Sex-specific Role of Sigma-1 Receptor in Alzheimer's Disease

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Thesis submitted to the University of Ottawa
in partial Fulfillment of the requirements for the
Master of Science degree in Neuroscience

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ABSTRACT

Alzheimer's disease (AD) is the leading cause of dementia in the elderly and affects a growing number of people every year, which are mostly women. Despite years of research, there is no cure and no treatment effectively alter disease progression. A failure to examine sex differences in AD research may be partially to blame. Thus, novel therapeutic interventions are greatly needed that account for potential sex differences in disease pathophysiology. One promising target for prevention and treatment of AD is the sigma 1 receptor (Sig1R), a protein regulated by sex hormones, synapse function, and cognition. Activation and overexpression of Sig1R is known to be anti-amnesic and neuroprotective, whereas loss or dysfunction in Sig1R is associated with neurodegeneration. Interestingly, brains from early AD patients exhibit a lower density of Sig1R and Sig1R are decreased in post-mortem AD hippocampus. Therefore, Sig1R reduction could lead to a hostile environment and contribute to AD progression. Here, we used two mouse model of AD, $A\beta_{25-35}$ infusion model and 3xTg-AD model, to investigate how Sig1R sex-specifically modulates AD pathology. We first looked at how Sig1R protein levels are altered by sex and AD pathology. We found that the triple transgenic mutation significantly alters Sig1R expression. Next, we examined how synapses are modified by sex and AD pathology and if changes can be rescued by Sig1R activation. Interestingly, we found differences in synaptic plasticity between the sexes. Furthermore, we observed that male and female mice respond differently to Sig1R activation. Our results also revealed a sex-specific baseline difference in surface GluA2 levels. Given that synapse loss is a morphological reflection of synaptic dysfunction, we finally studied the structural integrity of the hippocampus. We found that $A\beta_{25-35}$ infusion and triple transgenic mutation reduce hippocampal volumetry, with no apparent sex differences. Taken together, our findings support the idea that synaptic dysfunction is different between males and females.

RÉSUMÉ

La maladie d'Alzheimer (MA) est la principale cause de démence chez les personnes âgées et touche un nombre croissant de personnes chaque année, qui sont majoritairement des femmes. Malgré des années de recherche, aucun traitement ne peut guérir cette maladie. Le manque d'exploration des différences entre les sexes dans la recherche sur la MA peut être partiellement à blâmer. De nouvelles interventions thérapeutiques qui prennent en compte les différences entre les hommes et les femmes dans cette pathophysiologie sont désespérément nécessaires. Une cible prometteuse pour la prévention et le traitement de la MA est le récepteur sigma 1 (Sig1R), une protéine régulée par les hormones sexuelles, le fonctionnement des synapses et la cognition. L'activation et la surexpression de Sig1R sont connues pour être anti-amnésiques et neuroprotectrices, alors que la perte ou le dysfonctionnement de Sig1R est associé à la neurodégénérescence. Il est intéressant de noter que les cerveaux des patients atteints de MA ont une densité plus faible de Sig1R et que les niveaux de Sig1R sont diminués dans l'hippocampe des patients post-mortem. Par conséquent, il est probable que la réduction de Sig1R mène à un environnement hostile et contribue donc à la progression de la MA. Dans cette étude, nous avons utilisé deux modèles de souris qui récapitule la pathologie de la MA, un modèle d'infusion d'$A\beta_{25-35}$ et un modèle 3xTg-AD. Nous avons étudié comment Sig1R module spécifiquement la pathologie de la MA entre les sexes. Tout d'abord, nous avons examiné comment les niveaux de Sig1R sont modifiés par le sexe et la pathologie de la MA. Nous avons démontré que la mutation triple transgénique modifie de manière significative l'expression de Sig1R. Ensuite, nous avons examiné comment les synapses sont modifiées par le sexe et la pathologie de la MA et si les changements peuvent être sauvés par l'activation de Sig1R. Nous avons trouvé des différences de plasticité synaptique entre les sexes. En outre, nous avons observé que les souris mâles et femelles répondent différemment à l'activation de Sig1R. Nos résultats ont également révélé une différence de base spécifique au sexe dans les niveaux de GluA2 de surface. Étant donné que la perte de synapse est un reflet morphologique du dysfonctionnement synaptique, nous avons finalement étudié l'intégrité structurelle de l'hippocampe. Nous avons constaté que la perfusion d'$A\beta_{25-35}$ et la triple mutation transgénique réduisent la volumétrie de l'hippocampe, sans différence apparente entre les sexes. En résumé, nos résultats mettent en évidence l'idée que le dysfonctionnement synaptique est différent entre les sexes.

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Aβ	Amyloid Beta
ABP	AMPAR-binding protein
ACSF	Artifical cerebrospinal fluid
AD	Alzheimer's disease
ADNI	Alzheimer's Disease Neuroimaging Initiative
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxasolepropionic acid receptor
APH-1	Anterior pharynx defective 1
APoE	Apolipoprotein E
APP	Amyloid precursor protein
BACE1	β-secretase 1
BDNF	Brain-derived neurotrophic factor
BiP	Binding immoglobulin protein
CA	*Cornu Ammonis*
CaM	Calmodulin
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
CBP	CREB-binding Protein
Cdk5	Cyclin-dependent kinase 5
CNS	Central nervous system
CSF	Cerebrospinal Fluid
CTD	C-terminal domain
CTFα	C-terminal fragment α
C83	83-amino acid C-terminal fragment
C99	99-amino acid C-terminal fragment
DHEA	Dehydroepiandrostrerone
DHPG	Dihydroxyphenylglycine
eIF2α	Eukaryotic Initiation Factor 2
EOAD	Early-onset Alzheimer's Disease
ER	Endoplasmic Reticulum
ERα	Estrogen Receptor α
E2	17β-estradiol
FAD	Familial Alzheimer's Disease
fEPSP	Field Excitatory Postsynaptic Potential
GEF	Guanine-nucleotide exchange factor
GRIP	Glutamate receptor interacting protein
GSK3	Glycogen Synthase Kinase 3
HPC	Hippocalcin
i.c.v.	Intracerebroventricular
i.p.	Intraperitoneal
LFS	Low Frequency Stimulation
LOAD	Late-onset Alzheimer's disease
LTD	Long-term depression
LTP	Long-term potentiation
MAM	Mitochondrion-associated ER membrane
MAP	Microtubule-associated protein

MAPK	Mitogen-activated protein kinase
MCI	Mild Cognitive Impairment
mGluR	Metabotropic Glutamate Receptor
mGluR-LTD	mGluR-dependent LTD
MWM	Morris Water Maze
MR	Magnetic resonance
MRI	Magnetic resonance imaging
N	N-terminal domain
NFTs	Neurofibrillary tangles
NMDAR	N-methyl-D-aspartate receptor
NMDAR-LTD	NMDAR-dependent LTD
NonTg	Non-transgenic
NSF	*N*-ethylmaleimide-sensitive factor
O-GlcNAcylation	Addition of β-linked N-acetylglucosamine
OVX	Ovariectomized
PERK	PKR-like ER kinase
PET	Positron emission tomography
PEN2	Presenilin enhancer 2
PHF	Paired helical filament
PICK1	Protein interacting with C kinase 1
PKA	Protein kinase A
PKC	Protein kinase C
PP-LFS	Paired-pulse low frequency stimulation
PSD	Post-synaptic density
PSEN1	Presenilin 1
PSEN2	Presenilin 2
PTM	Post-translational modification
PTZ	(+)-pentazocine
R	Microtubule-binding domain
SCR	Scrambled
SEM	Standard Error of the Mean
Sig1R	Sigma-1 receptor
STEP	Striatal-enriched protein tyrosine phosphatase
TBS	Theta-burst stimulation
TrkB	Tropomyosin receptor kinase B
WHMIS	Women's Health Initiative Memory Study
WT	Wild-type

ACKNOWLEDGEMENTS

First and foremost, I would like to express my gratitude to my supervisor, Dr. Richard Bergeron, for giving me the opportunity to complete my master's degree in his laboratory. It has been an extraordinary experience in which I have not only acquired a strong background in neuroscience, but I also grew as an individual. Dr. Bergeron has truly been an inspiring and supportive mentor. His passion and accomplishments have pushed me towards reaching my goals.

I would also like to thank the members of my advisory committee, Dr. Johnny Ngsee and Dr. Paul Albert for the important role they played in carrying out my thesis. Throughout my project, they have provided me with insightful ideas and helped me become a better researcher. I would also like to extend my gratitude to all members of Dr Bergeron's laboratory, including Dr Melissa Snyder, Dr Prakash Chudalayandi, Dr Pamela Khacho, Boyang Wang, Thinh Nguyen, Kieran McCann, Julia Cappelli, Julian Pitney, Kayla Lynn Ferguson, and Sophie Raymond, for providing a great work environment and for helpful discussions. I would like to give a special thank you to Melissa and Thinh for their guidance and scientific expertise. Thank you, Melissa, for your help and for the time you spent proofreading my reports and thesis.

To my grandmother Murielle Danis, you are the main reason why I decided to learn more about dementia. Throughout my degree, you have been my greatest motivation. Finally, I cannot thank enough my father, Jocelyn Danis, and my partner, Payam Doryani, for believing in me and for supporting me. I thank them for listening to all my presentations and for showing interest in my research project.

1. INTRODUCTION

1.1 Alzheimer's Disease

1.1.1 Relevance of Studying Alzheimer's Disease

Dementia is a clinical syndrome in the elderly that is characterized by a deterioration in cognition, function and behavior. It involves a progressive impairment of memory and cognitive faculties. Over 50 million people worldwide are suffering from Alzheimer's disease (AD). AD is the leading cause of dementia in the elderly, representing 60-80% of all cases (Götz et al., 2018), with two-thirds of its patients being women (Hebert et al., 2013). By 2050, this number is projected to grow to 152 million (Association, 2019). In Canada alone, the care for people with AD, including the cost of unpaid care, was valued to 10.4 billion (CAD$) in 2016 and projected to rise to 16.6 billion (CAD$) by 2031 (Prince et al., 2016). AD doesn't only affect patients; its repercussion extends to family members by increasing their risk for emotional distress and negative mental and physical health outcomes. Furthermore, by 2038, long-term care beds are projected to have a shortfall, therefore increasing the number of AD patients living at home (Chang et al., 2015b; Chang et al., 2015a). Despite decades of research, the only available medication for AD treatment cannot cure the disease or reverse its progression. Therefore, there is an urgent need for AD research to broaden its horizons to include innovative approaches and novel areas of investigation.

In recent years, several clinical trials targeting beta-amyloid (Aβ) and tau individually have failed to find an effective therapeutic approach that could slow cognitive decline observed in AD patients. These failures highlight the urgent need for the development of new therapies. Recently, a novel area of investigation in AD research has been combination therapies involving both Aβ and tau, which have, so far, shown promising results (Stephenson et al., 2015; Gauthier et al., 2019; Morris, 2019). These combinatorial treatment strategies are likely to be the most successful (Long and Holtzman, 2019). AD pathogenesis is a complex disorder with multiple components that contribute to its progression including genetic susceptibility, lifestyle comorbidities, Aβ aggregation and vascular changes (Zetterberg and Bendlin, 2020). Moreover, there are numerous risk factors that significantly influence AD, one of them being sex differences. Indeed,

pathophysiological differences between men and women contributes to AD pathology (Pike, 2017; Ferretti et al., 2018). Unfortunately, few studies have investigated the impact of sex in AD research. Therefore, understanding the importance of sex differences in AD could be an interesting avenue for the discovery of disease-modifying therapies for AD.

1.1.2 Overview of Alzheimer's Disease

AD is a progressive neurodegenerative disorder characterized by initial cognitive impairment and memory loss followed by the inability to perform everyday activities, carry out basic bodily functions and ultimately resulting in death. AD was first described in 1901 by Alois Alzheimer. His post-mortem histological observations of his patient's brain, Auguste Deter, revealed two pathological hallmarks (Alzheimer, 1907), that are still used for diagnosis today. Neuropathologically, AD is characterized by the accumulation of Aβ in the form of senile plaques and the presence of neurofibrillary tangles (NFTs), which consist of aggregated hyperphosphorylated tau protein.

There are several forms of AD. About 1% - 2% of AD cases are inherited familial AD (FAD) caused from an autosomal dominant mutation (Van Cauwenberghe et al., 2016). These cases present with an earlier onset and faster progression of the disease. Individuals with FAD have inherited a genetic mutation in one of three genes: amyloid precursor protein (APP), presenilin 1 (PSEN1) or presenilin 2 (PSEN2). Patients who inherit a genetic mutation at the APP or PSEN1 are guaranteed to develop AD, while those who inherit the PSEN2 mutation have 95% chance of having the disease (Association, 2019). Early-onset Alzheimer's disease (EOAD) are cases that occur before the age of 65, making up less than 5% of all AD cases. Similarly, people affected by Down syndrome have an increased risk of developing AD. Having an extra copy of the chromosome 21, where the gene encoding the APP is located, makes them more vulnerable of developing the disease because it increases the production of Aβ produced in the brain (Association, 2019; DeTure and Dickson, 2019). Most AD cases are considered sporadic and occur after the age of 65, constituting late-onset Alzheimer's disease (LOAD). It can be caused by various factors: genetic, environmental or lifestyle. The greatest risk factors for sporadic AD is age, family history and apolipoprotein E (ApoE) ε4 genotype (Castro et al., 2017), with age being the strongest one. The percentage of people suffering from AD increases with age, with 3% of

people aged between 65 and 74 years old, 17% of people aged between 75 and 84 years old, and 32% of people aged 85 years old or older have AD (Hebert et al., 2013). Noteworthily, sex has also been shown to modulate the prevalence and the susceptibility to AD given by several risk factors of AD, including ApoE genotype.

1.1.3 Sex differences in Alzheimer's Disease

One of the least studied aspects of AD research is the consequences of sex on disease progression and therapeutic intervention. AD pathology impacts the male and female brain differently. For example, men and women affected by AD exhibit different cognitive and psychiatric symptoms. Men tend to demonstrate apathy, agitation and abusive behaviors, whereas women present depressive symptoms, emotional lability and affective symptoms (Ferretti et al., 2018). The largest study available documenting sex differences in psychiatric symptoms of AD showed that behavioral dysfunction and mood component score were worse in women than in men (Hollingworth et al., 2006). Furthermore, women who are diagnosed with AD lose their independence earlier than men (Sinforiani et al., 2010).

Interestingly, no clear sex differences in the absolute levels of diagnostic biomarkers, Aβ and tau, have been established (Ferretti et al., 2018; Toro et al., 2019). Post-mortem neuropathological analysis have revealed that sex doesn't seem to have an impact on Aβ burden (Barnes et al., 2005; Shinohara et al., 2016). Furthermore, studies from living patients with AD dementia have shown that cerebrospinal fluid (CSF) concentrations of $A\beta_{1-42}$ were not affected by sex (Mattsson et al., 2017). Post-mortem studies of brains from AD patients revealed that sex also doesn't have an effect on tau pathology, including neurofibrillary tangles and hyperphosphorylated tau (Barnes et al., 2005; Shinohara et al., 2016). Studies investigating tau accumulation via CSF analysis and positron emission tomography (PET) imaging, have shown similar results (Johnson et al., 2016; Jack Jr et al., 2017; Mattsson et al., 2017).

Sex differences in brain atrophy rates and patterns have been observed. A longitudinal study from the Alzheimer's Disease Neuroimaging Initiative (ADNI) indicated that women with mild cognitive impairment (MCI) have atrophy rates that are 1-1.5% faster than those in men (Hua et al., 2010; Ardekani et al., 2016). For example, the global brain atrophy (Sundermann et al.,

2016; Sundermann et al., 2017) and the hippocampal volume loss (Apostolova et al., 2006; Sundermann et al., 2017) are faster in women affected with MCI than men with MCI. However, another study reported that lower hippocampal volume was also present in men (Sundermann et al., 2016). These inconsistent findings could be due to the normalization method used. Men have a greater intracranial volume than women, thus if the volumetric measurements have been normalized to the intracranial volumes, the resulting data can be inaccurate (Perlaki et al., 2014). Together, these studies demonstrate that the patterns of decline and the atrophy rates differ between the sexes.

The development and progression of AD differ between men and women. Differences in brain structure and function have been reported. Moreover, absolute levels of diagnostic biomarkers have been carefully examined and revealed no sex-specific differences. Furthermore, brain atrophy rates have been shown to differ between the sexes. However, the reasons underlying these differences remain unknown. Many hypotheses have been made concerning these differences such as: differences in age-related sex hormone reduction, various of genetic risks, impact from risks of other diseases and sex differences in brain anatomy and function (Li and Singh, 2014). Taken together, the cumulative evidence highlights the importance of considering sex in AD research. Potential sex differences should be carefully examined in preclinical and clinical studies.

