer's-like pathology." *Brain* 139 (Pt 3):891-907. doi: 10.1093/brain/awv379.

Olton, D. S., T. J. Becker, and G. E. Handelmann. 1979. "Hippocampus, space, and memory." *Behavioral and Brain Sciences* 2 (3):313-322. doi: doi:10.1017/S0140525X00062713.

Olton, D. S., and M. A. Werz. 1978. "Hippocampal function and behavior: spatial discrimination and response inhibition." *Physiol Behav* 20 (5):597-605.

Otvos, L., Jr., L. Feiner, E. Lang, G. I. Szendrei, M. Goedert, and V. M. Lee. 1994. "Monoclonal antibody PHF-1 recognizes tau protein phosphorylated at serine residues 396 and 404." *J Neurosci Res* 39 (6):669-73. doi: 10.1002/jnr.490390607.

Ou-Yang, M. H., J. E. Kurz, T. Nomura, J. Popovic, T. W. Rajapaksha, H. Dong, A. Contractor, D. M. Chetkovich, W. G. Tourtellotte, and R. Vassar. 2018. "Axonal organization defects in the hippocampus of adult conditional BACE1 knockout mice." *Sci Transl Med* 10 (459). doi: 10.1126/scitranslmed.aao5620.

O'Brien, R. J., and P. C. Wong. 2011. "Amyloid Precursor Protein Processing and Alzheimer's Disease." *Annu Rev Neurosci* 34:185-204. doi: 10.1146/annurev-neuro-061010-113613.

Palmqvist, S., H. Zetterberg, N. Mattsson, P. Johansson, L. Minthon, K. Blennow, M. Olsson, and O. Hansson. 2015. "Detailed comparison of amyloid PET and CSF biomarkers for identifying early Alzheimer disease." *Neurology* 85 (14):1240-9. doi: 10.1212/wnl.0000000000001991.

Pardridge, W. M. 2005. "The blood-brain barrier: bottleneck in brain drug development." *NeuroRx* 2 (1):3-14. doi: 10.1602/neurorx.2.1.3.

Parvathy, S., I. Hussain, E. H. Karran, A. J. Turner, and N. M. Hooper. 1999. "Cleavage of Alzheimer's amyloid precursor protein by alpha-secretase occurs at the surface of neuronal cells." *Biochemistry* 38 (30):9728-34. doi: 10.1021/bi9906827.

Patel, N. S., D. Paris, V. Mathura, A. N. Quadros, F. C. Crawford, and M. J. Mullan. 2005. "Inflammatory cytokine levels correlate with amyloid load in transgenic mouse models of Alzheimer's disease." *J Neuroinflammation* 2 (1):9. doi: 10.1186/1742-2094-2-9.

Pearce, J.M., D.N. George, M. Haselgrove, J.T. Erichsen, and M.A. Good. 2005. "The Influence of Hippocampal Lesions on the Discrimination of Structure and on Spatial Memory in Pigeons (Columba Livia)." *Behavioral neuroscience* 119 (5). doi: 10.1037/0735-7044.119.5.1316.

Penninkilampi, R., H. M. Brothers, and G. D. Eslick. 2017. "Safety and Efficacy of Anti-Amyloid-beta Immunotherapy in Alzheimer's Disease: A Systematic Review and Meta-Analysis." *J Neuroimmune Pharmacol* 12 (1):194-203. doi: 10.1007/s11481-016-9722-5.

Perez-Grijalba, V., J. Romero, P. Pesini, L. Sarasa, I. Monleon, I. San-Jose, J. Arbizu, P. Martinez-Lage, J. Munuera, A. Ruiz, L. Tarraga, M. Boada, and M. Sarasa. 2019. "Plasma Abeta42/40 Ratio Detects Early Stages of Alzheimer's Disease and Correlates with CSF and Neuroimaging Biomarkers in the AB255 Study." *J Prev Alzheimers Dis* 6 (1):34-41. doi: 10.14283/jpad.2018.41.

Pervolaraki, E, Hall S. P, Foresteire. D, Saito. T, Saido. T, M. A Whittington, C Lever, and J Dachtler. 2019. "Insoluble Aβ overexpression in an App knock-in mouse model alters microstructure and gamma oscillations in the prefrontal cortex, causing anxiety-related behaviours." *Preprint*. doi: 10.1101/494443.

Petersen, R. C., G. E. Smith, S. C. Waring, R. J. Ivnik, E. G. Tangalos, and E. Kokmen. 1999. "Mild cognitive impairment: clinical characterization and outcome." *Arch Neurol* 56 (3):303-8. doi: 10.1001/archneur.56.3.303.

Petrache, A. L., A. Rajulawalla, A. Shi, A. Wetzel, T. Saito, T. C. Saido, K. Harvey, and A. B. Ali. 2019. "Aberrant Excitatory-Inhibitory Synaptic Mechanisms in Entorhinal Cortex Microcircuits During the Pathogenesis of Alzheimer's Disease." *Cereb Cortex* 29 (4):1834-1850. doi: 10.1093/cercor/bhz016.

Petry, F. R., J. Pelletier, A. Bretteville, F. Morin, F. Calon, S. S. Hebert, R. A. Whittington, and E. Planel. 2014. "Specificity of anti-tau antibodies when analyzing mice models of Alzheimer's disease: problems and solutions." *PLoS One* 9 (5):e94251. doi: 10.1371/journal.pone.0094251.

Phinney, A. L., M. E. Calhoun, D. P. Wolfer, H. P. Lipp, H. Zheng, and M. Jucker. 1999. "No hippocampal neuron or synaptic bouton loss in learning-impaired aged beta-amyloid precursor protein-null mice." *Neuroscience* 90 (4):1207-16.

Pike, C. J., A. J. Walencewicz, C. G. Glabe, and C. W. Cotman. 1991. "In vitro aging of beta-amyloid protein causes peptide aggregation and neurotoxicity." *Brain Res* 563 (1-2):311-4. doi: 10.1016/0006-8993(91)91553-d.

Plant, L. D., J. P. Boyle, I. F. Smith, C. Peers, and H. A. Pearson. 2003. "The production of amyloid beta peptide is a critical requirement for the viability of central neurons." *J Neurosci* 23 (13):5531-5.

Plant, L. D., N. J. Webster, J. P. Boyle, M. Ramsden, D. B. Freir, C. Peers, and H. A. Pearson. 2006. "Amyloid beta peptide as a physiological modulator of neuronal 'A'-type K+ current." *Neurobiol Aging* 27 (11):1673-83. doi: 10.1016/j.neurobiolaging.2005.09.038.

Portelius, E., E. Price, G. Brinkmalm, M. Stiteler, M. Olsson, R. Persson, A. Westman-Brinkmalm, H. Zetterberg, A. J. Simon, and K. Blennow. 2011. "A novel pathway for amyloid precursor protein processing." *Neurobiol Aging* 32 (6):1090-8. doi: 10.1016/j.neurobiolaging.2009.06.002.

Prox, J., C. Bernreuther, H. Altmeppen, J. Grendel, M. Glatzel, R. D'Hooge, S. Stroobants, T. Ahmed, D. Balschun, M. Willem, S. Lammich, D. Isbrandt, M. Schweizer, K. Horre, B. De Strooper, and P. Saftig. 2013. "Postnatal disruption of the disintegrin/metalloproteinase ADAM10 in brain causes epileptic seizures, learning deficits, altered spine morphology, and defective synaptic functions." *J Neurosci* 33 (32):12915-28, 12928a. doi: 10.1523/jneurosci.5910-12.2013.

Purushothuman, S., L. Marotte, S. Stowe, D. M. Johnstone, and J. Stone. 2013. "The response of cerebral cortex to haemorrhagic damage: experimental evidence from a penetrating injury model." *PLoS One* 8 (3):e59740. doi: 10.1371/journal.pone.0059740.

Puzzo, D., W. Gulisano, O. Arancio, and A. Palmeri. 2015. "The Keystone of Alzheimer Pathogenesis Might Be Sought in Aβ Physiology." *Neuroscience* 307:26-36. doi: 10.1016/j.neuroscience.2015.08.039.

Puzzo, D., L. Privitera, M. Fa, A. Staniszewski, G. Hashimoto, F. Aziz, M. Sakurai, E. M. Ribe, C. M. Troy, M. Mercken, S. S. Jung, A. Palmeri, and O. Arancio. 2011. "Endogenous Amyloid-β is Necessary for Hippocampal Synaptic Plasticity and Memory." *Ann Neurol* 69 (5):819-30. doi: 10.1002/ana.22313.