1.1.4 Risk factors

1.1.4.1 Apolipoprotein E

Located on chromosome 19, the ApoE gene is the strongest genetic risk factor for LOAD, which accounts for over 95% of AD cases. This gene encodes for a 34-kDa glycoprotein (Mahley, 1988) composed of 299 amino acids involved in lipid homeostasis. In the central nervous system (CNS), ApoE is highly expressed in astrocytes and to a lesser extent microglia (Xu et al., 2006; Kang et al., 2018). ApoE is found in lipoprotein particles where its main function is to transport cholesterol and other lipids to neurons (Yamazaki et al., 2019).

ApoE comprises three major isoforms: ε2, ε3 and ε4. Within exon 4 of the ApoE gene, the differential expression of these isoforms is dependent on two point mutations, rs429358 and rs7412

(Sims et al., 2020). The majority of the human population is homozygous for ApoE ε3. The ε4 allele, which is present in about 16% of the population (Balietti et al., 2012), has been shown to increase the risk of AD by 3-fold to 4-fold for a single inherited copy, while two inherited copies increases the risk by 15-fold (Sims et al., 2020). Inheritance of the ε2 allele is slightly protective against AD (Strittmatter et al., 1993; Corder et al., 1994; Dumitrescu et al., 2019).

ApoE ε4 modulate both amyloid and tau pathologies, which in turn affects the risk of AD. As shown by PET studies, carriers of the ApoE ε4 allele develop cerebral Aβ at a younger age (Fleisher et al., 2013) and the amyloid pathology progresses faster than in non-carriers. Indeed, the ApoE ε4 allele is associated with a diminished clearance of cerebral Aβ deposition (Fu et al., 2016; Liu et al., 2016) and an increased production of soluble and aggregated Aβ (Pietrzik et al., 2004; Zerbinatti et al., 2004). On the other hand, much less is known about the relationship between ApoE ε4 and tau pathology. Previous work in mice has shown that ApoE ε4 affects tau-mediated neurodegeneration independently of Aβ pathology (Shi et al., 2017). However, Zhao et al. found that ApoE ε2 was associated with increased tau pathology (Zhao et al., 2018b). A possible explanation for this discrepancy is that Shi et al. focused on tau-mediated neurodegeneration, whereas Zhao et al. looked at overall tau pathology. In addition, both studies used different mouse models. In humans, the findings regarding the relationship of ApoE with tau also have some inconsistencies. According to a post-mortem study, carriers of two ApoE ε4 alleles have increased tau aggregates compared to carriers of one allele or noncarriers (Tiraboschi et al., 2004). Furthermore, ApoE ε4 carriers who suffer from AD have been shown to have greater tau pathology than noncarriers (Zhao et al., 2018a). The effects of ApoE ε4 on tau-PET uptake have been controversial (Cho et al., 2016; Johnson et al., 2016; Ossenkoppele et al., 2016). However, a recent study has demonstrated that the interaction between ApoE ε4 and Aβ increases tau pathology (Therriault et al., 2020).

Interestingly, ApoE genotype has been shown to interact with sex in AD pathology, although the mechanisms underlying this interaction remains unclear. The risk of AD onset has been reported to be higher in female ApoE ε4 carriers than among their male counterparts (Ungar et al., 2014b). ApoE ε4 women carriers have a faster cognitive decline than women non-carriers

and men of any genotype (Mortensen and Høgh, 2001; Beydoun et al., 2012). They also have greater levels of AD pathology compared to men (Nebel et al., 2018). Evidence has indicated that women ε4 carriers with MCI have a greater hippocampal atrophy than men affected by MCI (Ungar et al., 2014a). While female ApoE ε4 carriers are more vulnerable against AD, female ε2 carriers are surprisingly more protected against AD than male ε2 carriers (Dumitrescu et al., 2019). Overall, ApoE status clearly interacts with sex and can also affect the risk of AD. Additional studies are needed to understand how sex interacts with ApoE status to influence AD risk.

1.1.4.2 Vascular risk factors

Vascular diseases have been shown to contribute the risk of AD. They also have an additive effect with amyloid pathology on cognitive decline (Ferretti et al., 2018). Several cardiovascular risk factors have been associated with an increased risk of cognitive decline and dementia, including diabetes, smoking, obesity and hypertension (Association, 2019). Indeed, factors leading to an increased risk of cardiovascular disease are also related to a greater risk of dementia (Samieri et al., 2018). Evidence has shown that the vasculature system differ between men and women (Toro et al., 2019). The prevalence of vascular diseases and risk factors are generally higher in men under the age of 60 years old. However, after menopause or the age of 60 years, the prevalence for cardiovascular risk factors and cerebrovascular events are equal or greater in women (Longstreth Jr et al., 2009; Gibson, 2013; Cordonnier et al., 2017; Madsen et al., 2017). Interestingly, men have a higher incidence of coronary disease than women (Kivipelto et al., 2001), whereas women have an increased risk of diabetic complications such as myocardial infarction and depression (Kautzky-Willer et al., 2016), which are known AD risk factors. Unfortunately, it is still unclear whether cognitive decline and the risk of AD are mediated by an interaction of sex with cerebrovascular pathology and cardiovascular risk. Even if men and women were included in most of these studies, only a fraction of them investigated their data to determine the impact of sex on cardiovascular diseases and how it affects AD risk.

1.1.4.3 Sleep

Sleep impairment are common among patients suffering from AD and can also influence the development of AD pathology. The production of Aβ is associated with wakefulness, whereas the clearance of Aβ occurs during sleep (Cedernaes et al., 2017). Numerous groups of researchers have investigated the potential link between AD and sleep. For example, two studies have shown in humans that the levels of CSF Aβ42 is increased when the amount of slow-wave sleep is decreased (Varga et al., 2016; Ju et al., 2017). Furthermore, sleep deprivation increases levels of $A\beta_{1\text{-}42}$ (Ooms et al., 2014) and tau (Holth et al., 2019) in both human CSF and animal model brains (Long and Holtzman, 2019).

Sex differences in sleep disorders have been reported and represent an interesting avenue of research. Women have more sleep problems than men, such as insomnia and inadequate sleep (Toro et al., 2019). Furthermore, following a short night's sleep, women are more prone to show symptoms then men. Moreover, age is also a risk factor for sleep disorders, they also have been shown to reach their peak during menopause for women (Bixler et al., 2001). Additional studies are needed to understand the link between sleep and the risk of developing AD and whether this is mediated by sex.

1.1.4.5 Psychiatric comorbidity

Depression is a well-established risk factor for AD (Ownby et al., 2006). Women have twofold greater risk of developing depression than men (Bromberger et al., 2011; Nebel et al., 2018). Because of this clinical profile, one would think that women affected by depression would be more prone to developing AD than men affected by depression. Surprisingly, there are some discrepancies present in the literature concerning the effect of sex on depression as a risk factor in AD pathology. Some studies suggest that women with depressive symptoms are associated with an increased risk of developing AD, while others suggest that men would have a greater risk of developing AD. In the Women's Health Initiative Memory Study (WHMIS), clinically significant depressive symptoms and a past history of depression were associated with a twofold increased risk of dementia (Goveas et al., 2011a). Interestingly, the same group also found that depression in women was associated with hippocampal volume loss, an hallmark in AD (Goveas et al.,

2011b). Studies have also shown that the risk of cognitive impairment is higher among women than among men suffering from depression (Sachdev et al., 2012; Kim et al., 2015). Conversely, others studies indicated that only men exhibiting depressive symptoms have an increased risk for AD pathology (Fuhrer et al., 2003; Dal Forno et al., 2005).

1.1.4.6 Lifestyle risk factors

Some risk factors cannot be changed, such as age and genetics, while others can be influenced by our lifestyle to reduce risk of cognitive impairment and AD. For example, studies have shown that regular physical activity and management of cardiovascular risk factors can lower the risk of cognitive decline and dementia (Baumgart et al., 2015; Guure et al., 2017; Stephen et al., 2017; Tan et al., 2017). Nutritional habits have also been shown to affect AD risk (Morris et al., 2015b; Morris et al., 2015a; Hardman et al., 2016). Indeed, a healthy diet has been established to reduce AD risk. Furthermore, education influences AD risk. Being more educated has been reported beneficial against AD risk (Sando et al., 2008), while a lower level of education has been associated with lower socioeconomic status (McDowell et al., 2007), higher cardiovascular risk factors and increased risk of AD. Similarly, lifelong learning and social engagement has also been linked to a decreased risk of cognitive decline (Association, 2019). Further studies are needed to elucidate the underlying mechanisms of how these factors reduce AD risk.

1.1.5 Pathological Hallmarks

1.1.5.1 Amyloid pathology

A key pathological feature of AD is the extracellular deposition of Aβ into senile plaques. Aβ peptides results from the sequential proteolytic cleavage of APP by different secretases. APP, a type I integral protein expressed at the cell surface, is processed through the non-amyloidogenic pathway or the amyloidogenic pathway. In the non-amyloidogenic pathway, APP is cleaved within the Aβ sequence near the extracellular plasma membrane by α-secretase, thus generating soluble α-APP fragments (sAPPα) and C-terminal fragment α (CTFα, C83). C83 is then divided by γ-secretase, generating non-toxic P3 and AICD fragments. Pathogenic Aβ peptides, on the other hand, are generated through the sequential cleavage of APP by β-secretase (BACE1) and γ-secretase. The γ-secretase is a protease complex consisting of presenilin 1 or 2, nicastrin, anterior

pharynx defective 1 (APH-1) and presenilin enhancer 2 (PEN2) (Haass et al., 2012). In the amyloidogenic pathway, β-secretase first cleaves APP at the N-terminal of the Aβ sequence to produce the sAPPβ fragment and the 99-amino acid C-terminal fragment (C99), which remains in the membrane. C99 is then successively cleaved by γ-secretase until the Aβ peptide is released. Interestingly, only $A\beta_{1-40}$ and $A\beta_{1-42}$ aggregate *in vivo*. Together, they are the main components of soluble oligomers and senile plaques. $A\beta_{1-42}$, the more neurotoxic form, is more prone to aggregation than $A\beta_{1-40}$ because of its increased hydrophobicity, which is granted by the two extra amino acids (Ahmed et al., 2010).

Under physiological conditions, most of the Aβ peptides are the 40-amino acid form ($A\beta_{1-40}$). In AD, $A\beta_{1-42}$ self-aggregates, forms insoluble amyloid fibrils and then accumulates in plaques, which is present in higher concentration in AD patients (Mayeux et al., 1999). Senile plaques are associated with disrupted neurite morphology, gliosis and oxidative stress (Spires-Jones and Hyman, 2014). They propagate in a stereotypical manner, they begin in the neocortex and progress through the allocortex, then to the diencephalon, striatum, and basal forebrain cholinergic nuclei. They then reach the brainstem nuclei and finally the cerebellum (Spires-Jones and Hyman, 2014).

Amyloid fibrils were long thought to be toxic and to be the component disrupting synaptic integrity. However, studies from the past decade have shown that soluble forms of Aβ, which accumulates around plaques, are more toxic than fibrils. Interestingly, oligomeric forms of Aβ contribute to synapse dysfunction and increased phosphorylation and secretion of tau, a pathological hallmark of AD (Spires-Jones and Hyman, 2014).

1.1.5.2 Tau pathology

Tau protein is a microtubule-associated protein (MAP) mainly localized in axons (Morris et al., 2011) and in significantly smaller amounts in somatodendritic compartments (Tashiro et al., 1997), including mitochondria (Li et al., 2016), nucleus, plasma membrane, as well as dendritic spines (Ksiezak-Reding et al., 2003; Kimura et al., 2014). It is encoded by the *MAPT* gene located on the chromosome 17. In the human brain, there are six distinct isoforms of tau (0N3R, 0N4R, 1N3R, 2N3R, and 2N4R), which are derived from the alternative splicing of the *MAPT* gene at the

N-terminal domain (N) and microtubule-binding domain (R), composed of repeats of tubulin-binding motif (Lee et al., 1989) . Tau isoforms can be differentiated in the presence of either zero, one or two 29 amino-acid-long inserts at the N-terminal portion of the protein and in the number of tubulin-binding repeats (Ballatore et al., 2007). The six tau isoforms are differentially expressed during brain development. In most regions of the adult brain, the 3R and 4R tau isoforms are expressed in a one-to-one ratio. Both 3R and 4R isoforms are found in NFTs in AD. Under physiological conditions, the main functions of tau is to regulate microtubule stabilization and axonal transport (Götz et al., 2006). Following Braak staging, tau propagation occurs in a stereotyped fashion. It begins in the enthorinal cortex, then progresses to the hippocampus and finally reaches the primary sensory areas in late stages of AD (Braak and Braak, 1991). Unlike Aβ, the evolution of tau pathology strongly correlate with cognitive impairment (Giannakopoulos et al., 2003).

Tau is subject to several post-translational modifications (PTM), including phosphorylation, acetylation, glycosylation, addition of β-linked N-acetylglucosamine (O-GlcNAcylation), glycation, ubiquitination and methylation (Guo et al., 2017; Marcelli et al., 2018). These modifications play a pivotal role in the regulation of tau, thus disruption in these modifications have a serious impact in the physiological role of tau. In AD, phosphorylation, acetylation and glycosylation have been shown to have neuroprotective and neurodegenerative effects by modulating tau aggregation (Hoover et al., 2010; Cook et al., 2014; Wang et al., 2016; Ryan et al., 2019). Excitingly, modulating these PTMs could represent promising therapeutic strategies to prevent and treat NFTs in AD.

Phosphorylation is the most commonly described tau PTM in the literature especially in the context of AD. Tau contains 85 different phosphorylation sites (Guo et al., 2017), 29 of which are involved in the AD brain (Hanger et al., 2009). Tau is a substrate for several kinases that mediate its phosphorylation, such as mitogen-activated protein kinase (MAPK), cyclin-dependent kinase-5 (Cdk5), cAMP-dependent protein kinase A (PKA), glycogen synthase kinase 3 (GSK3) and calcium-calmodulin-dependent protein kinase II (CaMKII). These kinases are divided into three groups: proline-directed serine-threonine protein kinases, non-proline directed serine-threonine kinases, and tyrosine-specific protein kinases (Long and Holtzman, 2019). One

particularly important kinase in AD progression is GSK3. This kinase plays a role in several cellular processes, including microtubule dynamics, gene transcription as well as cell proliferation (Frame and Cohen, 2001; Frame et al., 2001; Martinez, 2008). More importantly, GSK3 is highly involved in tau hyperphosphorylation. Its activity strongly correlates with NFTs (Leroy et al., 2002), and one of its isoenzymes, GSK3β, colocalizes with tau pathology in AD brain (Hanger et al., 1992). Interestingly, GSK3β inhibitors have been suggested as potential therapeutic strategies for AD pathology (Guo et al., 2017; Griebel et al., 2019).

When tau becomes hyperphosphorylated, it detaches from microtubules (Ballatore et al., 2007), and accumulates in the somatodendritic compartment in the form of paired helical and straight filaments (Spillantini and Goedert, 2013; Spires-Jones and Hyman, 2014). This self-aggregation of tau leads to the formation of tau oligomers, which are known to disrupt synapse function. Furthermore, the accumulation of tau in the somatodendritic compartment also results in synaptic dysfunction via the inhibition of synapse anchoring and glutamate receptor trafficking (Hoover et al., 2010). Moreover, phosphorylation alters the interaction of tau with its binding partners, thus disturbing the function of tau in numerous signaling pathways (Drewes et al., 1995).

First described by Min and colleagues (Min et al., 2010), tau acetylation is a novel post-translational modification mediated by CREB-binding protein (CBP). It has been shown to influence the capacity of tau to become phosphorylated and aggregated. Min et al. found that tau acetylation prevents the degradation of phosphorylated tau and increases tau pathology (Min et al., 2010). Interestingly, acetylation of tau by CBP at lysine residues 259, 290, 321 and 353 prevents phosphorylation of these same residues and tau aggregation (Cook et al., 2014). Acetylation of those specific lysine residues have been found decreased in AD brains. Tau acetylation has also been shown to disrupt α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPAR) insertion in AD brain (Guo et al., 2017), thus leading to synaptic dysfunction and cognitive impairment.

Tau glycosylation is also another emerging post-translational modification. Intriguingly, tau protein has been found to be modified by N-glycosylation in AD brain, but not in control brain. Additional studies are needed to elucidate the role N-glycosylation in AD. N-glycosylation of tau

possibly plays an important role in the maintenance of the paired helical filament (PHF) structure (Wang et al., 1996). On the other hand, O-GlcNAcylation of tau inversely regulate tau phosphorylation and hinders tau aggregation (Ryan et al., 2019). In AD brains, O-GlcNAcylation is reduced, thus possibly contributing to the increased phosphorylation and aggregation of tau (Liu et al., 2004). This reduction could be explained by the loss of O-GlcNAc transferase, the enzyme responsible for O-GlcNAcylation, which have also been reported in AD (Wang et al., 2016).

1.1.5.3 Biomarkers

Early diagnosis of AD is important, so that the patients can benefit from symptomatic drugs and will be necessary when disease-modifying therapies will be available. The hope is that future treatments will target the disease before the appearance of symptoms. One of the most promising paths to achieve this is to use biomarkers. Amyloid pathology is found prior to impairment. Biomarkers studies have shown that extracellular Aβ deposition occurs 15-20 years before the clinical onset (Jagust, 2018; DeTure and Dickson, 2019; Vermunt et al., 2019). In recent years, fluid and imaging biomarkers have been used to measure Aβ pathology *in vivo*. These tools have improved our understanding of the role of Aβ in AD pathology and are useful for diagnosis of preclinical AD. Several studies have consistently reported that AD CSF is characterized by a decrease in Aβ42 of approximatively 50% (Olsson et al., 2016). These reduced CSF levels are due to the aggregation of Aβ42 in the brain parenchyma (Zetterberg and Bendlin, 2020). The CSF Aβ42/40 ratio, on the other hand, has been shown to perform better as a diagnostic tool compared to the use of CSF Aβ42 alone (Zetterberg and Bendlin, 2020), because it accounts for interindividual differences the amyloidogenic APP-processing. Interestingly, Aβ42/40 ratio can also be measured in plasma. In the past, it was thought that plasma Aβ42 did not reflect cerebral Aβ pathology (Olsson et al., 2016). Conversely, recent findings reported that plasma Aβ biomarkers are correlated with the Aβ status in the brain. However, even though blood biomarkers are a great minimally invasive and cost-effective option compared to CSF and PET biomarkers, they still need extensive standardization. Plasma Aβ biomarkers results differ from those obtained via CSF. For example, the decrease in Aβ42/40 ratio in plasma is 14-20% (Janelidze et al., 2016; Ovod et al., 2017; Nakamura et al., 2018; Schindler et al., 2019), whereas in the CSF, it is 50% (Olsson et al., 2016). This could be explained by the production of Aβ by platelets and other tissues (Ashton et al., 2018; Zetterberg and Bendlin, 2020). Concerning imaging biomarkers, amyloid

PET has been validated against neuropathology and is the most widely used biomarker in current clinical trials. To biologically define AD in patients, researchers also use CSF total tau (t-tau) and phosphorylated tau (P-tau) in combination of CSF Aβ42/40 ratio as diagnostic tools. However, these tau biomarkers do not allow mapping and quantifying of tau pathology in a temporal and spatial manner, whereas tau PET imaging does.

1.1.6 Synaptic Plasticity

1.1.6.1 Hippocampus

One of the first brain region to be affected by AD pathology is the hippocampus (Braak et al., 1993), which is crucial for learning and memory. Located in the medial temporal lobe, the hippocampal formation can be divided in three distinct subregions: the dentate gyrus, the hippocampus proper (i.e. *cornu ammonis*) and the subiculum. The hippocampus proper is further divided in four anatomical subdivisions: CA1, CA2, CA3 and CA4. Mostly composed of pyramidal neurons, areas CA1 and CA3 are the most prominent.

The tri-synaptic circuit of the hippocampus is composed of three glutamatergic pathways. The perforant paths inputs from the enthorinal cortex make synaptic connections with the granule cells of the dentate gyrus. The axons from the granule cells, which constitute the mossy fibers, then projects to the pyramidal cells of the CA3 region. After synapsing in CA3, the axons from the CA3 pyramidal neurons, the Schaffer collaterals, forms a synapse in the CA1 area. The outputs of the hippocampus are the axons from CA1 that projects to the subiculum and the layers V-VI of the enthorinal cortex. The tri-synaptic circuit is highly preserved in coronal slices across the long axis of the hippocampus, which makes the hippocampal formation an attractive target for *in vitro* electrophysiological experiments.

Intriguingly, the hippocampus has been shown to be sexually dysmorphic, which makes it an interesting target for our investigation. Changes in steroid hormones levels, such as gonadal hormone manipulation as well as the estrous cycle phase, impact synaptic structure, synaptic physiology and cognition. Furthermore, sex steroid hormones, especially the potent estrogen 17β-estradiol (E_2), have been shown to be neuroprotective (Frick et al., 2015). Sex differences have

been reported in the morphology and electrophysiological properties of hippocampal neurons. Female rodents have a smaller volume than males and exhibit a lower cellular density in the CA1 region (Madeira and Lieberman, 1995). With regard to electrophysiological properties, males exhibit larger hippocampal long-term potentiation (LTP) in CA1 region than females (Maren et al., 1994; Yang et al., 2004; Harte-Hargrove et al., 2015; Monfort et al., 2015). LTP in females is also influenced by the estrous cycle phase at the CA3-CA1 synapse. During the proestrus phase, where estradiol and progesterone levels peak, LTP amplitude is at its greatest (Warren et al., 1995). Moreover, AMPA/NMDA ratio is different between males and females in the CA1 region, with females showing greater AMPA/NMDA ratio than males (Monfort et al., 2015; Qi et al., 2016).

1.1.6.2 AMPAR

AMPAR is an ionotropic receptor for glutamate, the major excitatory transmitter in the CNS. They play a crucial role in learning, memory and cognition. Composed of four subunits, GluA1-GluA4, AMPARs are tetramers that mediate the majority of fast excitatory transmission in the brain. The expression of AMPARs varies by age and brain region. GluA1, GluA2 and GluA3 subunits are abundantly expressed in the majority of neurons in the CNS, including in the hippocampus, the outer layer of the cortex, basal ganglia, olfactory bulb, lateral septum and amygdala. GluA4, on the other hand, is mostly expressed during early life. In the mature brain, GluA4 expression is absent from excitatory pyramidal neurons and is restricted to the cerebellum, thalamus and brain stem (Yadav et al., 2017; Diering and Huganir, 2018). Interestingly, after P14, GluA2-lacking receptors and GluA4 homomers are exchanged for GluA2-containing receptors (Pellegrini-Giampietro et al., 1992; Henley and Wilkinson, 2016). Additionally, by P21, GluA3 subunit levels decrease, whereas GluA1 levels are reduced (Henley and Wilkinson, 2016). Furthermore, AMPARs biophysical properties, number and composition alter in the postsynaptic membrane. Moreover, AMPARs subunit composition dictates their trafficking behaviour, which directly impacts synaptic plasticity. Additionally, AMPARs have highly dynamic nature. They are subject to continuous endocytosis, exocytosis, lateral diffusion and several posttranslational modifications, including phosphorylation, palmitoylation, ubiquitnation, S-nitrosylation and O-GlyNAcylation (Diering and Huganir, 2018).

One interesting property of AMPARs is that their subunit composition affects their permeability to calcium. GluA2-containing receptors are impermeable to Ca^{2+} and have a linear current-voltage-relationship, which means that they pass equal outward current than inward current at positive and negative potential, respectively. GluA2-lacking receptors, on the other hand, are permeable to Ca^{2+}, have a high conductance for Na^{+} and have an inward-rectifying current-voltage relationship. Most AMPARs are made up from GluA1/GluA2 and GluA2/GluA3 subunit combinations in the hippocampus (Wenthold et al., 1996; Lu et al., 2009; Diering and Huganir, 2018).

Interestingly, AMPAR dysfunction has been reported in multiple AD mouse model (Hsia et al., 1999; Chang et al., 2006; Hsieh et al., 2006). More specifically, the GluA2 subunit has been reported to play a key role in AD pathology and has been shown to be significantly altered. Indeed, GluA2 expression levels are reduced in both AD models and patients (Ikonomovic et al., 1997; Carter et al., 2004; Gong et al., 2009; Gaisler-Salomon et al., 2014; Savas et al., 2017). Similarly, a recent study using co-expression analysis reported a significant decrease in GluA2 in the hippocampus across several AD model (Savas et al., 2017). The same study also identified GluA2 as a crucial protein in AD hippocampus (Savas et al., 2017).

1.1.6.3 NMDAR

The *N*-methyl-D-aspartate receptor (NMDAR) is also a glutamate ionotropic receptor that plays a central role in CNS function. NMDARs are permeable to Na^{+}, K^{+} and Ca^{2+}. They also have a complex current-voltage relationship. Near the resting membrane potential, the pore of the channel is blocked by magnesium, thus preventing other ions to enter the post-synaptic neuron. NMDARs are known as coincidence detectors, because their opening depends on membrane voltage and transmitter release. In order to allow the passage of ions through the channel, glutamate has to be bound, and the post-synaptic neuron need to be depolarized. They are heterotetrameric receptors consisting of two obligatory GluN1 subunits and two GluN2 regulatory subunits or a mixture of GluN2 and GluN3 subunits (Paoletti et al., 2013). NMDARs have in total seven regulatory subunits: GluN1, GluN2A, GluN2B, GluN2C, GluN2D, GluN3A and GluN3B. Out of those seven subunits, GluN2A and GluN2B have been the most extensively studied subunits because of the crucial role they play in synaptic function and plasticity. They are also the

predominant subunits in higher brain structures (Liu et al., 2019), including the cortex and the hippocampus, which are the most affected regions in AD. Moreover, the majority of synaptic NMDARs found in these brain regions are the di-heteromeric GluN1/GluN2A and tri-heteromeric GluN1/GluN2A/GluN2B receptors (Al-Hallaq et al., 2007; Gray et al., 2011; Rauner and Köhr, 2011). However, in aged mice, di-heteromeric GluN1/GluN2A receptors are predominant due to the loss of GluN2B. This decrease in GluN2B expression could be linked to impaired synaptic plasticity and memory function (Paoletti et al., 2013).

Several neurodegenerative diseases, including AD, involve NMDAR dysfunction. NMDAR hyperactivity and NMDAR hypofunction can lead to neuronal death. In the case of AD, excessive activation of NMDARs leads to excitotoxicity, which may contribute to synaptic and neuronal loss. Evidence has also shown that Aβ oligomers (Snyder et al., 2005) and tau (Hoover et al., 2010) contribute to the synaptic dysfunction in AD through the removal of synaptic NMDARs via endocytosis. Changes in the NMDAR subunits composition has been shown to be implicated in AD. For example, activation of extrasynaptic GluN2B-containing NMDARs disrupts synaptic plasticity and leads to synaptic loss (Paoletti et al., 2013). Moreover, it increases the Aβ- and tau excitotoxicity caused by Aβ and tau (Paoletti et al., 2013). The GluN2B subunit has drawn significant interest in the context of AD because it plays a crucial role in cognition, memory formation and the induction of LTP (Paoletti et al., 2013). Interestingly, therapeutic strategies targeting the GluN2B subunit may improve cognitive function in AD patients. The blockade of GluN2B-containing receptors by NMDAR antagonist, such as APV and ifenprodil, have led to the rescue of Aβ-induced insults, including synaptic depression (Hu et al., 2009; Li et al., 2011; Rönicke et al., 2011), synaptic loss (Rönicke et al., 2011) and facilitation of LTD (Li et al., 2009).

1.1.6.4 Long-term Potentiation

How we learn and store information has always been an intriguing topic for mankind. Since the Age of Enlightenment in the 17th century, philosophers were already making hypothesis about the mechanism of memory. Today, LTP is widely believed to be the molecular mechanism underlying learning and memory (Bliss and Collingridge, 1993). It was first discovered by Bliss and Lomo in 1973 in the dentate gyrus of anaesthetized rabbits, by stimulating the perforant path from the enthorinal cortex (Bliss and Lomo, 1973). Most of the data collected on LTP comes from

extracellular recordings in the CA1 region of the hippocampus from acute hippocampal slices, which is a form of NMDA Receptor-Dependent LTP (NMDAR-LTP). Later, in 1990, another component of LTP was reported in the CA1 region (Grover and Teyler, 1990). This component is NMDAR-independent and requires the activation of voltage-dependent calcium channels. Furthermore, there is another form of LTP in the hippocampus at the mossy fibre-CA3 synapse. This form of LTP differ from the one found in the CA1 region on several aspects: it is NMDAR-independent and entirely expressed presynaptically (Nicoll and Schmitz, 2005). However, this thesis will solely focus on NMDAR-LTP because it is the most affected form in AD pathology (Spires-Jones and Hyman, 2014)

LTP in the CA1 region of the hippocampus is the most extensively studied form of synaptic plasticity. Furthermore, LTP has several basic properties that makes it an interesting cellular mechanism such as input specificity, associativity and cooperativity. In the CA1 region of the hippocampus, LTP is input-specific, which indicates that LTP is only induced at activated synapses and not at other synapses from the same postsynaptic neuron. This property increases the storage capacity of individual neurons. LTP is also associative, which means that a strong input can facilitate LTP of a weaker input when it is activated in association. Additionally, LTP is cooperative, it can only be induced if enough synapses are activated at the same time (Citri and Malenka, 2008).

1.1.6.4.1 LTP induction

To induce LTP, the pre- and postsynaptic neuron must be active at the same time so that the postsynaptic neuron is depolarized when glutamate is released from the presynaptic bouton to relieve the Mg^{2+} block of the NMDAR. Na^{+}, K^{+} and Ca^{2+} can then enter the cell through NMDARs. This rise in intracellular Ca^{2+} in the postsynaptic neuron is the critical trigger for LTP. Because NMDARs only conduct currents when glutamate is present in the synaptic cleft and when the postsynaptic neuron is depolarized, NMDARs are referred to as coincidence detectors (Citri and Malenka, 2008; Luscher and Malenka, 2012).

The increase in intracellular calcium concentrations in the postsynaptic neuron leads to the activation of intracellular signaling cascades. LTP involves preferential activation of protein kinases such as CaMKII, PKA, protein kinase C (PKC), MAPK and Src kinase.

CaMKII, which is found at high concentration in dendritic spines, is required as a mediator for NMDAR-LTP. Calcium binds to calmodulin (CaM), then Ca^{2+}/CaM activates CaMKII. Following this stimulation, CaMKII undergoes autophosphorylation at Thr 286/287, thus making this holoenzyme autonomously active and partially insensitive to changes in Ca^{2+}/CaM concentrations. The association of the activated CaMKII with NMDARs leads to the persistent activation of the enzyme at the post-synaptic density (PSD). The activation of CaMKII also leads to the phosphorylation of AMPARs, which causes an increase in single-channel conductance of AMPARs (Luscher and Malenka, 2012). It also contributes to the incorporation of additional AMPARs into the postsynaptic membrane (Lisman et al., 2012).

1.1.6.4.2 LTP expression

In the past, a major controversy concerning the expression mechanisms of LTP was whether LTP was mediated presynaptically as a change in probability of neurotransmitter release, postsynaptically, as a change of glutamate responsiveness, or both. Several studies found an increase in the probability of release using whole-cell patch clamp recordings in hippocampal slices (Dolphin et al., 1982; Bekkers and Stevens, 1990; Malinow and Tsien, 1990; Malinow, 1991; Malgaroli et al., 1995; Zakharenko et al., 2001). The results of these studies were consistent with Tim Bliss's findings suggesting an increase in glutamate release in LTP (Dolphin et al., 1982).

The discovery of silent synapses put an end to the "pre-" versus "post-" debate. Silent synapses are synapses that contain only NMDARs with few or no AMPARs. These synapses are capable of synaptic plasticity mediated by NMDAR activation but are functionally silent under baseline conditions. They can be activated following an LTP induction protocol because of the insertion of AMPARs into their postsynaptic membrane (Isaac et al. 1995, Liao et al. 1995). In the context of AD, Aβ has been shown to reduce the number of synaptic AMPARs (Hsieh et al., 2006; Ting et al., 2007), including the number of silent synapses in the CA1 region of the hippocampus (Bie et al., 2018), and the number of synaptic NMDARs (Snyder et al., 2005; Hsieh et al., 2006).

It is likely that the AMPA silencing occurring in AD is of previously functional synapses (Hanse et al., 2013). The loss of AMPARs and NMDARs leads to synapse dysfunction and synapse elimination. Interestingly, the prevalence of silence synapses has been shown to decrease in the aged brain (Nicoll, 2017).

The widely accepted mechanism of expression of LTP at in the CA1 region of the hippocampus involves an increase in the number of AMPARs within the PSD, driven through activity-dependent changes in AMPAR trafficking. Most of the inserted AMPARs during LTP are heteromeric GluA1/GluA2 (Adesnik and Nicoll, 2007).

1.1.6.5 Long-term Depression

Long-term depression (LTD) is a form of long-lasting plasticity in the mammalian brain. It is characterized by a long-lasting decrease of synaptic strength following a long-lasting low frequency stimulus (Collingridge et al., 2010; Spires-Jones and Hyman, 2014). There are two major forms of LTD in the hippocampus, both resulting in the reduction of the number of AMPARs at the post-synaptic membrane. LTD involves the preferential activation of protein phosphatases.