Puzzo, D., L. Privitera, E. Leznik, M. Fa, A. Staniszewski, A. Palmeri, and O. Arancio. 2008. "Picomolar amyloid-β positively modulates synaptic plasticity and memory in hippocampus." *J Neurosci* 28 (53):14537-45. doi: 10.1523/jneurosci.2692-08.2008.

Puzzo, D., L. Privitera, and A. Palmeri. 2012. "Hormetic effect of amyloid-beta peptide in synaptic plasticity and memory." *Neurobiol Aging* 33 (7):1484.e15-24. doi: 10.1016/j.neurobiolaging.2011.12.020.

Qiu, W. Q., A. Ferreira, C. Miller, E. H. Koo, and D. J. Selkoe. 1995. "Cell-surface beta-amyloid precursor protein stimulates neurite outgrowth of hippocampal neurons in an isoform-dependent manner." *J Neurosci* 15 (3 Pt 2):2157-67.

Quiroz, Y. T., A. P. Schultz, K. Chen, H. D. Protas, M. Brickhouse, A. S. Fleisher, J. B. Langbaum, P. Thiyyagura, A. M. Fagan, A. R. Shah, M. Muniz, J. F. Arboleda-Velasquez, C. Munoz, G. Garcia, N. Acosta-Baena, M. Giraldo, V. Tirado, D. L. Ramirez, P. N. Tariot, B. C. Dickerson, R. A. Sperling, F. Lopera, and E. M. Reiman. 2015. "Brain Imaging and Blood Biomarker Abnormalities in Children With Autosomal Dominant Alzheimer Disease: A Cross-Sectional Study." *JAMA Neurol* 72 (8):912-9. doi: 10.1001/jamaneurol.2015.1099.

Rabinovich-Nikitin, I., I. S. Rakover, M. Becker, and B. Solomon. 2012. "Beneficial Effect of Antibodies against beta- Secretase Cleavage Site of App on Alzheimer's-Like Pathology in Triple-Transgenic Mice." *Plos One* 7 (10):9. doi: 10.1371/journal.pone.0046650.

Radde, R., T. Bolmont, S. A. Kaeser, J. Coomaraswamy, D. Lindau, L. Stoltze, M. E. Calhoun, F. Jaggi, H. Wolburg, S. Gengler, C. Haass, B. Ghetti, C. Czech, C. Holscher, P. M. Mathews, and M. Jucker. 2006. "Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology." *EMBO Rep* 7 (9):940-6. doi: 10.1038/sj.embor.7400784.

Rakover, I., M. Arbel, and B. Solomon. 2007. "Immunotherapy against APP beta-secretase cleavage site improves cognitive function and reduces neuroinflammation in Tg2576 mice without a significant effect on brain A beta levels." *Neurodegenerative Diseases* 4 (5):392-402. doi: 10.1159/000103250.

Ramser, E. M., K. J. Gan, H. Decker, E. Y. Fan, M. M. Suzuki, S. T. Ferreira, and M. A. Silverman. 2013. "Amyloid-beta oligomers induce tau-independent disruption of BDNF axonal transport via calcineurin activation in cultured hippocampal neurons." *Mol Biol Cell* 24 (16):2494-505. doi: 10.1091/mbc.E12-12-0858.

Ravi, L. B., J. G. Mohanty, F. J. Chrest, R. Jayakumar, E. Nagababu, P. V. Usatyuk, V. Natarajan, and J. M. Rifkind. 2004. "Influence of beta-amyloid fibrils on the interactions between red blood cells and endothelial cells." *Neurol Res* 26 (5):579-85. doi: 10.1179/016164104225016227.

Reaume, A. G., D. S. Howland, S. P. Trusko, M. J. Savage, D. M. Lang, B. D. Greenberg, R. Siman, and R. W. Scott. 1996. "Enhanced amyloidogenic processing of the beta-amyloid precursor protein in gene-targeted mice bearing the Swedish familial Alzheimer's disease mutations and a "humanized" Abeta sequence." *J Biol Chem* 271 (38):23380-8. doi: 10.1074/jbc.271.38.23380.

Reddy, P. H. 2009. "Amyloid beta, mitochondrial structural and functional dynamics in Alzheimer's disease." *Exp Neurol* 218 (2):286-92. doi: 10.1016/j.expneurol.2009.03.042.

Reddy, P. H. 2011. "Abnormal tau, mitochondrial dysfunction, impaired axonal transport of mitochondria, and synaptic deprivation in Alzheimer's disease." *Brain Res* 1415:136-48. doi: 10.1016/j.brainres.2011.07.052.

Redwine, J. M., B. Kosofsky, R. E. Jacobs, D. Games, J. F. Reilly, J. H. Morrison, W. G. Young, and F. E. Bloom. 2003. "Dentate gyrus volume is reduced before onset of plaque formation in PDAPP mice: a magnetic resonance microscopy and stereologic analysis." *Proc Natl Acad Sci U S A* 100 (3):1381-6. doi: 10.1073/pnas.242746599.

Reed, M. N., J. J. Hofmeister, L. Jungbauer, A. T. Welzel, C. Yu, M. A. Sherman, S. Lesne, M. J. LaDu, D. M. Walsh, K. H. Ashe, and J. P. Cleary. 2011. "Cognitive effects of cell-derived and synthetically derived Abeta oligomers." *Neurobiol Aging* 32 (10):1784-94. doi: 10.1016/j.neurobiolaging.2009.11.007.

Regehr, W. G. 2012. "Short-Term Presynaptic Plasticity." In *Cold Spring Harb Perspect Biol*.

Reilly, J. F., D. Games, R. E. Rydel, S. Freedman, D. Schenk, W. G. Young, J. H. Morrison, and F. E. Bloom. 2003. "Amyloid deposition in the hippocampus and entorhinal cortex: quantitative analysis of a transgenic mouse model." *Proc Natl Acad Sci U S A* 100 (8):4837-42. doi: 10.1073/pnas.0330745100.

Reitz, C., and R. Mayeux. 2014. "Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers." *Biochem Pharmacol* 88 (4):640-51. doi: 10.1016/j.bcp.2013.12.024.

Ring, S., S. W. Weyer, S. B. Kilian, E. Waldron, C. U. Pietrzik, M. A. Filippov, J. Herms, C. Buchholz, C. B. Eckman, M. Korte, D. P. Wolfer, and U. C. Muller. 2007. "The secreted beta-amyloid precursor protein ectodomain APPs alpha is sufficient to rescue the anatomical, behavioral, and electrophysiological abnormalities of APP-deficient mice." *J Neurosci* 27 (29):7817-26. doi: 10.1523/jneurosci.1026-07.2007.

Roberson, E. D., K. Scearce-Levie, J. J. Palop, F. Yan, I. H. Cheng, T. Wu, H. Gerstein, G. Q. Yu, and L. Mucke. 2007. "Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model." *Science* 316 (5825):750-4. doi: 10.1126/science.1141736.

Roberts, G. W., S. M. Gentleman, A. Lynch, and D. I. Graham. 1991. "beta A4 amyloid protein deposition in brain after head trauma." *Lancet* 338 (8780):1422-3. doi: 10.1016/0140-6736(91)92724-g.

Rodgers, R. J., and J. C. Cole. 1993. "Anxiety enhancement in the murine elevated plus maze by immediate prior exposure to social stressors." *Physiol Behav* 53 (2):383-8.

Rogers, K., K. M. Felsenstein, L. Hrdlicka, Z. Tu, F. Albayya, W. Lee, S. Hopp, M. J. Miller, D. Spaulding, Z. Yang, H. Hodgdon, S. Nolan, M. Wen, D. Costa, J. F. Blain, E. Freeman, B. De Strooper, V. Vulsteke, L. Scrocchi, H. Zetterberg, E. Portelius, B. Hutter-Paier, D. Havas, M. Ahlijanian, D. Flood, L. Leventhal, G. Shapiro, H. Patzke, R. Chesworth, and G. Koenig. 2012. "Modulation of gamma-secretase by EVP-0015962 reduces amyloid deposition and behavioral deficits in Tg2576 mice." *Mol Neurodegener* 7:61. doi: 10.1186/1750-1326-7-61.

Roses, A. D., M. W. Lutz, H. Amrine-Madsen, A. M. Saunders, D. G. Crenshaw, S. S. Sundseth, M. J. Huentelman, K. A. Welsh-Bohmer, and E. M. Reiman. 2010. "A TOMM40 variable-length polymorphism predicts the age of late-onset Alzheimer's disease." *Pharmacogenomics J* 10 (5):375-84. doi: 10.1038/tpj.2009.69.