NMDAR-dependent LTD (NMDAR-LTD) is the most studied form of LTD and is usually induced by prolonged periods of low-frequency stimulation (LFS, typically single or pairs of pulses, 200 ms apart, delivered at 1 Hz for 15 min) (Marchetti and Marie, 2011). The modest depolarization of the postsynaptic neuron leads to the activation of NMDARs, which results in the rise of intracellular calcium in the postsynaptic cell. Furthermore, the phosphorylation state of GluA2 AMPAR subunit is important to regulate the clathrin-mediated endocytosis of AMPARs during NMDAR-LTD. GluA2-containing AMPARs are stabilised on the post-synaptic membrane by an interaction with *N*-ethylmaleimide-sensitive factor (NSF), an ATPase involved in membrane fusion events (Collingridge et al., 2004). The internalization of surface AMPARs is initiated when the neuronal calcium sensor protein hippocalcin (HPC) forms a complex with the clathrin adaptor protein AP2 and GluA2 subunit, which then displaces NSF, thus breaking the stabilization between GluA2 and NSF (Palmer et al., 2005). Another protein involved in NMDAR-LTD is the protein interacting with C kinase 1 (PICK1). PICK1 competes with the scaffolding proteins AMPAR-binding protein (ABP) and glutamate receptor interacting protein (GRIP) for binding to the C-

terminal region of GluA2. Together, these scaffolding proteins anchors AMPARs at non-synaptic sites. When this binding ability is impaired, NMDAR-LTD becomes unstable, thus suggesting that ABP-GRIP is crucial for the expression of NMDAR-LTD. PICK1 aid the dissociation of AMPARs from ABP-GRIP through the phosphorylation of Ser880 of GluA2 by protein kinase Cα (PKCα). Another important role of PICK1 in NMDAR-LTP is to modify neuronal architecture by enabling actin depolymerization via an interaction with F-actin and the actin-related protein 2/3 (Arp2/3 complex) (Rocca et al., 2008).

mGluR-LTD is the second major form of LTD studied in the hippocampus and can be induced by either prolonged paired-pulse low frequency stimulation protocol (1-3 Hz; 5-15 min), or by the application of dihydroxyphenylglycine (DHPG). mGluR-LTD at the CA1 synapse is triggered predominantly through activation of mGluR5. Numerous signaling pathways have been found to be involved in mGluR-LTD, most of them implicating MAPK such as JNK, p38 (Bolshakov et al., 2000; Rush et al., 2002; Moult et al., 2008) and ERK 1/2 (Collingridge et al., 2010).

1.1.6.6 Synaptic Dysfunction and Synapse Loss

Early in the course of AD, the normal function of synapses is impaired and leads to synaptic loss, which is correlated with cognitive decline observed in AD pathology. In the early 1990s, synapse loss in AD was first observed in the frontal cortex, temporal cortex and dentate gyrus of the hippocampus (DeKosky and Scheff, 1990; Terry et al., 1991; Masliah et al., 1994; DeKosky et al., 1996). Interestingly, synaptic loss correlates better with cognitive decline than histopathological hallmarks.

It is widely believed that Aβ and tau act in concert to contribute to synaptic loss and that their soluble forms strongly contribute synaptotoxicity. Several studies have shown that soluble species of Aβ are toxic to synaptic function. Indeed, oligomeric forms of Aβ enhances LTD, impairs LTP and cognitive function. This particular form of Aβ is physically present at synapses around plaques in the brains of AD mouse model (Koffie et al., 2009) and post-mortem AD brain tissue (Spires-Jones and Hyman, 2014). Noteworthily, synapse degeneration is greatest near senile plaques. Oligomeric forms of Aβ are thought to increase intracellular calcium in dendrites and dendritic spines, which then activates calcineurin. Interestingly, calcineurin activation has been

proven necessary and sufficient for synaptic degeneration (Wu et al., 2010; Rozkalne et al., 2011; Cavallucci et al., 2013). Similarly, Aβ induces the internalization of AMPAR and NMDAR via the same pathways involving calcineurin in NMDAR-LTD (Snyder et al., 2005). Furthermore, soluble Aβ activates pro-apoptotic pathways involving caspase-3 (Chen et al., 2013), thus promoting neuronal death.

Less is known about how pathological changes in tau lead to synaptic loss. Tau is believed to act downstream of Aβ and its pathological forms are transferred through synaptic circuits. Interestingly, because of its microtubule-stabilizing role, pathological modifications in tau results in impaired cellular transport to the mitochondria and synaptic receptors (Kopeikina et al., 2013). Soluble forms of tau have also been suggested to be the culprit in synaptotoxicity. Oligomeric tau is found in synapses of human AD brain (DeKosky and Scheff, 1990; DeKosky et al., 1996; Tai et al., 2012). Mice containing the MAPT P301L mutation, rTg4510 mice, exhibit altered synaptic function and synaptic degeneration. Furthermore, in the same mouse model, memory deficits can be reversible even in the presence of tangles (Santacruz et al., 2005) and are correlated with soluble oligomers of tau (Berger et al., 2007). Together, these studies highlight how Aβ and tau disrupt synaptic function, which then leads to synaptic loss and promotes neuronal death.

1.1.7 Rodent models of Alzheimer's disease

1.1.7.1 Transgenic AD models

Several animal models have been created in order to model and study AD. Most of them have used a genetic approach, by targeting the genes involved in autosomal dominant AD. Transgenic AD models are obtained by integration of specific gene encoding for proteins involved in AD pathology. Over two decades ago, the first AD mouse models were overexpressing genes implicated in AD. The goal of this approach was to recapitulate the key histopathological lesions of AD, senile plaques and NFTs, found in human brains.

In 1995, Games et al. created the first transgenic model of AD, the PDAPP mouse, which expressed human APP with the Indiana familial AD mutation (V717F) (Games et al., 1995). These

mice exhibit several features of human AD, including memory loss, extracellular Aβ deposition, dystrophic neurites, synaptic loss and gliosis. Developed in 1996 by Hsiao et al., the Tg2576 model is one of the most characterized and extensively used mouse models of AD. These mice express human APP (isoform 695) with the double mutation K670N/M671L, also known as Swedish mutation, under the control of the hamster prion protein promoter (Hsiao et al., 1996). They display progressive cognitive impairments and elevated levels of Aβ by the age of 11-13 months. In addition, the Swedish mutation was also expressed in another mouse model created in 1997, the APP23 mouse model (Sturchler-Pierrat et al., 1997), driven by the murine Thy1 promoter. Even though these early transgenic models develop Aβ deposits, they all present a serious limitation: none of them develop NFTs or display hyperphosphorylated tau.

Transgenic mouse models were also used to better understand the role of tau in AD pathology. The first NFT-developing mouse was created by expressing the P301L mutation driven by the mouse prion promoter (Lewis et al., 2000). These mice develop NFTs in an age and gene-dose dependent manner. Several other tau transgenic mice were created in the early 2000s, such as the Tau P301L mouse driven by the neuron-specific murine Thy1 promoter (Terwel et al., 2005), the Tau V337M mouse under the control of PDGF-β promoter (Tanemura et al., 2002) and the Tau R406W transgenic mouse driven by the CaMKII promoter (Tatebayashi et al., 2002). Although these transgenic mouse models recapitulate several pathological hallmarks of AD, such as tangles, changes in LTP and cognitive impairments, they all lack amyloid plaques deposition.

Combinations of AD- related genes have also been used to overcome the shortcomings of previous models. For example, Tg2576 mice develop plaques only at 9 months of age. Because of this late onset of AD phenotype, researchers usually use these mice at the age of 12 months. One advantage to use the APP/PS1 mice instead of the Tg2576 mice is that they develop plaques at the age of 6 months. The onset of the AD phenotype occurs earlier in the APP/PS1 mice. This mouse model was made by co-injecting two plasmid vectors, one encoding the mutant APP and the other one encoding the mutant PS1. APP/PS1 mice are extensively used in AD research. They also exhibit a higher amyloid production and deposition than Tg2576 mice.

In 2003, the LaFerla lab generated the widely used 3xTg-AD mice, which harbors 3 mutations associated with familial Alzheimer's Disease. These mice were generated by injecting the single-cell embryos from mice with knock-in of PSEN1 with the $PS1_{M146V}$ mutation with two human transgenes, APP with the Swedish mutation and $MAPT_{P301L}$, both driven by the mouse Thy1.2 promoter (Oddo et al., 2003). This mouse model was the first to develop both plaques and tangles in an age- and regional-specific manner. Extracellular Aβ deposition is detectable in the frontal cortex at the age of 6 months and eventually progresses to the hippocampus when amyloid pathology becomes more extensive. Tau pathology, on the other hand, isn't present at the age of 6 months and occurs later in the hippocampus around the age of 12 months. Cognitive impairments first manifest as retention deficits at the age of 4 months. Learning deficits occurs later around the age of 6.5 months, as shown in the Barnes maze (Stover et al., 2015). Moreover, by the age of 6 months, these mice exhibit basal synaptic transmission impairment. Compared to WT controls, male 3xTg-AD mice show a decreased LTP and paired-pulse facilitation (Oddo et al., 2003). Overall, these mice display several neuropathological and behavioural features of AD pathology such as extracellular Aβ deposits, NFTs, gliosis, synaptic dysfunction and cognitive impairments. Sex differences in cognition, behavioral measures and amyloid pathology have been reported in several publications in this mouse strain, which makes it an interesting target for our investigation (Clinton et al., 2007; Hirata-Fukae et al., 2008; Carroll et al., 2010; Yang et al., 2018). However, to our knowledge, no study has investigated at whether the ability to modify synaptic connections (*i.e.* LTP and LTD) was different between males and females.

Although transgenic mouse models do not fully recapitulate human AD pathology, they have proven to be an indispensable tool for AD research, especially for the design of human clinical trials. They also helped elucidating the role of several genes encoding proteins involved in AD. Today, most mouse models of AD are overexpressing a mutated form of the human APP gene, with or without mutated tau. The main limitation of genetic mouse models is that they mostly target the genetically driven autosomal dominant forms of AD, which only represents 1-2% of all AD cases.

1.1.7.2 Non-genetic AD models

Non-genetic AD models have been used on their own and in combination with transgenic models to overcome their limitations. Non-transgenic AD models are mostly obtained by injecting either Aβ or tau in the ventricles or the hippocampus via intracranial stereotaxic injections. These models have been shown to better recapitulate sporadic AD (Bird, 2008; Puzzo et al., 2014), which accounts for the majority of AD cases. Additionally, they have several advantages compared to genetic models. Intracranial injections are useful to study the role of Aβ or tau species in animals at different concentrations. They can also be used to study the role of Aβ in the absence of APP and its fragments. Moreover, non-transgenic models allow the investigation of the differences between acute or chronic administration. However, the time-course seen in human AD is not replicated in non-transgenic models (Puzzo et al., 2015). Other major drawbacks include indirect effects of the invasive intracerebral injection on surrounding brain tissue and the inability to propagate the mice as lines (Götz et al., 2018).

1.1.7.2.1 $A\beta_{25-35}$ infusion model

The $A\beta_{25-35}$ infusion model was first used in 1996 by Maurice et al. in male mice to induce AD dementia. They performed an acute intracerebroventricular (i.c.v.) injection of an aggregated $A\beta_{25-35}$ peptide into mouse brain (Maurice et al., 1996). They found that i.c.v injection of $A\beta_{25-35}$ led to impaired spontaneous alternation behavior, water-maze learning, and passive avoidance in male mice (Maurice et al., 1998a). These findings were replicated several times (Stepanichev et al., 2003; Meunier et al., 2006; D'Agostino et al., 2012; Zussy et al., 2013; Maurice, 2016). In rodent brains, acute injection of aggregated $A\beta_{25-35}$ peptide, the highly amyloidogenic region of Aβ, recapitulates several aspects of AD pathology, including impairments in learning and memory, neuronal loss, oxidative stress, Aβ deposition and tau hyperphosphorylation (Maurice et al., 1996; Delobette et al., 1997; Villard et al., 2009; Chavant et al., 2010; Lahmy et al., 2013). Interestingly, this peptide is physiologically present in AD patients and is generated by the proteolysis of $A\beta_{1-40}$ *in vivo* (Kubo et al., 2002). $A\beta_{25-35}$ also retains the toxicity and aggregation properties of full-length $A\beta_{1-42}$ (Tarozzi et al., 2010). Furthermore, administration of $A\beta_{25-35}$ has been shown to alter APP processing and to promote the amyloidogenic pathway, thus resulting in the accumulation of endogenous $A\beta_{1-42}$ in the hippocampus (Meunier et al., 2013).

1.1.7.3 Sex differences in animal models of Alzheimer's disease

Just as sex differences are seen in AD patients, sex differences are also observed in AD mouse models. Increased cognitive impairment and earlier onset of deficits in female mice compared to male mice have been characterized in several mouse models, including Tg2576, APP/PS1 and 3xTg-AD lines (Dubal et al., 2012). Most cognition and behavioral studies have compared gonadally intact males and females.

In the Tg2576 mouse model, King et al. reported sex-related cognitive deficits at 3 months and 9 months of age (King et al., 1999). Interestingly, these deficits were progressive in nature and occurred before amyloid deposition in the brain. Another study also found that females exhibit more senile plaques and higher levels of $A\beta_{40}$ than males at the age of 15 and 19 months (Callahan et al., 2001). Regarding cognitive decline, a recent study indicated that Tg2576 females have a worse reference memory than males at 12, 14 and 16 months of age (Schmid et al., 2019). Schmid et al. also found a sex-dependent correlation between amyloid pathology and cognitive impairments in Tg2576 mice. They suggested that females are more vulnerable to the effects of Aβ deposition than males (Schmid et al., 2019). The higher amyloid pathology in Tg2576 female mice may be due to differential levels of transgene expression (Clinton et al., 2007).

Sex differences have also been found in the several APP/PS1 mouse models. Wang et al. reported that female APP/PS1 (A246E) mice had a higher amyloid burden and number of plaques than age-matched males, at 12 and 17 months of age (Wang et al., 2003). Another study has indicated that 8-month-old APP/PS1(dE9) females traveled a longer distance compared to non-transgenic females in the open field test. APP/PS1(dE9) males, on the other hand, did not show any cognitive deficits (Melnikova et al., 2006).

In the triple transgenic mouse model of AD (3xTg-AD), females show poorer cognitive performance than males in the MWM and inhibitory avoidance (IA) task at 6 and 9 months (Clinton et al., 2007). Additionally, at that age, Pietropaolo et al. revealed that female mice exhibit a decrease in activity, spatial memory novel arm preference compared to female control mice. Male 3xTg-AD mice, on the hand, did not show any deficits compared to male controls

(Pietropaolo et al., 2008). Conversely, another study found that female 3xTg-AD mice had an increased activity in the open field test compared to female non-transgenic mice. Curiously, male 3xTg-AD mice had the opposite results: they had a decreased activity in the open field test (Giménez-Llort et al., 2010). In the light-dark box test, used to measure anxiety levels, transgenic females had higher number of entries into light then non-transgenic females, while transgenic males had the opposite results, thus suggesting that females were more anxious. Among 3xTg-AD mice, females showed a decrease in spontaneous alternating behavior in the Y-maze test compared to non-transgenic female and male 3xTg-AD mice at the age of 12-14 months (Carroll et al., 2010). Additionally, aged 3xTg-AD mice show deficits in short-term and working memory. Interestingly, sex differences are reflected by increased cognitive deficits in females and increased behavioral inhibitions in males (Blázquez et al., 2014). Furthermore, aged 3xTg-AD females exhibit increased pathological hallmarks, neuroinflammation and spatial cognitive deficits compared to males (Yang et al., 2018). Female 3xTg-AD mice also have higher levels of Aβ peptides and plaques, higher β-secretase activity and lower neprilysin activity compared to males (Carroll et al., 2010). However, no sex differences have been found regarding tau pathology (Hirata-Fukae et al., 2008).

Several studies have compared AD-related measures in male versus female mice in various AD mouse models. However, they have reported varying results, thus making overall conclusions challenging. This variation in data could be mostly due to the complex comparison of gonadally intact males with gonadally intact cycling females. Even within the same AD model, reports of sex differences in cognition, behavior, baseline Aβ levels and amyloid plaque deposition, differ considerably (Dubal et al., 2012).

Taken together, cumulative evidence has shown that sex differences in disease severity and progression are present in several AD mouse models. Female AD mice exhibit a more rapid cognitive decline than their male counterparts. The reasons underlying this faster disease progression observed in females are still unknown. We believe that Aβ- and tau-induced deficits may also sex specific. As mentioned above, there is an urgent need for the development of novel therapies to prevent and treat AD pathophysiology. An emerging and promising avenue involves the sigma-1 receptor (Sig1R), which has shown anti-amnesic and neuroprotective effects in both AD mouse models and AD patients. Sig1R has been shown to modulate voltage-gated ion channels

and NMDARs, which could impact LTP. Sig1R agonist, PTZ, has been shown to potentiate LTP (Martina et al., 2007), whereas its Sig1R ablation reduces LTP magnitude (Snyder et al., 2016). These findings suggest that Sig1R seems to alter synaptic plasticity and neuronal network. However, the effects of Sig1R manipulation are still unknown in the female neuronal network. The same is true for Sig1R activation in combination with AD pathology in females. Interestingly, steroid hormones modulate the Sig1R and thus its functionality may differ between males and females, which makes the Sig1R an attractive target for our investigation.

1.2 Sigma-1 Receptor

1.2.1 History

In the 1960s and 1970s, opioid receptors subtypes were divided into four classes and were named from the first letter of their selective ligands, leading to the name of μ (morphine), δ (vas deferens), κ (ketazocine), and σ (SKF-10,047). Discovered in 1976 by Martin et al., sigma receptors were first classified as opioid receptors. In his experiment in chronic spinal dogs, Martin et al. attributed the psychotomimetic effects of SKF-10,047 (N-allylnormetazocine) to a new type of receptor at the time, which differed from the known μ and κ opioid receptors. Interestingly, this novel receptor was also blocked by naloxone, a universal opioid antagonist. Martin et al. named this receptor based on its ligand-binding properties. Later, sigma receptors were classified in two subtypes, sigma-1 and sigma-2 (Sig2R). They are mostly distinguished by their binding affinity to specific ligands, such as benzomorphans, and their molecular characteristics (Hellewell et al., 1994).