Rudy, C. C., H. C. Hunsberger, D. S. Weitzner, and M. N. Reed. 2015. "The role of the tripartite glutamatergic synapse in the pathophysiology of Alzheimer's disease." *Aging Dis* 6 (2):131-48. doi: 10.14336/ad.2014.0423.

Sabec, M. H., S. Wonnacott, E. C. Warburton, and Z. I. Bashir. 2018. "Nicotinic Acetylcholine Receptors Control Encoding and Retrieval of Associative Recognition Memory through Plasticity in the Medial Prefrontal Cortex." *Cell Rep* 22 (13):3409-3415. doi: 10.1016/j.celrep.2018.03.016.

Sabo, S. L., A. F. Ikin, J. D. Buxbaum, and P. Greengard. 2003. "The amyloid precursor protein and its regulatory protein, FE65, in growth cones and synapses in vitro and in vivo." *J Neurosci* 23 (13):5407-15.

Sadigh-Eteghad, S., J. Mahmoudi, S. Babri, and M. Talebi. 2015. "Effect of alpha-7 nicotinic acetylcholine receptor activation on beta-amyloid induced recognition memory impairment. Possible role of neurovascular function." *Acta Cir Bras* 30 (11):736-42. doi: 10.1590/s0102-865020150110000003.

Sadigh-Eteghada, S., M. Talebia, M. Farhoudia, S.E.J. Golzarib, B. Sabermaroufa, and J. Mahmoudi. 2014. "Beta-amyloid exhibits antagonistic effects on alpha 7 nicotinic acetylcholine receptors in orchestrated manner." *Journal of Medical Hypotheses and Ideas* 8 (2):49-52.

Saido, T. C., T. Iwatsubo, D. M. Mann, H. Shimada, Y. Ihara, and S. Kawashima. 1995. "Dominant and differential deposition of distinct beta-amyloid peptide species, A beta N3(pE), in senile plaques." *Neuron* 14 (2):457-66.

Saint-Aubert, L., E. J. Barbeau, P. Peran, F. Nemmi, C. Vervueren, H. Mirabel, P. Payoux, A. Hitzel, F. Bonneville, R. Gramada, M. Tafani, C. Vincent, M. Puel, S. Dechaumont, F. Chollet, and J. Pariente. 2013. "Cortical florbetapir-PET amyloid load in prodromal Alzheimer's disease patients." *EJNMMI Res* 3 (1):43. doi: 10.1186/2191-219x-3-43.

Saito, T., Y. Matsuba, N. Mihira, J. Takano, P. Nilsson, S. Itohara, N. Iwata, and T. C. Saido. 2014. "Single App knock-in mouse models of Alzheimer's disease." *Nat Neurosci* 17 (5):661-3. doi: 10.1038/nn.3697.

Saito, T., Y. Matsuba, N. Yamazaki, S. Hashimoto, and T. C. Saido. 2016. "Calpain Activation in Alzheimer's Model Mice Is an Artifact of APP and Presenilin Overexpression." *J Neurosci* 36 (38):9933-6. doi: 10.1523/jneurosci.1907-16.2016.

Saito, T., N. Mihira, Y. Matsuba, H. Sasaguri, S. Hashimoto, S. Narasimhan, B. Zhang, S. Murayama, M. Higuchi, V. M. Y. Lee, J. Q. Trojanowski, and T. C. Saido. 2019. "Humanization of the entire murine Mapt gene provides a murine model of pathological human tau propagation." *J Biol Chem* 294 (34):12754-12765. doi: 10.1074/jbc.RA119.009487.

Sakakibara, Y., M. Sekiya, T. Saito, T. C. Saido, and K. M. Iijima. 2018. "Cognitive and emotional alterations in App knock-in mouse models of Aβ amyloidosis." *BMC Neurosci* 19 (46). doi: 10.1186/s12868-018-0446-8.

Sakakibara, Y., M. Sekiya, T. Saito, T. C. Saido, and K. M. Iijima. 2019. "Amyloid-beta plaque formation and reactive gliosis are required for induction of cognitive deficits in App knock-in mouse models of Alzheimer's disease." *BMC Neurosci* 20 (1):13. doi: 10.1186/s12868-019-0496-6.

Salas, I. H., Z. Callaerts-Vegh, R. D'Hooge, T. C. Saido, C. G. Dotti, and B. De Strooper. 2018. "Increased Insoluble Amyloid-beta Induces Negligible Cognitive Deficits in Old AppNL/NL Knock-In Mice." *J Alzheimers Dis* 66 (2):801-809. doi: 10.3233/jad-180410.

Sasaguri, H., P. Nilsson, S. Hashimoto, K. Nagata, T. Saito, B. De Strooper, J. Hardy, R. Vassar, B. Winblad, and T. C. Saido. 2017. "APP mouse models for Alzheimer's disease preclinical studies." *Embo j* 36 (17):2473-2487. doi: 10.15252/embj.201797397.

Satlin, A., J. Wang, V. Logovinsky, S. Berry, C. Swanson, S. Dhadda, and D. A. Berry. 2016. "Design of a Bayesian adaptive phase 2 proof-of-concept trial for BAN2401, a putative disease-modifying monoclonal antibody for the treatment of Alzheimer's disease." *Alzheimers Dement (N Y)* 2 (1):1-12. doi: 10.1016/j.trci.2016.01.001.

Savonenko, A. V., T. Melnikova, F. M. Laird, K. A. Stewart, D. L. Price, and P. C. Wong. 2008. "Alteration of BACE1-dependent NRG1/ErbB4 signaling and schizophrenia-like phenotypes in BACE1-null mice." *Proc Natl Acad Sci U S A* 105 (14):5585-90. doi: 10.1073/pnas.0710373105.

Schenk, D., R. Barbour, W. Dunn, G. Gordon, H. Grajeda, T. Guido, K. Hu, J. Huang, K. Johnson-Wood, K. Khan, D. Kholodenko, M. Lee, Z. Liao, I. Lieberburg, R. Motter, L. Mutter, F. Soriano, G. Shopp, N. Vasquez, C. Vandevert, S. Walker, M. Wogulis, T. Yednock, D. Games, and P. Seubert. 1999. "Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse." *Nature* 400 (6740):173-7. doi: 10.1038/22124.

Schettini, G., S. Govoni, M. Racchi, and G. Rodriguez. 2010. "Phosphorylation of APP-CTF-AICD domains and interaction with adaptor proteins: signal transduction and/or transcriptional role--relevance for Alzheimer pathology." *J Neurochem* 115 (6):1299-308. doi: 10.1111/j.1471-4159.2010.07044.x.

Schilling, S., J. U. Rahfeld, I. Lues, and C. A. Lemere. 2018. "Passive Aβ Immunotherapy: Current Achievements and Future Perspectives." In *Molecules*.

Schindler, S. E., J. G. Bollinger, V. Ovod, K. G. Mawuenyega, Y. Li, B. A. Gordon, D. M. Holtzman, J. C. Morris, T. L. S. Benzinger, C. Xiong, A. M. Fagan, and R. J. Bateman. 2019. "High-precision plasma beta-amyloid 42/40 predicts current and future brain amyloidosis." *Neurology*. doi: 10.1212/wnl.0000000000008081.

Schmidt, S. D., R. A. Nixon, and P. M. Mathews. 2005. "ELISA method for measurement of amyloid-beta levels." *Methods Mol Biol* 299:279-97. doi: 10.1385/1-59259-874-9:279.

Schneeberger, A., M. Mandler, O. Otawa, W. Zauner, F. Mattner, and W. Schmidt. 2009. "Development of AFFITOPE vaccines for Alzheimer's disease (AD)--from concept to clinical testing." *J Nutr Health Aging* 13 (3):264-7.

Seabrook, G. R., D. W. Smith, B. J. Bowery, A. Easter, T. Reynolds, S. M. Fitzjohn, R. A. Morton, H. Zheng, G. R. Dawson, D. J. Sirinathsinghji, C. H. Davies, G. L. Collingridge, and R. G. Hill. 1999. "Mechanisms contributing to the deficits in hippocampal synaptic plasticity in mice lacking amyloid precursor protein." *Neuropharmacology* 38 (3):349-59.

Seabrook, T. J., K. Thomas, L. Jiang, J. Bloom, E. Spooner, M. Maier, G. Bitan, and C. A. Lemere. 2007. "Dendrimeric Abeta1-15 is an effective immunogen in wildtype and APP-tg mice." *Neurobiol Aging* 28 (6):813-23. doi: 10.1016/j.neurobiolaging.2006.04.007.