The Sig1R gene was first cloned in 1996 from guinea pig liver (Hanner et al., 1996) and revealed that this receptor is not part of the opioid receptor family, because of its dissimilar sequence. Sig1R consists of 223 amino acids and its unique sequence does not resemble of any other mammalian protein. Sig1R was later cloned from mouse kidney, human cell lines, rat brain and mouse brain (Kekuda et al., 1996; Seth et al., 1997; Seth et al., 1998). Notably, Sig2R was recently cloned from calf liver (Alon et al., 2017). For a long time, it was thought that Sig1R to had two transmembrane domains (Su et al., 2010; Brune et al., 2014; Ortega-Roldan et al., 2015). However, the recent crystal structure of the human Sig1R proved otherwise. Sig1R has a trimeric architecture with one transmembrane domain for every subunit (Schmidt et al., 2016). Since the

cloning of the Sig1R and its subsequent crystallization, our understanding of the Sig1R has greatly expanded in both physiological and pathological processes. The Sig1R has been implicated in a variety of diseases, including cocaine addiction, neuropathic pain, Huntington's disease, juvenile amyotrophic lateral sclerosis and more importantly, AD. The interest surrounding the Sig1R is driven by the hope that its modulation could result in a novel therapeutic strategy for various conditions and diseases.

1.2.2 Localization and function

Sig1R is a transmembrane protein located specifically at the mitochondrion-associated endoplasmic reticulum (ER) membrane (MAM) where it acts as an unusual molecular chaperone. Under physiological conditions, Sig1R is sequestered with binding immoglobulin protein (BiP) at the MAM. This transmembrane protein interacts with various targets, including a wide number of ion channels, receptors and kinases (Kourrich et al., 2012; Nguyen et al., 2015). Furthermore, Sig1R can directly modulate voltage-gated channels, including sodium channels, potassium channels and more importantly, calcium channels.

Interestingly, the localization of Sig1R is highly dynamic. Upon overexpression or stimulation by an agonist, Sig1R can dissociate from BiP and either acts within the MAM or translocate from the MAM to the plasma membrane. Sig1R moves within the cell via translocation and protein-protein interactions. At the plasma membrane, it influences synaptic function by acting on ion channels and neurotransmitter release (Hayashi and Su, 2003, 2007). Activation of Sig1R has been shown to have neuroprotective actions through regulation of intracellular calcium homeostasis and glutamate activity. Sig1R has also been shown to regulate synaptic plasticity. For example, stimulation of Sig1R by dehydroepiandrosterone (DHEA), the most abundant neurosteroid in the CNS, promotes hippocampal LTP in the CA1 region (Moriguchi et al., 2011).

1.2.3 Pharmacology

One of the interesting properties of Sig1R is that it has several endogenous and exogenous ligands that modulate its activity. Pharmacological studies investigating the function of Sig1R have identified specific ligands either as agonist or antagonist of Sig1R. For some of these compounds,

there is clear definition regarding their designation. However, some commonly cited Sig1R agonists are: (+)-pentazocine (PTZ), PRE-084, SKF-10,047 and SA4503. These ligands recapitulate the phenotype of receptor activation or overexpression. On the other hand, compounds that are defined as antagonists mimic the gene knockdown phenotype. Common Sig1R antagonists found in the literature include: BD1047, BD1063 and NE-100. Furthermore, several drugs have been shown to interact with Sig1R, including benzomorphans, antidepressants, antipsychotics and drugs of abuse. Noteworthily, neurosteroids have been shown to modulate Sig1R. For example, sex hormones like DHEA and pregnenolone sulfate acts as a Sig1R agonist, whereas testosterone and progesterone act as an antagonist and reverse the potentiation induced by DHEA (Bergeron et al., 1996; Monnet and Maurice, 2006). Together, this indicates that the functionality of Sig1R may differ between males and females. Excitingly, because of its numerous modulating actions, Sig1R is an interesting target for neuroprotection in many neurodegenerative diseases, including AD.

1.2.4 Sigma-1 Receptor and Alzheimer's Disease

Sig1R binding sites have been found to be decreased in AD. An early postmortem study indicated that Sig1R were reduced in the hippocampus region of AD patients (Jansen et al., 1993). Similarly, Sig1R protein levels were significantly lower in human postmortem cortical tissue from AD patients compared to controls (Hedskog et al., 2013). Another study also reported loss of Sig1R in the early phase of AD using PET imaging (Mishina et al., 2008). These observations suggest that decreased Sig1R levels may affect vulnerability to AD or contribute to AD progression. Interestingly, a genetic polymorphism of Sig1R has been associated with reduced levels of Sig1R, which may modify risk of AD (Miyatake et al., 2004; Huang et al., 2011). Genetic combinations of Sig1R and ApoE ε4 allele have been found to interact with each other and increase the risk of AD (Uchida et al., 2005; Maruszak et al., 2007; Huang et al., 2011; Fehér et al., 2012). More importantly, Sig1R agonists have been shown to improve cognitive function and have anti-amnesic and neuroprotective effects *in vitro* and in animal models of AD, including the $A\beta_{25-35}$ infusion model. Sig1R agonists, such PRE-084, ANAVEX2-73, ANAVEX1-41, DHEA, DHEA sulfate and pregnenolone sulfate, have been shown to decrease $A\beta_{1-42}$, hyperphosphorylated tau, oxidative stress and cognitive impairment in that specific mouse model (Meunier et al., 2006; Maurice and Goguadze, 2017). Moreover, the activation of GSK3β, the main kinase involved in tau

phosphorylation, is also decreased following Sig1R agonist treatment (Lahmy et al., 2013). Conversely, loss or dysfunction in Sig1R are associated with neurodegeneration (Nguyen et al., 2015) and could influence AD progression. Noteworthily, several Sig1R agonists are currently used in preclinical trials and have shown exciting and promising results. For example, administration of the mixed muscarinic/Sig1R agonist AF710B treated AD hallmarks by reducing $A\beta_{1\text{-}42}$, plaques, tau phosphorylation, GSK3β and BACE1 activity in 3xTg-AD mice (Fisher et al., 2016). Furthermore, rodent AD models treated with AF710B showed improved cognitive performance (Fisher et al., 2016; Hall et al., 2018). Together, these studies indicate that Sig1R agonists demonstrate neuroprotective properties against AD. Additionally, these reports suggest that treatment with Sig1R agonists can alleviate pathological hallmarks, thus supporting Sig1R as a potential therapeutic target in AD.

2. HYPOTHESIS

We hypothesize that the activation of Sig1R, a known neuroprotector, may sex-specifically reduce the synaptic deficits induced by Aβ and tau.

3. OBJECTIVES

1. Determine how Sig1R protein levels are altered by sex and AD pathology
2. Determine how hippocampal plasticity is altered by sex and AD pathology and if changes can be rescued by Sig1R activation
3. Evaluate the structural integrity of the hippocampus in AD mouse models

4. MATERIAL AND METHODS

4.1 Animals

All procedures were performed in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the University of Ottawa Animal Care Committee. Non-transgenic (NonTg) control mice (B6129SF1/J) (101043 JAX, Bar Harbour, ME), and Alzheimer's model mice (3xTg-AD) homozygous for the Psen1 mutation and homozygous for the co-injected APPSwe and tauP301L transgenes (B6/129-Psen1^{tm1Mpm} Tg (APPSwe, tauP301L)1Lfa/Mmjax), obtained from the MMRRC (MMRRC Stock No: 34830-JAX) were bred in house. Another strain of mice, Sig1R wild-type (WT) (B6;129S5-*Sigmar1*$^{Gt(OST422756)Lex}$/Mmucd, MMRRC Stock No: 011750-UCD) (Jackson Laboratory, Maine, USA), herein after WT mice, was also used for western blot, electrophysiology and MRI experiments. To mimic the hormone deprived state of the human female AD population, all female WT mice were ovariectomized (OVX) one week prior to lateral *i.c.v.* injections of $A\beta_{25\text{-}35}$ (Sigma, St-Louis, USA) or $A\beta_{25\text{-}35}$ scrambled (SCR) (AnaSpec, California, USA). All strains of animals were kept on a 12-hour light/dark cycle, with *ad libitum* access to food and water. Experiments were conducted when animals ranged from 3 months to 12 months of age, as noted.

4.1.1 Triple transgenic mouse model

3xTg-AD mice mimic the neuropathology of AD. This animal model progressively develops Aβ and tau pathology in a temporal- and regional-specific manner that closely resemble the development in the human AD brain (Oddo et al., 2003). At the age of 6 months, extracellular Aβ deposits initially accumulate in the frontal cortex and progress to the hippocampus. Hyperphosphorylated tau starts its deposition at the age of 12 months in the hippocampus and progresses to the cortex (Oddo et al., 2003). By the age of 6 months, 3xTg-AD mice show an impairment in basal synaptic transmission. They have a smaller LTP magnitude compared to wild-type controls (Oddo et al., 2003). These mice also exhibit cognitive impairments as early as 4 months of age. At this time point, 3xTg-AD mice show retention deficits during the acquisition phase of the Morris Water Maze (MWM) task (Billings et al., 2005).

4.1.2 $A\beta_{25-35}$-infusion mouse model

Infusion models have been shown to better recapitulate the process of sporadic AD, which accounts for 95% of all AD cases (Bird, 2008; Puzzo et al., 2014). $A\beta_{25-35}$, the highly amyloidogenic region of Aβ, is a synthetic peptide of 11 amino acids that is often used as a model for full-length Aβ because it retains its toxicity. $A\beta_{25-35}$ has been shown to be physiologically present in elderly people and playing a relevant role in AD because of its distinct aggregation properties. This infusion model has been shown to recapitulate the main characteristics of AD such as impairments in learning and memory, Aβ plaques deposits, tau hyperphosphorylation and cell loss in the hippocampus (Maurice et al., 1998a; Zussy et al., 2013).

The *i.c.v.* $A\beta_{25-35}$ injections (-0.3 mm AP, +1.0 mm ML, -2.7 mm DV) were performed at a rate of 1µL/min using a 10 µL Hamilton syringe (Hamilton, Reno, Nevada, USA). The mice were infused with 3µL of SCR or $A\beta_{25-35}$ (9nmol). The animals were used 2-3 weeks post-surgery.

4.2 Western Blot

4.2.1 Isolation of whole-cell fraction

Cortex and hippocampus from male and female NonTg and 3xTg-AD mice were collected at 3, 6, and 9 months of age. NonTg and 3xTg-AD cortical and hippocampal tissues were homogenized in 9 volumes of RIPA buffer and protease inhibitor (Roche, Bale, Switzerland). Each sample were then centrifuged at 10,000 × *g* for 10 minutes at 4°C. The supernatant is kept as the whole-cell fraction and stored at -80°C.

4.2.2 Isolation of crude synaptosomal fraction

Differential centrifugations were performed as previously described (**Figure 3**; (Hallett et al., 2008) with minor modifications in order to isolate lysed synaptosomal membrane fraction (LP1). Hippocampal tissues from all strains (male and female NonTg, 3xTg-AD and WT mice) of animals are homogenized in 9 volumes of sucrose homogenization buffer. Samples are then centrifuged at 1000 × *g* for 10 minutes at 4°C. The supernatant (S1) is subsequently centrifuged at 15,000 × *g* for

15 minutes at 4°C. The pellet containing crude synaptosomal fraction (P2) is resuspended in sucrose homogenization buffer and then spun at 15,000 × *g* for 15 minutes at 4°C. In order to lyse the pellet via hypoosmotic shock, crude synaptosomal fraction (P2') are resuspended in 9 volumes of ice-cold ddH_2O containing protease and phosphatase inhibitors. Samples were adjusted to 4mM using 1M HEPES, pH 7.4 (1.4 μL HEPES). Tissues are then rehomogenenized and incubated for 30 minutes at 4°C, mixing continuously. Lysates are later centrifuged at 25,000 × *g* for 30 minutes. Pellets (LP1) are resuspended in 9 volumes of sucrose homogenization buffer and then stored at -80°C.

4.2.3 Antibodies

The following antibodies and dilutions were used: rabbit monoclonal anti-GluR1 (1:2000, cat. #05-855R, Millipore, MA, USA), rabbit polyclonal anti-GluR2 (1:2000, cat. #PA1-4659; Thermo Fisher Scientific, MA, USA), mouse monoclonal anti-GluN2A (1:8000, cat. #LS-C152944-100; LifeSpan Biosciences, WA, USA), mouse monoclonal anti-GluN1 (1:2000, cat. #114 011; Synaptic Systems, Göttingen, Germany), mouse monoclonal anti-GluN2B (1:4000, cat. #LS-C16904-100; LifeSpan Biosciences, WA, USA), rabbit polyclonal anti-σ-1R (1:2000; cat. #HPA018002; Atlas Antibodies, Stockholm, Sweden) and mouse monoclonal anti-β-actin (1:10000, cat. #A00730; GenScript, NJ, USA). HRP-conjugated secondary antibodies (mouse, rabbit) were purchased from Jackson ImmunoResearch (West Grove, PA, USA).

4.2.4 Western blotting

The protein concentration for all samples was determined by DC protein assay (Bio Rad). 10-15 μg of sample was loaded on 10% SDS-PAGE, transferred onto PVDF membrane and developed using Luminata Crescendo (Millipore, Darmstadt, Germany). The bands were detected using film and band intensities were quantified using NIH ImageJ (Schindelin et al., 2012).Western blot experiments were repeated 3 times. Band intensities were normalized to β-actin before comparison. β-actin was used as a loading control.

4.2.5 Statistical analysis

Differences between NonTg and 3xTg-AD mice, and between WT and $WT_{A\beta}$ mice were analyzed using a two-way ANOVA followed by Tukey's *post hoc* test ($P < 0.05$). All values are expressed as mean ± SEM. N indicates the number of animals used.

4.3 MRI acquisitions

Male and female NonTg and 3xTg-AD mice were imaged at 3 months intervals, starting at the age of 3 months (3 months, 6 months and 9 months). Male and female WT mice infused with Aβ were imaged one week before surgery and 2 weeks after. Mouse brain MRI was performed at the University of Ottawa pre-clinical imaging core using a 7 Tesla GE/Agilent MR 901. Animals were maintained at ≤ 2% isoflurane. A 2D fast spin echo (FSE) pulse sequence was used, with the following parameters: slice thickness=0.3 mm, field of view=2 cm, matrix=256x256, echo time=25 ms, repetition time=7000 ms, echo train length=8, bandwidth = 15.63 kHz, fat saturation, scan time=3.75 minutes. Respiratory gating was employed as necessary. To improve image signal-to-noise ratio, FSE was repeated several times (n≥4), then the images were motion corrected (Thevenaz et al., 1998) and averaged.

Difference between NonTg and 3xTg-AD mice, and between WT and $WT_{A\beta}$ mice were analyzed using a two-way ANOVA with the Sidak multiple comparisons test ($P < 0.05$). All values are expressed as means ± SEM. N indicates the number of animals used.

4.4 Slice preparation

Animals were anaesthetized by isoflurane inhalation (Stoelting, WoodDale, IL, USA) and sacrificed by decapitation. The brain was then quickly removed and placed in ice-cold choline chloride cutting solution containing (in mM): 119 Choline Chloride, 2.5 KCl, 4.3 MgSO4-7H20, 1 CaCl2-2H2O, 1 NaH2PO4, 1.3 Na-Ascorbate, 11 glucose, 26.2 NaHCO3 (pH 7.3, osmolarity

295 mOsm, 95% O2/5% CO2). A block of brain tissue is blocked and mounted to a stage with superglue. Coronal brain slices (300 μm thick) containing the hippocampus were cut with a vibrating microtome (Leica VT 1000S, Germany) and incubated for an hour at 37°C in an oxygenated chamber of artificial cerebral fluid (ACSF) containing (in mM): 119 NaCl, 2.5 KCl, 1.3 MgSO4-7H2O, 2.5 CaCl2-2H2O, 1 NaH2PO4, 26.2 NaHCO3, and 11 glucose. They were then allowed to equilibrate at room temperature for an hour. Following recovery, slices were transferred to a submerged recording chamber and continuously perfused with oxygenated ACSF at 33°C with the use of a perfusion chamber heater (dual channel heater controller TC-344B; Warner Instrument Corp. Hamden, CT, USA). Field recordings are usually performed at physiological ACSF temperature, which is 31-34°C. At higher temperatures, the slice quality can suffer (Abrahamsson et al., 2016). However, it is possible to induce LTP at room temperature (Feldman, 2000).