Selkoe, D. J., and J. Hardy. 2016. "The amyloid hypothesis of Alzheimer's disease at 25 years." *EMBO Mol Med* 8 (6):595-608. doi: 10.15252/emmm.201606210.

Senechal, Y., P. H. Kelly, J. F. Cryan, F. Natt, and K. K. Dev. 2007. "Amyloid precursor protein knockdown by siRNA impairs spontaneous alternation in adult mice." *J Neurochem* 102 (6):1928-1940. doi: 10.1111/j.1471-4159.2007.04672.x.

Seo, J., P. Giusti-Rodriguez, Y. Zhou, A. Rudenko, S. Cho, K. T. Ota, C. Park, H. Patzke, R. Madabhushi, L. Pan, A. E. Mungenast, J. S. Guan, I. Delalle, and L. H. Tsai. 2014. "Activity-dependent p25 generation regulates synaptic plasticity and Abeta-induced cognitive impairment." *Cell* 157 (2):486-98. doi: 10.1016/j.cell.2014.01.065.

Sereno, L., M. Coma, M. Rodriguez, P. Sanchez-Ferrer, M. B. Sanchez, I. Gich, J. M. Agullo, M. Perez, J. Avila, C. Guardia-Laguarta, J. Clarimon, A. Lleo, and T. Gomez-Isla. 2009. "A novel GSK-3beta inhibitor reduces Alzheimer's pathology and rescues neuronal loss in vivo." *Neurobiol Dis* 35 (3):359-67. doi: 10.1016/j.nbd.2009.05.025.

Serneels, L., J. Van Biervliet, K. Craessaerts, T. Dejaegere, K. Horre, T. Van Houtvin, H. Esselmann, S. Paul, M. K. Schafer, O. Berezovska, B. T. Hyman, B. Sprangers, R. Sciot, L. Moons, M. Jucker, Z. Yang, P. C. May, E. Karran, J. Wiltfang, R. D'Hooge, and B. De Strooper. 2009. "gamma-Secretase heterogeneity in the Aph1 subunit: relevance for Alzheimer's disease." *Science* 324 (5927):639-42. doi: 10.1126/science.1171176.

Sevigny, J., P. Chiao, T. Bussiere, P. H. Weinreb, L. Williams, M. Maier, R. Dunstan, S. Salloway, T. Chen, Y. Ling, J. O'Gorman, F. Qian, M. Arastu, M. Li, S. Chollate, M. S. Brennan, O. Quintero-Monzon, R. H. Scannevin, H. M. Arnold, T. Engber, K. Rhodes, J. Ferrero, Y. Hang, A. Mikulskis, J. Grimm, C. Hock, R. M. Nitsch, and A. Sandrock. 2016. "The antibody aducanumab reduces Abeta plaques in Alzheimer's disease." *Nature* 537 (7618):50-6. doi: 10.1038/nature19323.

Shaftel, S. S., S. Kyrkanides, J. A. Olschowka, J. N. Miller, R. E. Johnson, and M. K. O'Banion. 2007. "Sustained hippocampal IL-1 beta overexpression mediates chronic neuroinflammation and ameliorates Alzheimer plaque pathology." *J Clin Invest* 117 (6):1595-604. doi: 10.1172/jci31450.

Shah, D., E. Jonckers, J. Praet, G. Vanhoutte, Y. Palacios R. Delgado, C. Bigot, D. V. D'Souza, M. Verhoye, and A. Van der Linden. 2013. "Resting state FMRI reveals diminished functional connectivity in a mouse model of amyloidosis." *PLoS One* 8 (12):e84241. doi: 10.1371/journal.pone.0084241.

Shah, D., J. Praet, A. Latif Hernandez, C. Hofling, C. Anckaerts, F. Bard, M. Morawski, J. R. Detrez, E. Prinsen, A. Villa, W. H. De Vos, A. Maggi, R. D'Hooge, D. Balschun, S. Rossner, M. Verhoye, and A. Van der Linden. 2016. "Early pathologic amyloid induces hypersynchrony of BOLD resting-state networks in transgenic mice and provides an early therapeutic window before amyloid plaque deposition." *Alzheimers Dement* 12 (9):964-976. doi: 10.1016/j.jalz.2016.03.010.

Shah, Disha, Amira Latif-Hernandez, Bart De Strooper, Takashi Saito, Takaomi Saido, Marleen Verhoye, Rudi D'Hooge, and Annemie Van der Linden. 2018. "Spatial reversal learning defect coincides with hypersynchronous telencephalic BOLD functional connectivity in APP NL-F/NL-F knock-in mice." *Scientific Reports* 8 (1):6264. doi: doi:10.1038/s41598-018-24657-9.

Shankar, G. M., B. L. Bloodgood, M. Townsend, D. M. Walsh, D. J. Selkoe, and B. L. Sabatini. 2007. "Natural oligomers of the Alzheimer amyloid-beta protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway." *J Neurosci* 27 (11):2866-75. doi: 10.1523/jneurosci.4970-06.2007.

Shankar, G. M., S. Li, T. H. Mehta, A. Garcia-Munoz, N. E. Shepardson, I. Smith, F. M. Brett, M. A. Farrell, M. J. Rowan, C. A. Lemere, C. M. Regan, D. M. Walsh, B. L. Sabatini, and D. J. Selkoe. 2008. "Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory." *Nat Med* 14 (8):837-42. doi: 10.1038/nm1782.

Shariati, S. A., and B. De Strooper. 2013. "Redundancy and divergence in the amyloid precursor protein family." *FEBS Lett* 587 (13):2036-45. doi: 10.1016/j.febslet.2013.05.026.

Shen, Y., H. Wang, Q. Sun, H. Yao, A. P. Keegan, M. Mullan, J. Wilson, S. Lista, T. Leyhe, C. Laske, D. Rujescu, A. Levey, A. Wallin, K. Blennow, R. Li, and H. Hampel. 2018. "Increased Plasma Beta-Secretase 1 May Predict Conversion to Alzheimer's Disease Dementia in Individuals With Mild Cognitive Impairment." *Biol Psychiatry* 83 (5):447-455. doi: 10.1016/j.biopsych.2017.02.007.

Sherrington, R., S. Froelich, S. Sorbi, D. Campion, H. Chi, E. A. Rogaeva, G. Levesque, E. I. Rogaev, C. Lin, Y. Liang, M. Ikeda, L. Mar, A. Brice, Y. Agid, M. E. Percy, F. ClergetDarpoux, S. Piacentini, G. Marcon, B. Nacmias, L. Amaducci, T. Frebourg, L. Lannfelt, J. M. Rommens, and P. H. StGeorgeHyslop. 1996. "Alzheimer's disease associated with mutations in presenilin 2 is rare and variably penetrant." *Human Molecular Genetics* 5 (7):985-988. doi: 10.1093/hmg/5.7.985.

Sherrington, R., E. I. Rogaev, Y. Liang, E. A. Rogaeva, G. Levesque, M. Ikeda, H. Chi, C. Lin, G. Li, K. Holman, T. Tsuda, L. Mar, J. F. Foncin, A. C. Bruni, M. P. Montesi, S. Sorbi, I. Rainero, L. Pinessi, L. Nee, I. Chumakov, D. Pollen, A. Brookes, P. Sanseau, R. J. Polinsky, W. Wasco, H. A. Da Silva, J. L. Haines, M. A. Perkicak-Vance, R. E. Tanzi, A. D. Roses, P. E. Fraser, J. M. Rommens, and P. H. St George-Hyslop. 1995. "Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease." *Nature* 375 (6534):754-60. doi: 10.1038/375754a0.

Shin, I. S., M. Carter, D. Masterman, L. Fairbanks, and J. L. Cummings. 2005. "Neuropsychiatric symptoms and quality of life in Alzheimer disease." *Am J Geriatr Psychiatry* 13 (6):469-74. doi: 10.1176/appi.ajgp.13.6.469.

Siemers, E. R., K. L. Sundell, C. Carlson, M. Case, G. Sethuraman, H. Liu-Seifert, S. A. Dowsett, M. J. Pontecorvo, R. A. Dean, and R. Demattos. 2016. "Phase 3 solanezumab trials: Secondary outcomes in mild Alzheimer's disease patients." *Alzheimers Dement* 12 (2):110-120. doi: 10.1016/j.jalz.2015.06.1893.

Simard, A. R., D. Soulet, G. Gowing, J. P. Julien, and S. Rivest. 2006. "Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease." *Neuron* 49 (4):489-502. doi: 10.1016/j.neuron.2006.01.022.