In order to produce optimal slices for recording in the 6-month-old mice, a cardiac perfusion technique was used. Once deeply anaesthetized, a lateral incision through the integument and abdominal wall is made to expose the heart. An incision is thereafter made to the animal's right atrium to create as large an outlet as possible. The animal is then perfused with 10 mL of ice-cold choline chloride solution via the left ventricle of the heart. After perfusion, the head is dissected, and the brain is removed and affixed as previously mentioned.

4.5 Extracellular field recordings

Hippocampal neurons were visualized using an upright microscope Olympus BX51WI (Hamburg, Germany) using differential interference contrast and infrared video microscopy. Extracellular field recordings were performed using borosilicate glass patch electrodes (3-5 MΩ; World Precision Instruments) pulled on a Narishige PC-10 pipette puller (Tritech Research Inc., CA, USA).

To evoke LTP, hippocampal slices were presented with a Theta-Burst Stimulation (TBS) protocol consisting of three trains of stimuli delivered at 200-millisecond intervals, each train composed of ten stimulus bursts delivered at 5 Hz, with each burst consisting of four pulses at 100 Hz. LTD was induced by a paired-pulse low-frequency stimulation (PP-LFS) protocol (50 ms

paired-pulse interval, 900 pairs of stimuli). For each hippocampal slice, stimulus intensity was set below the threshold for evoking a population spike, usually 50% of the intensity necessary to evoke a maximum fEPSP response. Field potentials were recorded in the stratum radiatum from the CA1 region of hippocampal slices from 6-month-old male and female NonTg, 3xTg-AD and WT mice. LTP and LTD recordings were performed for 75 minutes in each individual hippocampal slice (15 minutes of baseline recording and 60 minutes after the LTP/LTD induction).

4.6 Data analysis

Data were collected with a Multiclamp 700B amplifier (Axon Instruments) and acquired by a Digidata 1550 digitizer (Molecular Devices). Analysis was performed offline using Clampfit 10.3 software (Molecular Devices). Differences between NonTg and 3xTg-AD mice, and between WT and $WT_{A\beta}$ mice were analyzed using a two-way ANOVA followed by Tukey's *posthoc* test ($P < 0.05$). All values are expressed as means ± SEM. N indicates the number of slices recorded.

4.7 Drugs

Picrotoxin (10 μM) was purchased from Abcam (Cambridge, MA, USA). (+)-pentazocine (PTZ) was purchased from Sigma-Aldrich (MO, USA). PTZ was initially dissolved in warm 0.1 N HCl and was then diluted in PBS. The final concentration of the stock solution was 0.5 mg/mL.

4.8 Drug administration

Based on previous publications, mice (6 months of age) were injected intraperitoneally (*i.p.*) with 2 mg/kg PTZ (Steinfels et al., 1988; Miller et al., 1992; Beskid et al., 1998), or saline as a vehicle control. Hippocampi and cortex were isolated and collected 90 minutes following *i.p.* injection, as brain concentrations of PTZ are maximal 20-30 minutes post-injection and negligible after 120 minutes (Medzihradsky and Ahmad, 1971).

5. RESULTS

5.1 Determine how Sig1R protein levels are altered by sex and AD pathology

5.1.1 Altered expression of Sig1R protein levels in 3xTg-AD mouse model

We investigated the expression levels of Sig1R at different ages and compared these levels between males and females. In this investigation, we assumed that 3xTg-AD mice would have different disease states at different ages. Disease progression was followed in the 3xTg-AD mouse model, in which western blot analysis was performed at different time points: no sign of pathology (3 months of age), when Aβ deposits are developing (6 months of age) and when intraneuronal tau pathology starts to emerge (9 months of age) (Oddo et al., 2003; Billings et al., 2005; Oddo et al., 2006; Oh et al., 2010). In the 3xTg-AD mouse model, the expression levels were analyzed in the cortex and hippocampus.

At 3 months of age, the triple transgenic mutation significantly altered Sig1R expression levels in the cortex (**Figure 4A**, two-way ANOVA, $F(1,28) = 13.61$, $n = 8$, $p=0.0010$). However, sex did not change Sig1R protein levels in the cortex (**Figure 4A**, two-way ANOVA, $F(1,28) = 1.557$, $n = 8$, $p = 0.2224$). Tukey's *post hoc* analysis revealed that Sig1R expression levels are significantly decreased only in 3xTg-AD F compared to NonTg F (**Figure 4A**, $n = 8$, $p=0.0310$). We did not observe any changes in Sig1R expression the males. Interestingly, this change in Sig1R expression is sex-specific, even though there is no sex difference. As shown in **Figure 4B**, there was no change in Sig1R levels in the hippocampus (genotype: $F(1,20) = 1.678$, $p = 0.2099$; sex: $F(1,20) = 1.769$, $n = 6$, $p = 0.1985$). When comparing the expression of Sig1R between cortex and hippocampus, no significant differences was found between brain regions (**Figure 4C**, two-way ANOVA, $F(1,48) = 0.01210$, $n = 6$-8, $p = 0.9129$).

At 6 months of age, the triple transgenic mutation significantly altered Sig1R expression levels in the cortex (**Figure 4D**, two-way ANOVA, $F(1,19) = 5.459$, $n = 6$, $p=0.0306$). Tukey's *post hoc* multiple comparisons test revealed that Sig1R expression levels is significantly decreased in 3xTg-AD M compared to NonTg M (**Figure 4D**, $n = 6$, $p=0.0230$). Interestingly, sex significantly altered Sig1R expression levels in the hippocampus (**Figure 4E**, two-way ANOVA, $F(1,20) = 7.628$), $n = 6$, $p=0.0120$). Furthermore, there was a significant interaction between sex

and the triple transgenic mutation (**Figure 4E**, two-way ANOVA, F(1,20)= 9.372), n = 6, p= 0.0062). Tukey's *post hoc* multiple comparisons test revealed that Sig1R expression levels were significantly increased in NonTg F compared to NonTg M (n = 6, p=0.0028). Moreover, Sig1R protein levels were significantly decreased in 3xTg-AD F compared to NonTg F (p=0.0083). When comparing Sig1R expression levels between cortex and hippocampus, we found that Sig1R is significantly increased in the hippocampus compared to the cortex (**Figure 4F**, two-way ANOVA, F (1.39) =27.13, n = 6, p<0.0001).

At 9 months of age, the triple transgenic mutation significantly altered Sig1R expression levels in the cortex (two-way ANOVA, F (1,12) = 4.791, n = 4, p=0.0491) and in the hippocampus (F(1,19)= 5.289), n = 5-6, p=0.0330). Sex did not affect Sig1R protein levels in the cortex (two-way ANOVA, F (1,12) = 2.130, n = 4, p = 0.1701) or in the hippocampus (two-way ANOVA, F (1,19) = 0.6035, n = 5-6, p = 0.4468). We were expecting that the decrease in Sig1R levels observed in the cortex at 6 months in 3xTg-AD M would also be present at the age of 9 months. A possible explanation is that the n at 9 months (n = 4) was lower than at 6 months (n = 6). We were also expecting that the decrease in Sig1R levels observed in the hippocampus at 6 months in 3xTg-AD F would be present at 9 months. We can see in **Figure 4H** that Sig1R protein levels in 3xTg-AD F showed a trend compared to NonTg F, however it was not significant (p = 0.2191). When comparing the expression of Sig1R between cortex and hippocampus, no significant differences were found (two-way ANOVA, F (1,31) = 1.138, p = 0.2943).

Taken together, our results show that AD pathology significantly alters Sig1R expression, even before Aβ deposition. Furthermore, sex only had a significant effect at mild Aβ pathology in the hippocampus. There was also a significant difference in Sig1R expression between the cortex and the hippocampus at this disease stage. Surprisingly, this effect was lost at more severe Aβ pathology.

5.2 Determine how hippocampal plasticity is altered by sex and AD pathology and if changes can be rescued by Sig1R activation

5.2.1 Male mice exhibit larger LTP magnitude

In 2003, when Oddo and colleagues characterized the 3xTg-AD mouse model, they reported that LTP is impaired at 6 months (Oddo et al., 2003). However, their study only included male mice. To investigate how basal synaptic transmission is affected by sex and AD pathology, we first performed extracellular field recordings in CA1 region of the hippocampus in 6-month-old male and female NonTg and 3xTg-AD mice. LTP was induced by stimulating Schaffer's collateral with the TBS protocol. LTP was then measured in the *stratum radiatum* region. **Figure 5A** shows representative fEPSPs from male and female NonTg and 3xTg-AD mice. Unexpectedly, we found no overall differences between NonTg and 3xTg-AD (**Figure 5**) (two-way ANOVA, $F(1, 22) = 0.01990$, $p = 0.8891$). Interestingly, we found a sex-specific baseline difference in LTP magnitude, male mice exhibit a larger LTP magnitude than female mice (**Figure 5B**, two-way ANOVA, $F(1, 22) = 10.49$, $P=0.0038$).

5.2.2 LTP is impaired in male and female WT mice following Aβ infusion

It is well established that $A\beta_{25-35}$ reduces LTP and induces memory deficit (Chen et al., 2000; Haass and Selkoe, 2007; Zhang et al., 2009). Previous reports have demonstrated that i.c.v. injections of $A\beta_{25-35}$ impairs LTP in the CA1 region of the rat hippocampus *in vivo* (Freir et al., 2001) and *in vitro* (Freir et al., 2003). However, these studies only used male animals. To further examine whether basal synaptic transmission is affected by sex and AD pathology, we performed extracellular field recordings in the CA1 hippocampal region. LTP was investigated in 6-month-old male and female mice infused with $A\beta_{25-35}$. LTP was measured in the *stratum radiatum* region after theta-burst stimulation. **Figure 6A** shows representative fEPSPs from male and female WT and $WT_{A\beta}$. We found that Aβ infusion significantly reduces LTP in male and female WT mice (**Figure 6B**, two-way ANOVA, $F(1,27) = 17.12$, $p= 0.0003$). To our surprise, sex did not affect LTP magnitude ($F(1,27) = 0.02618$, $p=0.9727$). **Figure 6C** shows the average fEPSP in the last 5-min post-tetanus for M-WT, M-$WT_{A\beta}$, F-WT and F-$WT_{A\beta}$. Tukey's *post hoc* multiple comparisons test revealed that LTP is significantly decreased in M-$WT_{A\beta}$ compared to M-WT ($p=0.0268$) and in F-$WT_{A\beta}$ compared to F-WT ($p=0.0399$).

5.2.3 Triple transgenic mutation and Aβ infusion facilitates LTD in male mice

LTD has been shown to be unaffected or enhanced in the presence of Aβ (Wang et al., 2002; Hsieh et al., 2006; Shankar et al., 2007; Shankar et al., 2008). To further our study on whether $A\beta_{25-35}$ infusion alters synaptic plasticity, we also compared LTD in the male and female 3xTg-AD mice and WT mice infused with $A\beta_{25-35.}$ LTD was measured in the *stratum radiatum* region after paired-pulse low-frequency stimulation. **Figure 7A** shows representative fEPSPs from male and female NonTg and 3xTg-AD mice. To our surprise, we observed in the male NonTg mice an initial depression followed by a facilitated response over time, even though the stimulation protocol used meant to induce LTD. However, in the male 3xTg, we found that the initial depression lessened after a few minutes (~15 min) but resulted in a sustained depression over time. Two-way ANOVA revealed that LTD did not differ by sex (**Figure 7B**, F (1,18) = 1.068, p=0.3151). However, triple transgenic mutation showed a trend on LTD magnitude, which did not reach significance (F (1,16) = 3.734, p=0.0712). Tukey's *post hoc* multiple comparisons test revealed that LTD was significantly different between male NonTg and male 3xTg-AD (p=0.0263). **Figure 8A** shows representative fEPSPs from male and female WT and $WT_{A\beta}$. We found that LTD is significantly facilitated in male WT mice infused with Aβ. Two-way ANOVA revealed that LTD did not differ by sex (**Figure 8B**, F (1,18) = 1.068, p=0.3151). However, Aβ infusion showed a trend on LTD magnitude, which did not reach significance (F (1,18) = 3.800, p=0.0670). Tukey's *post hoc* multiple comparisons test revealed that LTD was significantly different between M-WT and M-$WT_{A\beta}$ (p=0.05). Taken together, our results suggest that long-term depression is sex-specifically altered in AD mice.

5.2.4 Sig1R agonist, PTZ, rescues LTP in Aβ-infused mice

Considering the interest of our laboratory towards the Sig1R and knowing that several publications have reported that Sig1R agonists can ameliorate Aβ-induced deficits (Maurice et al., 1998b) , we wanted to test if PTZ, a selective Sig1R agonist, could rescue the Aβ-induced deficits observed in LTP in male and female WT mice (**Figure 9**). Three-way ANOVA analysis was used, with the 3 factors being: sex, Aβ treatment and PTZ treatment. We found that PTZ rescues Aβ-induced deficits in LTP in male and female WT mice (**Figure 9C,** three-way ANOVA, F(1,28)= 10.94, p=0.0026). Tukey's *post hoc* multiple comparisons analysis revealed that LTP magnitude

in F-$WT_{A\beta}$ compared to F-WT mice is significantly greater (p=0.0016). Interestingly, our analysis showed a trend for the effect of sex which did not reach significance (three-way ANOVA, F(1,20)=3.201, p=0.0888). LTP magnitude between M-$WT_{A\beta}$ and F-$WT_{A\beta}$ when treated with PTZ was significant (p=0.0442). These findings suggest that PTZ may differently act on male and female mice.

5.2.5 Sig1R agonist, PTZ, doesn't affect LTD in female Aβ-infused WT mice

Next, we looked at the effect of PTZ on LTD in female WT mice. We compared LTD in F-WT and F-$WT_{A\beta}$ mice following PTZ treatment. We performed extracellular field recordings in CA1 hippocampal region. LTD was again induced by using a paired-pulse low-frequency. **Figure 10A** shows representative fEPSPs in slices from F-WT, F-$WT_{A\beta}$, F-WT PTZ and F-$WT_{A\beta}$ PTZ mice. We found no difference in LTD magnitude between all experimental groups. **Figure 10B** shows that the average fEPSP in the last 5-min post-tetanus for F-WT was 1.46 ± 0.18 compared to 1.38 for F-WT PTZ and 1.38 ± 0.09 for F-$WT_{A\beta}$ compared to 1.15± 0.08 for F-$WT_{A\beta}$ PTZ (F-WT $n = 5$, F-$WT_{A\beta}$ $n = 5$, F-WT PTZ $n = 6$, F-$WT_{A\beta}$ PTZ $n = 6$, two-way ANOVA).

5.2.6 Increased GluA2 expression levels in female mice

Aβ alters the function of AMPARs and NMDARs (Ondrejcak et al., 2010; Paula-Lima et al., 2013; Tu et al., 2014). However, the importance of sex remains unclear. To further our investigation about how synapses are altered by sex and AD pathology, we looked at the synaptic protein levels of AMPAR subunits (GluA1, GluA2), NMDAR subunits (GluN1, GluN2A, GluN2B) and Sig1R in the hippocampus at 6 months of age. For this purpose, we used both the 3xTg-AD mouse model and the $A\beta_{25\text{-}35}$ infusion model. The analysis of NMDARs subunits protein levels revealed no difference between AD phenotype compared to controls (**Figure 11B & 12B, Table 3**). Furthermore, western blot analysis revealed no change in GluA1 protein expression in either mouse model (**Figure 11B & 12B**, **Table 3**). Two-way ANOVA revealed no significant effect for both sex (F (1,20) = 0.4677, p=0.8857) and Aβ treatment (F (1,20) = 1.253, p = 0.2762) in the $A\beta_{25\text{-}35}$ infusion model. Similarly, in the 3xTg-AD mice, three-way ANOVA revealed no

significant effect for both sex (F (1,16) = 0.1991, p=0.6614) and triple transgenic mutation (F (1,16) = 1.483, p = 0.2409)

Interestingly, in both mouse models, GluA2 protein levels were increased in females compared to males in the hippocampal synaptic fraction (**Figure 11B & 12B**, **Table 3**). In the $A\beta_{25-35}$ infusion model, two-way ANOVA showed a significant effect of sex (F (1,20) = 8.489, p=0.0086), but not for genotype (F (1,20) = 0.2148, p=0.6480). Similarly, in 3xTg-AD mice, statistical analysis showed a significant effect of sex (p = 0.0326), but not for genotype (p = 0.4833).

Taken together, GluA1 and NMDAR subunits (GluN1, GluN2A, GluN2B) protein levels did not differ by sex or by AD phenotype in both mouse models. Only GluA2 expression levels were affected by sex.

5.2.7 Sig1R agonist, PTZ has no effect on AMPARs and NMDARs subunits expression levels

Sig1R agonists have been shown to mediate trafficking of NMDARs to the cell surface. More specifically, the expression of GluN2A and GluN2B subunits have been found to be upregulated in the hippocampus following *in vivo* administration of SKF 10047, PRE-084 and PTZ (Pabba et al., 2014). However, the effects of Sig1R agonists on female has been unexplored. We looked at the effect of the well-known Sig1R agonist, PTZ, on the protein levels of AMPAR subunits (GluA1, GluA2), NMDAR subunits (GluN1, GluN2A, GluN2B) and Sig1R in the hippocampus at 6 months of age in the 3x-Tg-AD mouse model. Three-way ANOVA analysis was used, with the 3 factors being: sex, genotype and PTZ treatment. Surprisingly, following a 90-minute treatment with PTZ, statistical analysis revealed that GluN1 (p=0.6689), GluN2A (p=0.7225) and GluN2B (p=0.2068) subunits expression levels do not significantly change (**Figure 12B**, **Table 3**). Interestingly, the three-way ANOVA for GluN2A protein expression revealed a main effect of sex (p=0.0195) and an interaction between genotype and sex (p=0.0397). However, PTZ treatment did not affect the expression of GluA1.