Sinha, S., J. P. Anderson, R. Barbour, G. S. Basi, R. Caccavello, D. Davis, M. Doan, H. F. Dovey, N. Frigon, J. Hong, K. Jacobson-Croak, N. Jewett, P. Keim, J. Knops, I. Lieberburg, M. Power, H. Tan, G. Tatsuno, J. Tung, D. Schenk, P. Seubert, S. M. Suomensaari, S. Wang, D. Walker, J. Zhao, L. McConlogue, and V. John.

1999. "Purification and cloning of amyloid precursor protein beta-secretase from human brain." *Nature* 402 (6761):537-40. doi: 10.1038/990114.

Sisodia, SS, EH Koo, PN Hoffman, G Perry, and DL Price. 1993. "Identification and transport of full-length amyloid precursor proteins in rat peripheral nervous system." doi: 10.1523/JNEUROSCI.13-07-03136.1993.

Snyder, E. M., Y. Nong, C. G. Almeida, S. Paul, T. Moran, E. Y. Choi, A. C. Nairn, M. W. Salter, P. J. Lombroso, G. K. Gouras, and P. Greengard. 2005. "Regulation of NMDA receptor trafficking by amyloid-beta." *Nat Neurosci* 8 (8):1051-8. doi: 10.1038/nn1503.

Soba, P., S. Eggert, K. Wagner, H. Zentgraf, K. Siehl, S. Kreger, A. Löwer, A. Langer, G. Merdes, R. Paro, C. L. Masters, U. Müller, S. Kins, and K. Beyreuther. 2005. "Homo- and heterodimerization of APP family members promotes intercellular adhesion." In *EMBO J*, 3624-34.

Solomon, B., R. Koppel, E. Hanan, and T. Katzav. 1996. "Monoclonal antibodies inhibit in vitro fibrillar aggregation of the Alzheimer beta-amyloid peptide." *Proc Natl Acad Sci U S A* 93 (1):452-5. doi: 10.1073/pnas.93.1.452.

Song, D. K., M. H. Won, J. S. Jung, J. C. Lee, T. C. Kang, H. W. Suh, S. O. Huh, S. H. Paek, Y. H. Kim, S. H. Kim, and Y. H. Suh. 1998. "Behavioral and neuropathologic changes induced by central injection of carboxyl-terminal fragment of beta-amyloid precursor protein in mice." *J Neurochem* 71 (2):875-8. doi: 10.1046/j.1471-4159.1998.71020875.x.

Sperling, R. A., P. S. Aisen, L. A. Beckett, D. A. Bennett, S. Craft, A. M. Fagan, T. Iwatsubo, C. R. Jack, Jr., J. Kaye, T. J. Montine, D. C. Park, E. M. Reiman, C. C. Rowe, E. Siemers, Y. Stern, K. Yaffe, M. C. Carrillo, B. Thies, M. Morrison-Bogorad, M. V. Wagster, and C. H. Phelps. 2011. "Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease." *Alzheimers Dement* 7 (3):280-92. doi: 10.1016/j.jalz.2011.03.003.

Sperling, R., S. Salloway, D. J. Brooks, D. Tampieri, J. Barakos, N. C. Fox, M. Raskind, M. Sabbagh, L. S. Honig, A. P. Porsteinsson, I. Lieberburg, H. M. Arrighi, K. A. Morris, Y. Lu, E. Liu, K. M. Gregg, H. R. Brashear, G. G. Kinney, R. Black, and M. Grundman. 2012. "Amyloid-related imaging abnormalities in patients with Alzheimer's disease treated with bapineuzumab: a retrospective analysis." *Lancet Neurol* 11 (3):241-9. doi: 10.1016/s1474-4422(12)70015-7.

Spires-Jones, T. L., M. L. Mielke, A. Rozkalne, M. Meyer-Luehmann, A. de Calignon, B. J. Bacskai, D. Schenk, and B. T. Hyman. 2009. "Passive immunotherapy rapidly increases structural plasticity in a mouse model of Alzheimer disease." *Neurobiol Dis* 33 (2):213-20. doi: 10.1016/j.nbd.2008.10.011.

Srivareerat, M., T. T. Tran, K. H. Alzoubi, and K. A. Alkadhi. 2009. "Chronic psychosocial stress exacerbates impairment of cognition and long-term potentiation in beta-amyloid rat model of Alzheimer's disease." *Biol Psychiatry* 65 (11):918-26. doi: 10.1016/j.biopsych.2008.08.021.

Steinbach, J. P., U. Muller, M. Leist, Z. W. Li, P. Nicotera, and A. Aguzzi. 1998. "Hypersensitivity to seizures in beta-amyloid precursor protein deficient mice." *Cell Death Differ* 5 (10):858-66. doi: 10.1038/sj.cdd.4400391.

Svenningsson, A. L., E. Stomrud, P. S. Insel, N. Mattsson, S. Palmqvist, and O. Hansson. 2019. "beta-amyloid pathology and hippocampal atrophy are independently associated with memory function in cognitively healthy elderly." *Sci Rep* 9 (1):11180. doi: 10.1038/s41598-019-47638-y.

Swerdlow, R. H., and S. M. Khan. 2004. "A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease." *Med Hypotheses* 63 (1):8-20. doi: 10.1016/j.mehy.2003.12.045.

Tang, J. X., D. Baranov, M. Hammond, L. M. Shaw, M. F. Eckenhoff, and R. G. Eckenhoff. 2011. "Human Alzheimer and inflammation biomarkers after anesthesia and surgery." *Anesthesiology* 115 (4):727-32. doi: 10.1097/ALN.0b013e31822e9306.

Tariot, P. N., M. R. Farlow, G. T. Grossberg, S. M. Graham, S. McDonald, and I. Gergel. 2004. "Memantine treatment in patients with moderate to severe Alzheimer disease already receiving donepezil: a randomized controlled trial." *Jama* 291 (3):317-24. doi: 10.1001/jama.291.3.317.

Taylor, C. J., D. R. Ireland, I. Ballagh, K. Bourne, N. M. Marechal, P. R. Turner, D. K. Bilkey, W. P. Tate, and W. C. Abraham. 2008. "Endogenous secreted amyloid precursor protein-alpha regulates hippocampal NMDA receptor function, long-term potentiation and spatial memory." *Neurobiol Dis* 31 (2):250-60. doi: 10.1016/j.nbd.2008.04.011.

Teich, AF, M Patel, and O Arancio. 2013. "A Reliable Way to Detect Endogenous Murine β-Amyloid." *Plos One* 8 (2). doi: 10.1371/journal.pone.0055647.

Tharp, W. G., and I. N. Sarkar. 2013. "Origins of amyloid-beta." *BMC Genomics* 14:290. doi: 10.1186/1471-2164-14-290.

Theunis, C., N. Crespo-Biel, V. Gafner, M. Pihlgren, M. P. Lopez-Deber, P. Reis, D. T. Hickman, O. Adolfsson, N. Chuard, D. M. Ndao, P. Borghgraef, H. Devijver, F. Van Leuven, A. Pfeifer, and A. Muhs. 2013. "Efficacy and safety of a liposome-based vaccine against protein Tau, assessed in tau.P301L mice that model tauopathy." *PLoS One* 8 (8):e72301. doi: 10.1371/journal.pone.0072301.

Thomas, R. S., A. Henson, A. Gerrish, L. Jones, J. Williams, and E. J. Kidd. 2016. "Decreasing the expression of PICALM reduces endocytosis and the activity of beta-secretase: implications for Alzheimer's disease." *BMC Neurosci* 17 (1):50. doi: 10.1186/s12868-016-0288-1.

Thomas, R. S., M. Hvoslef-Eide, M. A. Good, and E. J. Kidd. 2013. "Inhibition of amyloid-beta production by anti-amyloid precursor protein antibodies in primary mouse cortical neurones." *Neuroreport* 24 (18):1058-1061. doi: 10.1097/wnr.0000000000000055.

Thomas, R. S., J. E. Liddell, and E. J. Kidd. 2011. "Anti-amyloid precursor protein immunoglobulins inhibit amyloid-beta production by steric hindrance." *Febs j* 278 (1):167-78. doi: 10.1111/j.1742-4658.2010.07942.x.

Thomas, R. S., J. E. Liddell, L. S. Murphy, D. M. Pache, and E. J. Kidd. 2006. "An antibody to the beta-secretase cleavage site on amyloid-beta-protein precursor inhibits amyloid-beta production." *Journal of Alzheimers Disease* 10 (4):379-390.

Thomsen, M. S., H. H. Hansen, D. B. Timmerman, and J. D. Mikkelsen. 2010. "Cognitive improvement by activation of alpha7 nicotinic acetylcholine receptors: from animal models to human pathophysiology." *Curr Pharm Des* 16 (3):323-43. doi: 10.2174/138161210790170094.

Tillement, L., L. Lecanu, W. Yao, J. Greeson, and V. Papadopoulos. 2006. "The spirostenol (22R, 25R)-20alpha-spirost-5-en-3beta-yl hexanoate blocks mitochondrial uptake of Abeta in neuronal cells and prevents Abeta-induced impairment of mitochondrial function." *Steroids* 71 (8):725-35. doi: 10.1016/j.steroids.2006.05.003.

Tong, M., K. Arora, M. M. White, and R. A. Nichols. 2011. "Role of key aromatic residues in the ligand-binding domain of alpha7 nicotinic receptors in the agonist

action of beta-amyloid." *J Biol Chem* 286 (39):34373-81. doi: 10.1074/jbc.M111.241299.

Townsend, M., G. M. Shankar, T. Mehta, D. M. Walsh, and D. J. Selkoe. 2006. "Effects of secreted oligomers of amyloid beta-protein on hippocampal synaptic plasticity: a potent role for trimers." *J Physiol* 572 (Pt 2):477-92. doi: 10.1113/jphysiol.2005.103754.

Trinchese, F., M. Fa', S. Liu, H. Zhang, A. Hidalgo, S. D. Schmidt, H. Yamaguchi, N. Yoshii, P. M. Mathews, R. A. Nixon, and O. Arancio. 2008. "Inhibition of calpains improves memory and synaptic transmission in a mouse model of Alzheimer disease." *J Clin Invest* 118 (8):2796-807. doi: 10.1172/jci34254.

Tsao, A., M. B. Moser, and E. I. Moser. 2013. "Traces of experience in the lateral entorhinal cortex." *Curr Biol* 23 (5):399-405. doi: 10.1016/j.cub.2013.01.036.

Turner, P. R., K. O'Connor, W. P. Tate, and W. C. Abraham. 2003. "Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory." *Progress in Neurobiology* 70 (1):1-32. doi: 10.1016/s0301-0082(03)00089-3.

Tyan, S. H., A. Y. Shih, J. J. Walsh, H. Maruyama, F. Sarsoza, L. Ku, S. Eggert, P. R. Hof, E. H. Koo, and D. L. Dickstein. 2012. "Amyloid precursor protein (APP) regulates synaptic structure and function." *Mol Cell Neurosci* 51 (1-2):43-52. doi: 10.1016/j.mcn.2012.07.009.

Van Broeckhoven, C., J. Haan, E. Bakker, J. A. Hardy, W. Van Hul, A. Wehnert, M. Vegter-Van der Vlis, and R. A. Roos. 1990. "Amyloid beta protein precursor gene and hereditary cerebral hemorrhage with amyloidosis (Dutch)." *Science* 248 (4959):1120-2. doi: 10.1126/science.1971458.

van Dyck, C. H., C. Sadowsky, G. Le Prince Leterme, K. Booth, Y. Peng, K. Marek, N. Ketter, E. Liu, B. T. Wyman, N. Jackson, M. Slomkowski, and J. M. Ryan. 2016. "Vanutide Cridificar (ACC-001) and QS-21 Adjuvant in Individuals with Early Alzheimer's Disease: Amyloid Imaging Positron Emission Tomography and Safety Results from a Phase 2 Study." *J Prev Alzheimers Dis* 3 (2):75-84. doi: 10.14283/jpad.2016.91.

Vandenberghe, R., J. O. Rinne, M. Boada, S. Katayama, P. Scheltens, B. Vellas, M. Tuchman, A. Gass, J. B. Fiebach, D. Hill, K. Lobello, D. Li, T. McRae, P. Lucas, I. Evans, K. Booth, G. Luscan, B. T. Wyman, L. Hua, L. Yang, H. R. Brashear, and R. S. Black. 2016. "Bapineuzumab for mild to moderate Alzheimer's disease in two global, randomized, phase 3 trials." *Alzheimers Res Ther* 8 (1):18. doi: 10.1186/s13195-016-0189-7.

Vandenberghe, R., M. E. Riviere, A. Caputo, J. Sovago, R. P. Maguire, M. Farlow, G. Marotta, R. Sanchez-Valle, P. Scheltens, J. M. Ryan, and A. Graf. 2017. "Active Aβ immunotherapy CAD106 in Alzheimer's disease: A phase 2b study." In *Alzheimers Dement (N Y)*, 10-22.

Vassar, R. 2014. "BACE1 inhibitor drugs in clinical trials for Alzheimer's disease." *Alzheimers Res Ther* 6 (89). doi: 10.1186/s13195-014-0089-7.

Vassar, R., B. D. Bennett, S. Babu-Khan, S. Kahn, E. A. Mendiaz, P. Denis, D. B. Teplow, S. Ross, P. Amarante, R. Loeloff, Y. Luo, S. Fisher, L. Fuller, S. Edenson, J. Lile, M. A. Jarosinski, A. L. Biere, E. Curran, T. Burgess, J. C. Louis, F. Collins, J. Treanor, G. Rogers, and M. Citron. 1999. "beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE." *Science* 286 (5440):735-741. doi: 10.1126/science.286.5440.735.

Vellas, B., R. Black, L. J. Thal, N. C. Fox, M. Daniels, G. McLennan, C. Tompkins, C. Leibman, M. Pomfret, and M. Grundman. 2009. "Long-term follow-up of patients

immunized with AN1792: reduced functional decline in antibody responders." *Curr Alzheimer Res* 6 (2):144-51.
Verret, L., E. O. Mann, G. B. Hang, A. M. Barth, I. Cobos, K. Ho, N. Devidze, E. Masliah, A. C. Kreitzer, I. Mody, L. Mucke, and J. J. Palop. 2012. "Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model." *Cell* 149 (3):708-21. doi: 10.1016/j.cell.2012.02.046.
Vlček, K., and J. Laczó. 2014. "Neural Correlates of Spatial Navigation Changes in Mild Cognitive Impairment and Alzheimer's Disease." *Front Behav Neurosci* 8. doi: 10.3389/fnbeh.2014.00089.
von Koch, C. S., H. Zheng, H. Chen, M. Trumbauer, G. Thinakaran, L. H. van der Ploeg, D. L. Price, and S. S. Sisodia. 1997. "Generation of APLP2 KO mice and early postnatal lethality in APLP2/APP double KO mice." *Neurobiol Aging* 18 (6):661-9.
Vorhees, Charles V., and Michael T. Williams. 2014. "Assessing Spatial Learning and Memory in Rodents." *ILAR Journal* 55 (2):310-332. doi: 10.1093/ilar/ilu013.
Wagner, S. L., K. D. Rynearson, S. K. Duddy, C. Zhang, P. D. Nguyen, A. Becker, U. Vo, D. Masliah, L. Monte, J. B. Klee, C. M. Echmalian, W. Xia, L. Quinti, G. Johnson, J. H. Lin, D. Y. Kim, W. C. Mobley, R. A. Rissman, and R. E. Tanzi. 2017. "Pharmacological and Toxicological Properties of the Potent Oral gamma-Secretase Modulator BPN-15606." *J Pharmacol Exp Ther* 362 (1):31-44. doi: 10.1124/jpet.117.240861.
Walsh, D. M., and D. J. Selkoe. 2007. "A beta oligomers - a decade of discovery." *J Neurochem* 101 (5):1172-84. doi: 10.1111/j.1471-4159.2006.04426.x.
Walsh, Dominic M., Igor Klyubin, Julia V. Fadeeva, William K. Cullen, Roger Anwyl, Michael S. Wolfe, Michael J. Rowan, and Dennis J. Selkoe. 2002. "Naturally secreted oligomers of amyloid |[beta]| protein potently inhibit hippocampal long-term potentiation in vivo." *Nature* 416 (6880):535-539. doi: doi:10.1038/416535a.
Wang, H., A. Megill, K. He, A. Kirkwood, and H. K. Lee. 2012. "Consequences of inhibiting amyloid precursor protein processing enzymes on synaptic function and plasticity." *Neural Plast* 2012:272374. doi: 10.1155/2012/272374.
Wang, H. Y., D. H. Lee, M. R. D'Andrea, P. A. Peterson, R. P. Shank, and A. B. Reitz. 2000. "beta-Amyloid(1-42) binds to alpha7 nicotinic acetylcholine receptor with high affinity. Implications for Alzheimer's disease pathology." *J Biol Chem* 275 (8):5626-32.
Wang, Z., B. Wang, L. Yang, Q. Guo, N. Aithmitti, Z. Songyang, and H. Zheng. 2009. "Presynaptic and postsynaptic interaction of the amyloid precursor protein promotes peripheral and central synaptogenesis." *J Neurosci* 29 (35):10788-801. doi: 10.1523/jneurosci.2132-09.2009.
Warburton, E., and M. Brown. 2015. "Neural circuitry for rat recognition memory." In *Behav Brain Res*, 131-9.
Warburton, E. C., G. R. Barker, and M. W. Brown. 2013. "Investigations into the involvement of NMDA mechanisms in recognition memory." *Neuropharmacology* 74:41-7. doi: 10.1016/j.neuropharm.2013.04.013.
Warburton, E. C., and M. W. Brown. 2010. "Findings from animals concerning when interactions between perirhinal cortex, hippocampus and medial prefrontal cortex are necessary for recognition memory." *Neuropsychologia* 48 (8):2262-72. doi: 10.1016/j.neuropsychologia.2009.12.022.
Webster, S. J., A. D. Bachstetter, P. T. Nelson, F. A. Schmitt, and L. J. Van Eldik. 2014. "Using mice to model Alzheimer's dementia: an overview of the clinical disease