5.3 Evaluate the structural integrity of the hippocampus in AD mouse models

5.3.1 Aβ infusion decreases hippocampal structural integrity in WT mice

Aβ infusion has been shown to lead to cell loss in the hippocampus (Zussy et al., 2013). However, no study has examined at the macroscopic level how the hippocampus as a whole was altered by sex and Aβ infusion. We evaluated the hippocampal volume of male and female WT mice following Aβ infusion using the MRI technique. **Figure 13A-H** shows representative coronal T2-weighted magnetic resonance images from M-WT, M-$WT_{A\beta}$, F-WT and F-$WT_{A\beta}$ one week prior surgery and 2 weeks after. Statistical analysis revealed that Aβ infusion reduced hippocampal volumetry in male and female WT mice (**Figure 13I-J**, two-way ANOVA, $F(1, 13) = 6.432$, $p=0.0248$). Interestingly, sex showed a trend, but did not reach significance (**Figure 13I-J**,two-way ANOVA, $F(1,13)= 4.433$, $p=0.0553$), thus suggesting that Aβ may differently lead to neuronal death in males and females.

5.3.2 The structural integrity of the hippocampus is impaired in aged male and female 3xTg-AD mice

Previous studies have shown that the volume of the hippocampus is decreased in 3xTg-AD mice (Chiquita et al., 2019; Güell-Bosch et al., 2020). However, none of these reports used male and female mice in their investigation. Here, we wanted to look at the hippocampal volume over time in the 3xTg-AD mice and determine if sex impacts hippocampal volumetry. To do so, we used the MRI technique to monitor the development of AD over time within the same mouse in a non-invasive manner.

We followed the progression of AD in the 3xTg-AD mouse model, which is known to develop Aβ and tau pathology is an age-dependent manner (Oddo et al., 2003). Disease progression was followed in the 3xTg-AD mouse model, in which MRI hippocampal volume analysis was performed at different time points: no sign of pathology (3 months of age), when Aβ deposits are developing (6 months of age) and when intraneuronal tau pathology starts to emerge (9 months of age).

The evolution over time of the hippocampal volume analyzed is shown in **Figure 14**. At 3 months of age, there was no significant difference in hippocampal volume between NonTg and 3xTg-AD mice. Significant differences between NonTg and 3xTg-AD mice started to emerge in

both males and females at 6 months of age and were still present at 9 months of age. This timeframe is in line with the appearance of extracellular plaques, synaptic dysfunction and LTP impairment reported by Oddo and colleagues (Oddo et al., 2003). No sex differences have been found regarding the hippocampal volume of NonTg and 3xTg-AD mice. Taken together, our results show that the volume of the hippocampus is impaired in both male and female 3xTg-AD mice over time.

6. DISCUSSION

The overall aim of this thesis was to understand how Sig1R sex-specifically modulates AD pathology using two separate AD mouse models: $A\beta_{25-35}$ infusion model and 3xTg-AD. We hypothesize that the activation of Sig1R, a known neuroprotector, may sex-specifically reduce the synaptic deficits induced by Aβ. We first investigated how Sig1R protein levels are altered by sex and AD pathology. Next, we aimed to determine how synapses are modified by sex and AD pathology and if these changes can be rescued by Sig1R activation. Given that synapse loss is a morphological reflection of synaptic dysfunction, we finally examined the structural integrity of the hippocampus in AD mouse models.

6.1 Altered expression of Sig1R protein levels in 3xTg-AD mouse model

Sig1R levels have been reported to be significantly reduced in AD patients and models (Jansen et al., 1993; Mishina et al., 2008; Hedskog et al., 2013). However, most of these studies did not include female in their investigation. Here, we observed that Sig1R is differently expressed in the cortex and hippocampus of male and female NonTg and 3xTg-AD mice at different ages. At 3 months, when there's no sign of pathology in the hippocampus (Oddo et al., 2003), we found that Sig1R protein levels were the same between all experimental groups. However, in the cortex, we observed a significant decrease in female 3xTg-AD compared to age-matched female NonTg. Interestingly, a previous report has shown that at the age of 2-4 months, female 3xTg-AD mice had more Aβ load than age-matched males (Carroll et al., 2010). This could potentially explain why we see a sex-specific decrease in Sig1R expression in female 3xTg-AD mice but not in males. Taken together, our results are contradictory compared to a similar previous study. Hedskog et al. examined Sig1R expression levels in the hippocampus and cortex of male $APP_{Swe/Lon}$ mice, another AD mouse model that develops Aβ plaques over time. They reported that in the hippocampus, Sig1R expression levels were increased in the $APP_{Swe/Lon}$ mice compared to WT mice at 2 months, before Aβ deposition occurs (Hedskog et al., 2013). Furthermore, at that same age, they observed no changes in Sig1R protein levels in the cortex. The discrepancy between our results might be explained by the fact that we are looking at 2 different mouse models. Indeed, in the $APP_{Swe/Lon}$ mice, Aβ deposits are mostly located in the neocortex (Hedskog et al., 2013), whereas in the 3xTg-

AD mice, Aβ pathology starts in the frontal cortex and reaches the hippocampus (Oddo et al., 2003).

As AD pathology progressed and reached the hippocampus around the age of 6 months (Oddo et al., 2003), a significant difference emerged between female NonTg and female 3xTg-AD mice. In the cortex, we were expecting to see a decrease female 3xTg-AD compared to age-matched female NonTg, as we previously observed at the 3-month time point. However, we curiously found a significant decrease in Sig1R expression in male 3xTg-AD compared to age-matched male NonTg mice, but no decrease in the females. The reason for this outcome is unclear.

At 9 months, when tau pathology begins (Oddo et al., 2003; Billings et al., 2005; Oddo et al., 2006; Oh et al., 2010), both male and female 3xTg-AD mice were expressing less Sig1R in the hippocampus. Together, this data suggests that Sig1R levels decreases in the hippocampus with AD progression, which is in agreement with previous studies. Furthermore, it demonstrates that Sig1R has a complex relationship with sex and AD. Future studies should investigate how Sig1R is altered by aging. Evidence shows that Sig1R expression does not change with aging (Phan et al., 2003; van Waarde et al., 2011; Hedskog et al., 2013). However, two of these reports used healthy subjects. Sig1R protein levels should be determined in aged animals (12-month-old, 18-month-old). At 12-month-old, 3xTg-AD mice show neurofibrillary tangles in the CA1 region (Oddo et al., 2003). Moreover, at that age, female mice are acyclic. It would be interesting to examine how Sig1R is altered in the presence of extensive Aβ and tau pathology. Furthermore, at 18-month-old, female mice are considered menopausal. How Sig1R is altered by hormone depletion and aging could be an exciting avenue to investigate.

6.2 Different mechanism of synaptic dysfunction between male and female mice

In this study, we first investigated how Sig1R protein levels are altered by sex and AD pathology. We found differences in Sig1R expression at 6 months of age. More importantly, we observed in the hippocampus a significant effect of sex as well as a significant interaction between sex and the triple transgenic mutation. This data made us wonder what synaptic changes occurred

at this specific time point. Therefore, we aimed to determine how hippocampal plasticity is modified by sex and AD pathology and if these changes can be rescued by Sig1R activation.

One of the major contributors to AD pathophysiology is synaptic dysfunction. Indeed, early in the course of the disease, the mechanisms of neural plasticity are disrupted, which eventually leads to synapse loss. Interestingly, loss of synapses is correlated with the cognitive decline observed in AD patients. Therefore, we strongly believe that it is important to examine how synapses are modified in male and female AD mice to begin understanding why women experience a more severe cognitive decline than men.

To determine if triple transgenic mutation and Aβ infusion sex-specifically alters synaptic plasticity, we first analyzed LTP and LTD using field recordings. LTP is considered the cellular basis of learning and memory (Bliss and Collingridge, 1993). Surprisingly, we found no LTP impairment in 3xTg-AD compared to NonTg mice. Although another group previously reported similar findings (Zhang et al., 2010), we were expecting a significant decrease in LTP magnitude in 3xTg-AD mice. In 2003, when Oddo et al. first characterized the 3xTg-AD mouse model, they reported that LTP was intact at 1 month and significantly reduced by the age of 6 months compared to age-matched NonTg mice (Oddo et al., 2003). One possible explanation for this discrepancy is that we are using a subline of the 3xTg-AD mouse model. Sublines of this specific mouse model have been shown to present a different onset and progression of the disease (Belfiore et al., 2019). Thus, it is possible that at a more advanced age, our 3xTg-AD mice will present impaired LTP. Another possibility may be the use of a different induction protocol. In their investigation, Oddo et al. induced LTP using high-frequency stimulation consisting of four trains of 100 Hz stimulation at 20s, while we induced LTP with a TBS protocol, which is the same protocol used in Zhang and colleagues' previous work, who also failed to see a decrease in LTP magnitude in 3xTg-AD mice.

Excitingly, we found that 6-month-old female NonTg and 3xTg-AD mice exhibit smaller LTP magnitude than their male counterparts in the CA1 region of the hippocampus. Few studies have attempted to look for sex differences in LTP magnitude and even fewer have done it in the context of AD. Investigations that have evaluated the effect of sex on hippocampal LTP have shown divergent results. Some report that there is no difference in LTP magnitude between male

and female mice (Yang et al., 2004; Qi et al., 2016), while others demonstrate greater LTP in males (Yang et al., 2004; Monfort et al., 2015; Qi et al., 2016). Comparing these studies is no trivial task because LTP magnitude can vary depending on several factors, including the synapse location, the induction protocol used and the estrous cycle phase. Indeed, LTP magnitude has been shown to vary across the estrous cycle (Warren et al., 1995). It is greatly influence by E2 level, which is at his highest during the proestrus phase and at its lowest during the estrus phase. Thus, several factors can contribute to varying results within the same mouse strain, making overall conclusion challenging.

To better understand why females had reduced LTP magnitude compared to males, we examined protein levels of AMPAR and NMDAR subunits. We hypothesized that this sex-specific baseline difference was likely due to changes in NMDAR protein levels. Indeed, several lines of evidence indicate that a reduction in synaptic NMDARs may contribute to impaired LTP (Kamenetz et al., 2003; Brigman et al., 2010). Regarding AMPAR subunits, our western blot analysis revealed no change in GluA1 protein expression, which is in agreement with what has been previously published (Mota et al., 2014). However, we found that female mice had increased GluA2 protein levels compared to their male counterparts. Regarding NMDAR subunits, we found no difference in GluN1 and GluN2B protein levels in 3xTg-AD compared to NonTg mice. How GluN1 protein levels are affected in AD hippocampus is contradictory. Our data show that there is no change GluN1 protein expression, which is consistent with previous studies (Chen et al., 2014; Mota et al., 2014). Conversely, Zhang et al. reported a decrease in GluN1 protein levels in 6-month-old male 3xTg-AD mice compared to age-matched control mice (Zhang et al., 2010). Moreover, our western blot analysis revealed no changes in GluN2B protein levels between NonTg and 3xTg-AD mice at 6 months of age, which is in agreement with previous publications in the literature (Mota et al., 2014; Revilla et al., 2014; Baglietto-Vargas et al., 2018). Interestingly, we found that synapses of female NonTg and 3xTg-AD mice contain more GluN2A receptors than their male counterparts. The physiological significance of an increase in synaptic GluN2A is unclear. However, our results suggest an increase in GluN2A/GluN2B ratio in females compared to males. Studies have shown that a higher GluN2A/GluN2B ratio requires a greater stimulation to induce LTP (Kopp et al., 2006; Yashiro and Philpot, 2008). Therefore, it is possible that this need for a higher threshold explains why our female mice had smaller LTP magnitude. Future

studies should investigate the intracellular mechanisms involved in LTP. For example, they could examine the phosphorylation sites of AMPAR and NMDAR subunits and their respective kinases.

It is important to note that because our female NonTg and 3xTg-AD mice were gonadally intact and that the estrous cycle was not determined, it is possible that the estrous cycle phase had an effect on hippocampal AMPAR and NMDAR expression (Foy et al., 2008).

In the $A\beta_{25-35}$ infusion model, we found that LTP magnitude was decreased in both male and female WT mice, which is consistent with multiple AD models (Sivanesan et al., 2013). However, we did not observe an effect of sex on LTP magnitude in the $A\beta_{25-35}$ infusion model, contrary to what we found in the 3xTg-AD mice. One possible explanation is that female mice in the $A\beta_{25-35}$ infusion model were ovariectomized, whereas the female NonTg and 3xTg-AD mice were gonadally intact. However, even if our female mice had different hormonal status, we still observed a disruption in LTP in both F-$WT_{A\beta}$ and female 3xTg-AD mice. This could suggest that hormones are not significantly contributing to the outcome. Future studies should compare LTP magnitude in ovariectomized versus gonadally intact mice in the context of AD. Another possible explanation why we did not observe an effect of sex on LTP magnitude in the $A\beta_{25-35}$ infusion model is that female mice do not use the same mechanisms of synaptic potentiation as male mice. A recent study reported that PKA is required for LTP induction only in female mice (Jain et al., 2019). Even though, this investigation showed that male and female mice had the same LTP amplitude, they used distinct molecular mechanisms. Interestingly, another study suggested that females could have differences in synaptic kinase activation. The authors showed that only female rodents require E2 and the membrane estrogen receptor α (ERα) to activate Src, ERK1/2 and TrkB, all of which are involved in LTP (Wang et al., 2018).

In addition to LTP, LTD is another form of synaptic plasticity underlying learning and memory that is altered in AD pathophysiology. Inducing LTD in rodents of intermediate ages is a challenging task (Kumar et al., 2007). This is due to several factors, including differences in animal strain, stimulation pattern as well as Ca^{2+}/Mg^{2+} ratio (Wasling et al., 2002; Temido-Ferreira et al., 2018). In this study, we used a PP-LFS stimulation protocol known to facilitate LTD induction in aged animals (Thiels et al., 1994; Kemp et al., 2000; Wasling et al., 2002; Foster and Kumar,

2007). The protocol that we utilized gave an immediate depression that wasn't sustained over time. To maintain the depression, we tried different conditions and several stimulation protocols. For example, we tried the widely used LFS protocol (1Hz, 900 stimuli). We also changed the ISI in our PP-LFS for 200 ms instead of 50 ms. Furthermore, we tried performing extracellular field recordings in different recording conditions. We added PTX to the ACSF and we heated the ACSF to physiological temperature. However, even with all these modifications to our induction protocol, the results were similar.

LTD has been shown to be unaffected or enhanced in the presence of Aβ (Wang et al., 2002; Hsieh et al., 2006; Shankar et al., 2007; Shankar et al., 2008). Here, we show that only male mice were significantly affected by the triple transgenic mutation and Aβ-induced toxicity. Few studies have investigated the effect of sex on LTD and found that females express enhanced LTD compared to their male counterparts (Titterness and Christie, 2008; Dursun et al., 2018). However, both of these reports induced LTD in anesthetized animals with a different stimulation protocol than the one used in this investigation. Furthermore, they recorded *in vivo* LTD, while we used hippocampal slices.

Surprisingly, Aβ infusion and 3xTg mutation did not affect LTD magnitude in female mice. A possible explanation for this observation is that female mice express increased GluA2 protein levels compared to their male counterparts. It is well established that GluA2 plays a crucial role in LTD (Huganir and Nicoll, 2013; Diering and Huganir, 2018). Interestingly, numerous proteins interact with the C-terminal domain (CTD) of GluA2. A disruption in these protein-protein interactions directly affects AMPAR internalization, thus LTD expression. One mechanism by which AMPAR endocytosis is promoted during hippocampal mGluR-LTD is through the dephosphorylation of GluA2 Y876. Indeed, when GluA2 Y876 is dephosphorylated at the same time as a ligand binds, BRAG2 is stimulated, which then activates Arf6. BRAG2 acts as a guanine-nucleotide exchange factor (GEF). This specific activation of Arf6 leads to the internalization of synaptic AMPARs upon LTD induction (Scholz et al., 2010). One suggested tyrosine phosphatase responsible for the dephosphorylation of GluA2 Y876 is $STEP_{61}$ (Zhang et al., 2011). Interestingly, $STEP_{61}$ is involved in the pathophysiology of AD and is elevated in the presence of Aβ oligomers (Kurup et al., 2010). Indeed, AD patients and several AD mouse models have increased $STEP_{61}$

activity due to the Aβ-mediated inhibition of the proteasome (Chin et al., 2005; Kurup et al., 2010). More importantly, increased $STEP_{61}$ promotes Aβ-mediated endocytosis of GluA1/GluA2 AMPARs (Zhang et al., 2011). Whether the expression of $STEP_{61}$ differs between males and females is unknown. Indeed, most investigations on $STEP_{61}$ have been done with male mice only. However, it is likely that females have higher $STEP_{61}$ activity than males because they have higher Aβ pathology. Future studies should investigate for possible sex differences in $STEP_{61}$ expression or activity in the context of AD.