and the preclinical behavioral changes in 10 mouse models." *Front Genet* 5:88. doi: 10.3389/fgene.2014.00088.

Webster, S. J., A. D. Bachstetter, and L. J. Van Eldik. 2013. "Comprehensive behavioral characterization of an APP/PS-1 double knock-in mouse model of Alzheimer's disease." *Alzheimers Res Ther* 5 (3):28. doi: 10.1186/alzrt182.

West, T., Y. Hu, P. B. Verghese, R. J. Bateman, J. B. Braunstein, I. Fogelman, K. Budur, H. Florian, N. Mendonca, and D. M. Holtzman. 2017. "Preclinical and Clinical Development of ABBV-8E12, a Humanized Anti-Tau Antibody, for Treatment of Alzheimer's Disease and Other Tauopathies." *J Prev Alzheimers Dis* 4 (4):236-241. doi: 10.14283/jpad.2017.36.

Westerman, M. A., D. Cooper-Blacketer, A. Mariash, L. Kotilinek, T. Kawarabayashi, L. H. Younkin, G. A. Carlson, S. G. Younkin, and K. H. Ashe. 2002. "The relationship between Abeta and memory in the Tg2576 mouse model of Alzheimer's disease." *J Neurosci* 22 (5):1858-67.

Weyer, S. W., M. Zagrebelsky, U. Herrmann, M. Hick, L. Ganss, J. Gobbert, M. Gruber, C. Altmann, M. Korte, T. Deller, and U. C. Muller. 2014. "Comparative analysis of single and combined APP/APLP knockouts reveals reduced spine density in APP-KO mice that is prevented by APPsalpha expression." *Acta Neuropathol Commun* 2:36. doi: 10.1186/2051-5960-2-36.

Whitson, J. S., D. J. Selkoe, and C. W. Cotman. 1989. "Amyloid beta protein enhances the survival of hippocampal neurons in vitro." *Science* 243 (4897):1488-90. doi: 10.1126/science.2928783.

Whyte, L. S., K. M. Hemsley, A. A. Lau, S. Hassiotis, T. Saito, T. C. Saido, J. J. Hopwood, and T. J. Sargeant. 2018. "Reduction in open field activity in the absence of memory deficits in the App(NL-G-F) knock-in mouse model of Alzheimer's disease." *Behav Brain Res* 336:177-181. doi: 10.1016/j.bbr.2017.09.006.

Wiessner, C., K. H. Wiederhold, A. C. Tissot, P. Frey, S. Danner, L. H. Jacobson, G. T. Jennings, R. Lüönd, R. Ortmann, J. Reichwald, M. Zurini, A. Mir, M. F. Bachmann, and M. Staufenbiel. 2011. "The Second-Generation Active Aβ Immunotherapy CAD106 Reduces Amyloid Accumulation in APP Transgenic Mice While Minimizing Potential Side Effects." In *J Neurosci*, 9323-31.

Willem, M., A. N. Garratt, B. Novak, M. Citron, S. Kaufmann, A. Rittger, B. DeStrooper, P. Saftig, C. Birchmeier, and C. Haass. 2006. "Control of peripheral nerve myelination by the beta-secretase BACE1." *Science* 314 (5799):664-6. doi: 10.1126/science.1132341.

Willem, M., S. Tahirovic, M. A. Busche, S. V. Ovsepian, M. Chafai, S. Kootar, D. Hornburg, L. D. Evans, S. Moore, A. Daria, H. Hampel, V. Muller, C. Giudici, B. Nuscher, A. Wenninger-Weinzierl, E. Kremmer, M. T. Heneka, D. R. Thal, V. Giedraitis, L. Lannfelt, U. Muller, F. J. Livesey, F. Meissner, J. Herms, A. Konnerth, H. Marie, and C. Haass. 2015. "eta-Secretase processing of APP inhibits neuronal activity in the hippocampus." *Nature* 526 (7573):443-7. doi: 10.1038/nature14864.

Wills, T. J., F. Cacucci, N. Burgess, and J. O'Keefe. 2010. "Development of the hippocampal cognitive map in preweanling rats." *Science* 328 (5985):1573-6. doi: 10.1126/science.1188224.

Wilson, D. I., S. Watanabe, H. Milner, and J. A. Ainge. 2013. "Lateral entorhinal cortex is necessary for associative but not nonassociative recognition memory." *Hippocampus* 23 (12):1280-90. doi: 10.1002/hipo.22165.

Winblad, B., N. Andreasen, L. Minthon, A. Floesser, G. Imbert, T. Dumortier, R. P. Maguire, K. Blennow, J. Lundmark, M. Staufenbiel, J. M. Orgogozo, and A. Graf.

2012. "Safety, tolerability, and antibody response of active Abeta immunotherapy with CAD106 in patients with Alzheimer's disease: randomised, double-blind, placebo-controlled, first-in-human study." *Lancet Neurol* 11 (7):597-604. doi: 10.1016/s1474-4422(12)70140-0.

Winters, B. D., S. E. Forwood, R. A. Cowell, L. M. Saksida, and T. J. Bussey. 2004. "Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe." *J Neurosci* 24 (26):5901-8. doi: 10.1523/jneurosci.1346-04.2004.

Wisniewski, T., and F. Goñi. 2015. "Immunotherapeutic Approaches for Alzheimer's Disease." *Neuron* 85 (6):1162-76. doi: 10.1016/j.neuron.2014.12.064.

Witter, M. P., T. P. Doan, B. Jacobsen, E. S. Nilssen, and S. Ohara. 2017. "Architecture of the Entorhinal Cortex A Review of Entorhinal Anatomy in Rodents with Some Comparative Notes." *Front Syst Neurosci* 11. doi: 10.3389/fnsys.2017.00046.

Wolfe, M. S., W. Xia, B. L. Ostaszewski, T. S. Diehl, W. T. Kimberly, and D. J. Selkoe. 1999. "Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity." *Nature* 398 (6727):513-7. doi: 10.1038/19077.

Wong, H. K., T. Sakurai, F. Oyama, K. Kaneko, K. Wada, H. Miyazaki, M. Kurosawa, B. De Strooper, P. Saftig, and N. Nukina. 2005. "beta subunits of voltage-gated sodium channels are novel substrates of beta-site amyloid precursor protein-cleaving enzyme (BACE1) and gamma-secretase." *Journal of Biological Chemistry* 280 (24):23009-23017. doi: 10.1074/jbc.M414648200.

Xie, Z., D. J. Culley, Y. Dong, G. Zhang, B. Zhang, R. D. Moir, M. P. Frosch, G. Crosby, and R. E. Tanzi. 2008. "The common inhalation anesthetic isoflurane induces caspase activation and increases amyloid beta-protein level in vivo." *Ann Neurol* 64 (6):618-27. doi: 10.1002/ana.21548.

Xie, Z., and Z. Xu. 2013. "General anesthetics and beta-amyloid protein." *Prog Neuropsychopharmacol Biol Psychiatry* 47:140-146. doi: 10.1016/j.pnpbp.2012.08.002.

Xiong, M., O. D. Jones, K. Peppercorn, S. M. Ohline, W. P. Tate, and W. C. Abraham. 2017. "Secreted amyloid precursor protein-alpha can restore novel object location memory and hippocampal LTP in aged rats." *Neurobiol Learn Mem* 138:291-299. doi: 10.1016/j.nlm.2016.08.002.

Xu, G., Y. Ran, S. E. Fromholt, C. Fu, A. T. Yachnis, T. E. Golde, and D. R. Borchelt. 2015. "Murine Aβ over-production produces diffuse and compact Alzheimer-type amyloid deposits." *Acta Neuropathol Commun* 3 (72). doi: 10.1186/s40478-015-0252-9.

Xu, W., S. Fitzgerald, R. A. Nixon, E. Levy, and D. A. Wilson. 2015. "Early hyperactivity in lateral entorhinal cortex is associated with elevated levels of AβPP metabolites in the Tg2576 mouse model of Alzheimer's disease." *Exp Neurol* 264:82-91. doi: 10.1016/j.expneurol.2014.12.008.

Yan, R. Q., M. J. Bienkowski, M. E. Shuck, H. Y. Miao, M. C. Tory, A. M. Pauley, J. R. Brashler, N. C. Stratman, W. R. Mathews, A. E. Buhl, D. B. Carter, A. G. Tomasselli, L. A. Parodi, R. L. Heinrikson, and M. E. Gurney. 1999. "Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity." *Nature* 402 (6761):533-537. doi: 10.1038/990107.

Yan, R., and R. Vassar. 2014. "Targeting the beta secretase BACE1 for Alzheimer's disease therapy." *Lancet Neurol* 13 (3):319-29. doi: 10.1016/s1474-4422(13)70276-x.

Yang, X, C Yao, T Tian, Li X, H Yan, Wu J, Li H, Pei L, Liu D, Tian Q, Zhu L-Q, and Y Lu. 2018. "A novel mechanism of memory loss in Alzheimer's disease mice via the degeneration of entorhinal–CA1 synapses." *Molecular Psychiatry* 23 (2):199-210. doi: doi:10.1038/mp.2016.151.

Yankner, B. A., L. K. Duffy, and D. A. Kirschner. 1990. "Neurotrophic and neurotoxic effects of amyloid beta protein: reversal by tachykinin neuropeptides." *Science* 250 (4978):279-82. doi: 10.1126/science.2218531.

Yeo, J. M., B. Waddell, Z. Khan, and S. Pal. 2015. "A systematic review and meta-analysis of (18)F-labeled amyloid imaging in Alzheimer's disease." *Alzheimers Dement (Amst)* 1 (1):5-13. doi: 10.1016/j.dadm.2014.11.004.

Yeung, L. K., R. K. Olsen, B. Hong, V. Mihajlovic, M. C. D'Angelo, A. Kacollja, J. D. Ryan, and M. D. Barense. 2019. "Object-in-place Memory Predicted by Anterolateral Entorhinal Cortex and Parahippocampal Cortex Volume in Older Adults." *J Cogn Neurosci* 31 (5):711-729. doi: 10.1162/jocn_a_01385.

Zetterberg, H., K. Tullhog, O. Hansson, L. Minthon, E. Londos, and K. Blennow. 2010. "Low incidence of post-lumbar puncture headache in 1,089 consecutive memory clinic patients." *Eur Neurol* 63 (6):326-30. doi: 10.1159/000311703.

Zhang, B., M. Tian, H. Zheng, Y. Zhen, Y. Yue, T. Li, S. Li, E. R. Marcantonio, and Z. Xie. 2013. "Effects of anesthetic isoflurane and desflurane on human cerebrospinal fluid Abeta and tau level." *Anesthesiology* 119 (1):52-60. doi: 10.1097/ALN.0b013e31828ce55d.

Zhang, H., S. Sun, L. Wu, E. Pchitskaya, O. Zakharova, K. Fon Tacer, and I. Bezprozvanny. 2016. "Store-Operated Calcium Channel Complex in Postsynaptic Spines: A New Therapeutic Target for Alzheimer's Disease Treatment." *J Neurosci* 36 (47):11837-11850. doi: 10.1523/jneurosci.1188-16.2016.

Zhang, H., L. Wu, E. Pchitskaya, O. Zakharova, T. Saito, T. Saido, and I. Bezprozvanny. 2015. "Neuronal Store-Operated Calcium Entry and Mushroom Spine Loss in Amyloid Precursor Protein Knock-In Mouse Model of Alzheimer's Disease." *J Neurosci* 35 (39):13275-86. doi: 10.1523/jneurosci.1034-15.2015.

Zhang, Y., and W. M. Pardridge. 2001. "Mediated efflux of IgG molecules from brain to blood across the blood-brain barrier." *J Neuroimmunol* 114 (1-2):168-72.

Zhang, Y. W., R. Thompson, H. Zhang, and H. Xu. 2011. "APP processing in Alzheimer's disease." *Mol Brain* 4:3. doi: 10.1186/1756-6606-4-3.

Zhang, Z., M. Song, X. Liu, S. Su Kang, D. M. Duong, N. T. Seyfried, X. Cao, L. Cheng, Y. E. Sun, S. Ping Yu, J. Jia, A. I. Levey, and K. Ye. 2015. "Delta-secretase cleaves amyloid precursor protein and regulates the pathogenesis in Alzheimer's disease." *Nat Commun* 6:8762. doi: 10.1038/ncomms9762.

Zhao, G., J. Tan, G. Mao, M. Z. Cui, and X. Xu. 2007. "The same gamma-secretase accounts for the multiple intramembrane cleavages of APP." *J Neurochem* 100 (5):1234-46. doi: 10.1111/j.1471-4159.2006.04302.x.

Zhao, J., Y. Fu, M. Yasvoina, P. Shao, B. Hitt, T. O'Connor, S. Logan, E. Maus, M. Citron, R. Berry, L. Binder, and R. Vassar. 2007. "Beta-site amyloid precursor protein cleaving enzyme 1 levels become elevated in neurons around amyloid plaques: implications for Alzheimer's disease pathogenesis." *J Neurosci* 27 (14):3639-49. doi: 10.1523/jneurosci.4396-06.2007.

Zhao, J., L. Paganini, L. Mucke, M. Gordon, L. Refolo, M. Carman, S. Sinha, T. Oltersdorf, I. Lieberburg, and L. McConlogue. 1996. "Beta-secretase processing of the beta-amyloid precursor protein in transgenic mice is efficient in neurons but inefficient in astrocytes." *J Biol Chem* 271 (49):31407-11.

Zhao, R., S. W. Fowler, A. C. A. Chiang, D. Ji, and J. L. Jankowsky. 2014. "Impairments in experience-dependent scaling and stability of hippocampal place fields limit

spatial learning in a mouse model of Alzheimer's disease." *Hippocampus* 24 (8):963-78. doi: 10.1002/hipo.22283.

Zheng, H, and E.H. Koo. 2011. "Biology and pathophysiology of the amyloid precursor protein." *Molecular Neurodegeneration* 6 (1):27. doi: doi:10.1186/1750-1326-6-27.

Zheng, H., M. Jiang, M. E. Trumbauer, D. J. Sirinathsinghji, R. Hopkins, D. W. Smith, R. P. Heavens, G. R. Dawson, S. Boyce, M. W. Conner, K. A. Stevens, H. H. Slunt, S. S. Sisoda, H. Y. Chen, and L. H. Van der Ploeg. 1995. "beta-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity." *Cell* 81 (4):525-31.

Zhu, K., F. Peters, S. Filser, and J. Herms. 2018. "Consequences of Pharmacological BACE Inhibition on Synaptic Structure and Function." *Biol Psychiatry* 84 (7):478-487. doi: 10.1016/j.biopsych.2018.04.022.

Zhuo, J. M., A. Prakasam, M. E. Murray, H. Y. Zhang, M. G. Baxter, K. Sambamurti, and M. M. Nicolle. 2008. "An increase in Abeta42 in the prefrontal cortex is associated with a reversal-learning impairment in Alzheimer's disease model Tg2576 APPsw mice." *Curr Alzheimer Res* 5 (4):385-91.

Zou, C., S. Crux, S. Marinesco, E. Montagna, C. Sgobio, Y. Shi, S. Shi, K. Zhu, M. M. Dorostkar, U. C. Muller, and J. Herms. 2016. "Amyloid precursor protein maintains constitutive and adaptive plasticity of dendritic spines in adult brain by regulating D-serine homeostasis." *Embo j* 35 (20):2213-2222. doi: 10.15252/embj.201694085.