Decreased Sig1R levels may affect vulnerability to AD or contribute to AD progression. Thus, we wanted to test if Sig1R activation could rescue Aβ-induced deficits. Here, we show that the acute administration of PTZ, a selective Sig1R agonist, rescues LTP impairment from Aβ-induced toxicity in both male and female mice. This outcome is in agreement with previous studies, including work from our laboratory. Indeed, Sig1R activation via agonists positively modulates LTP (Martina et al., 2007; Moriguchi et al., 2011; Solntseva et al., 2014; Ryskamp et al., 2019), whereas loss of Sig1R decreases LTP magnitude (Snyder et al., 2016). Furthermore, evidence shows that activation of Sig1R via agonist modulates cellular pathways involved in the induction and expression of hippocampal LTP. For example, BDNF, a trophic factor regulated by Sig1R, is upregulated following Sig1R activation (Xu et al., 2015). Additionally, upon Sig1R stimulation, several kinases are activated, including TrkB (Kikuchi-Utsumi and Nakaki, 2008; Peviani et al., 2014), CaMKII, PKC and ERK (Moriguchi et al., 2011), all of which are involved in LTP. Interestingly, the stimulation of these kinases leads to the modulation of calcium signaling, which may regulate synaptic plasticity.

Mounting evidence suggests that male and female mice use different molecular mechanisms during LTP (Jain et al., 2019). For example, differences in synaptic kinase activation have been observed (Wang et al., 2018). Given the potential difference in LTP mechanisms between the sexes, we find it intriguing that Sig1R activation rescued LTP magnitude in both males and females. This rescue could be explained by the fact that our female WT mice were ovariectomized, which could affect synaptic kinase activation. Indeed, it is possible that ERK and TrkB, which are both involved in LTP and activated upon Sig1R stimulation, are differently regulated in OVX mice. Gonadally intact female mice have been shown to have a greater ERα-

mediated kinase activation. Interestingly, OVX mice have significantly lower E2 levels than gonadally intact mice (Carroll et al., 2007). Therefore, it is possible to assume that less membrane ERα are activated in OVX mice. Thus, it is likely that ERK and TrkB, which are both activated by membrane ERα during LTP induction, are differently regulated in OVX mice compared to gonadally intact mice. However, it is unclear if gonadally intact female mice have higher postsynaptic ERα than OVX mice.

Sig1R has been shown to regulate AMPAR and NMDAR protein levels (Guitart et al., 2000). More importantly, several studies have shown that Sig1R positively modulates NMDARs (Maurice and Goguadze, 2017), including previous work from our laboratory. Thus, it is possible that the rescue of LTP impairment following acute PTZ administration was due to an increase in the surface expression of NMDAR subunits. Our laboratory has previously shown that PTZ increases the expression of GluN2A and GluN2B in addition to mediating the trafficking of NMDARs to the cell surface (Pabba et al., 2014). However, when we examined the effect of acute PTZ administration on surface protein levels of AMPARs and NMDARs receptors in 3xTg-AD mice, we surprisingly found that PTZ had no effect on surface AMPAR and NMDAR subunits. A possible explanation for this discrepancy is that the previous study was done with healthy Sprague Dawley rats, thus their hippocampal circuitry is not representative of a diseased state, such as AD pathophysiology. Additional experiments are required to fully investigate the effect of Sig1R activation on NMDAR subunits expression levels in male and female Aβ-infused mice.

Little is known about how Sig1R affects LTD. However, recent evidence has shown that regulation of the PERK pathway, which is modulated by Sig1R, affects hippocampal mGluR-LTD amplitude. Indeed, inhibition of eIF2α via PERK suppression rescues mGluR-LTD failure in the hippocampus (Yang et al., 2016). Interestingly, one way to decrease PERK expression is to activate Sig1R. Here, we observed that acute administration of PTZ, a selective Sig1R agonist, has surprisingly no effect on hippocampal mGluR-LTD magnitude in female WT mice infused with Aβ. A possible explanation for this unexpected outcome is that the PERK-eIF2α pathway is already overly activated (Ohno, 2018), thus the acute PTZ administration may not be enough to suppress it. Future studies should further investigate the role of PERK in mGluR-LTD in AD pathophysiology.

6.3 The structural integrity of the hippocampus is impaired by Aβ infusion and 3xTg mutation

In our investigation, we first looked at how synapses are altered by sex and AD pathology. We found a sex-specific baseline difference in LTP magnitude and surface GluN2A and GluA2 protein expression in 3xTg-AD mice. Interestingly, one morphological reflection of synaptic dysfunction is synapse loss, which is one of the best correlates of cognitive deficits in human AD. Thus, we aimed to evaluate the structural integrity of the hippocampus in AD mouse models.

The hippocampus is one of the first brain region to be affected by AD. It is well known to play a crucial role in learning and memory. Interestingly, hippocampal atrophy is present in AD patients (Hsu et al., 2015). Moreover, sex differences in hippocampal volume loss have been reported (Apostolova et al., 2006; Sundermann et al., 2017). Decrease in hippocampal volume is faster in women than in men. Here, we found that *in vivo* MRI measures of hippocampal volume were statistically different starting at the age of 6 months in 3xTg-AD mice, which is a consistent timeframe with the appearance of extracellular plaques, synaptic dysfunction and LTP impairment observed in this model (Oddo et al., 2003). Furthermore, we observed that Aβ infusion significantly reduced hippocampal volumetry in male and female WT mice. Surprisingly, we did not observe sex differences in structural integrity in either mouse model. Based on the human imaging data, we were expecting that female mice would have a smaller hippocampal volume than male mice. The discrepancy in hippocampal volumetry between our Aβ infusion model and human imaging studies might be explained by the fact that our mouse model lacks several human pathological hallmarks. Indeed, Aβ-infused mice do not present age-dependent accumulation of Aβ and tau. Regarding the 3xTg-AD mice, it would be interesting to take into consideration the estrous cycle phase of our female mice to reduce the variability of the data acquired from female mice. Interestingly, hippocampal volume has been shown to differ across mouse estrous cycle (Qiu et al., 2013). The volume of the hippocampus is at its smallest during the estrus phase, when the estradiol and progesterone levels are low. During the proestrus phase and during the diestrus phase, when the estradiol and progesterone levels reaches their peak, respectively, the hippocampal volume is at its highest. These changes in hippocampal volume can be detected within 24 hours.

Therefore, the volume of the hippocampus should be measured during the metestrus phase of the estrous cycle, when estradiol and progesterone are low. This is the best practice to compare gonadally intact male and female because it reduces the possibility of a hormone bias.

6.4 Limitations

The main limitation of this study is that we compared gonadally intact males with gonadally intact, cycling females. In our investigation, we used female mice aged between 3 and 9 months. Female WT mice were OVX whereas female NonTg and 3xTg-AD mice were gonadally intact. We did not take into consideration estrous cycle phase to avoid stressing the animals by vaginal smears. Based on a previous study (Meziane et al., 2007), we assumed that our gonadally intact female mice were in the same phase of their cycle if housed together in the same cage.

7. CONCLUSION

The overall aim of this thesis was to understand how Sig1R sex-specifically modulates AD pathology. As the population ages, the number of AD patients, who are mostly women, is expected to grow exponentially. Meanwhile, there is still no cure and no disease-modifying treatment available. A failure to examine sex differences in AD research may be partially to blame. Therefore, novel therapeutic interventions are tremendously needed that account for potential sex differences in disease pathophysiology. One promising target for prevention and treatment of AD is Sig1R, a protein regulated by sex hormones, synapse function, and cognition. We hypothesize that the activation of Sig1R may sex-specifically reduce the synaptic deficits induced by Aβ. By exploring the synaptic mechanisms that could account for the clinical observation that AD progresses faster in women, we have generated exciting data that deepens our understanding of sex differences in AD pathology. Our findings support the idea that synaptic plasticity is different between male and female mice, which may be because of distinct molecular mechanisms. Our data is also in agreement with the mounting evidence indicating that sex is an important factor in AD phenotype and severity. Therefore, sex must be considered in preclinical and clinical studies. Furthermore, our results suggest a complex interaction of Sig1R with sex and AD pathophysiology. Indeed, we observed that male and female mice respond differently to Sig1R activation, thus suggesting that Sig1R agonists may differentially benefit men and women if administered in a clinical setting. Finally, the work of this thesis supports that sex should not be neglected for therapeutic interventions and may encourage others to consider sex differences in their investigation.

8. TABLES

Model	Mice	Transgene	Pathology	Synaptic Changes	Cognitive Impairment	References
			MOUSE			
Amyloidopathy	PDAPP	hAPP Ind (V717F)	Extracellular Aβ deposition at 6 months	Reduced synaptic density in the dentate gyrus	Deficits in spatial working memory starting at 4 months Deficits in recognition memory	(Games et al., 1995)
	Tg2576	hAPP Swe (K670N/M671L)	Elevated levels of Aβ at 11-13 months	Dendritic spine loss starting at 4.5 months Impaired LTP at 5 months	Deficits in spatial learning, working memory and contextual fear conditioning before 6 months	(Hsiao et al., 1996)
	APP23	hAPP Swe (K670N/M671L)	Extracellular Aβ deposition at 6 months	No deficits in LTP	Deficits in spatial working memory starting at 3 months	(Strurchler-Pierrat et al., 1997)
	APP/PS1	hAPP Swe (K670N/M671L), PSEN1 A246E	Extracellular Aβ deposition at 9 months	Unknown	Deficits in spatial working memory at 11-12 months in male mice	(Wang et al., 2003) (Puoliväli et al., 2002)
Tauopathy	JNPL3	MAPT P301L	Neurofibrillary tangles at 4.5 months	Unknown	Unknown	(Lewis et al., 2001)
	Tau P301L	MAPT P301L	Neurofibrillary tangles at 8 months	Impaired LTP at 6 months	Deficits in the passive avoidance task at 5 months Deficits in recognition memory at 9 months	(Maurin et al., 2014)
	Tau V337M	MAPT V337M (Seattle)	Neurofibrillary tangles in the hippocampus at 11 months		No deficits in spatial working memory at 11 months Behavioral impairment in the elevated plus maze compared to control mice	(Tanemura et al., 2002)
	Tau R460W	MAPT R460W	Neurofibrillary tangles in the	Unknown	Deficits in associative memory retrieval in aged	(Tatebayashi et al., 2002)

			forebrain at 18 months		mice (16-23 months), but no sensorimotor deficits	
Amyloidopathy X Tauopathy	3xTg	APP Swe (K670N/M671L), MAPT P301L, PSEN1 M416V	Extracellular Aβ deposition at 6 months. Tau pathology appears around 12 months in the CA1 region of the hippocampus.	Impairment of basal synaptic transmission Decreased LTP at the age of 6 months.	Deficits in long-term retention at 4 months	(Oddo et al., 2003) (Billings et al., 2005)

Table 1. Phenotype characterization of rodent models of Alzheimer's disease

Mice	Age	Sex-dependent hallmarks	References
3xTg-AD	6 and 9 months	♀ ↓ cognitive performance in the MWM and IA	(Clinton et al., 2007)
	6 months	♀ ↓ spatial memory (MWM) ♀ ↓ novel arm preference (Y-maze) ♀ ↓ activity (open field test)	(Pietropaolo et al., 2008)
	9, 16 and 23 months	♀↑ levels of Aβ pathology ♀↑ β-secretase activity ♀↓ neprilysin activity No sex differences in tau phosphorylation	(Hirata-Fukae et al., 2008)
	6 months	♀ ↑ activity in open field test ♂ ↓ activity in open field test	(Giménez-Llort et al., 2010)
	12-14 months	♀↓ spontaneous alternating behaviour	(Carroll et al., 2010)
	12 and 15 months	♀↑ cognitive deficits ♂↑ behavioral inhibitions	(Blázquez et al., 2014)
	12 months	♀↑ pathological hallmarks ♀↑ neuroinflammation ♀↑ spatial cognitive deficits	(Yang et al., 2018)

Table 2. Sex differences in 3xTg-AD mouse model

	GluN1 (n=5)	GluN2A (n=5)	GluN2B (n=5)	GluA1 (n=5)	GluA2 (n=5)	Sig1R (n=5)
	F(1,16)	F(1,16)	F(1,16)	F(1,16)	F(1,16)	F(1,16)
PTZ	0.6689	0.7225	0.2068	0.4645	0.8694	0.8039
Genotype	0.3455	0.1048	0.1154	0.2409	0.4833	0.2858
Sex	0.3354	0.0195, *	0.7967	0.6614	0.0326, *	0.1619
PTZ x Genotype	0.8269	0.1287	0.9062	0.4377	0.4633	0.8222
PTZ x Sex	0.7144	0.3399	0.7394	0.9651	0.9656	0.4110
Genotype x Sex	0.5814	0.0397, *	0.8905	0.9502	0.9878	0.1694
PTZ x Genotype x Sex	0.7049	0.3257	0.4451	0.3022	0.7112	0.5514

Table 3. Statistical results corresponding to surface protein expression levels of NMDARs subunits, AMPARs subunits and Sig1R in male and female 3xTg-AD mice. *, p<0.05

	GluN1 (n=4-8)	GluN2A (n=4-8)	GluN2B (n=4-8)	GluA1 (n=4-8)	GluA2 (n=4-8)	Sig1R (n=4-8)
	F(1,20)	F(1,20)	F(1,20)	F(1,20)	F(1,20)	F(1,20)
Interaction	0.5936	0.2260	0.3745	0.5019	0.8427	0.0641
Sex	0.4180	0.6253	0.5580	0.8857	0.0086, **	0.1620
Aβ infusion	0.5441	0.4222	0.2376	0.2762	0.6480	0.9882

Table 4. Statistical results corresponding to surface protein expression levels of NMDARs subunits, AMPARs subunits and Sig1R in male and female WT mice following Aβ infusion. *, p<0.05

9. FIGURES

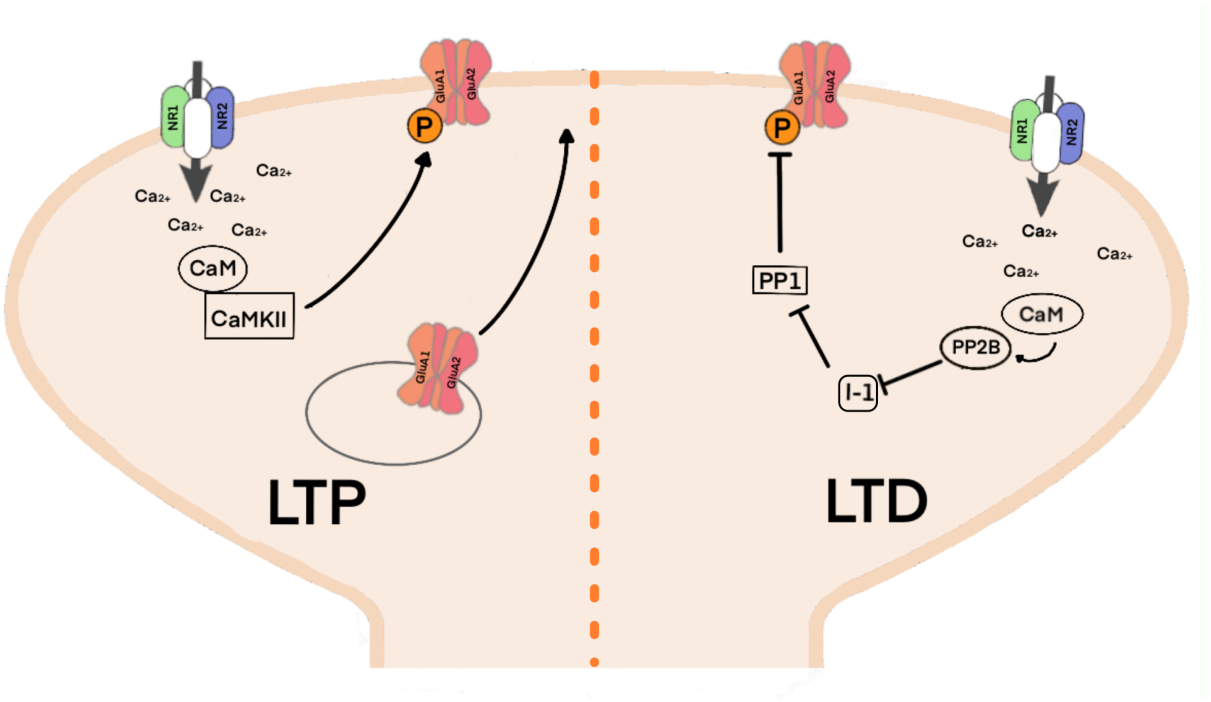

Figure 1. Postsynaptic NMDAR-dependent mechanism of LTP and LTD in the hippocampus

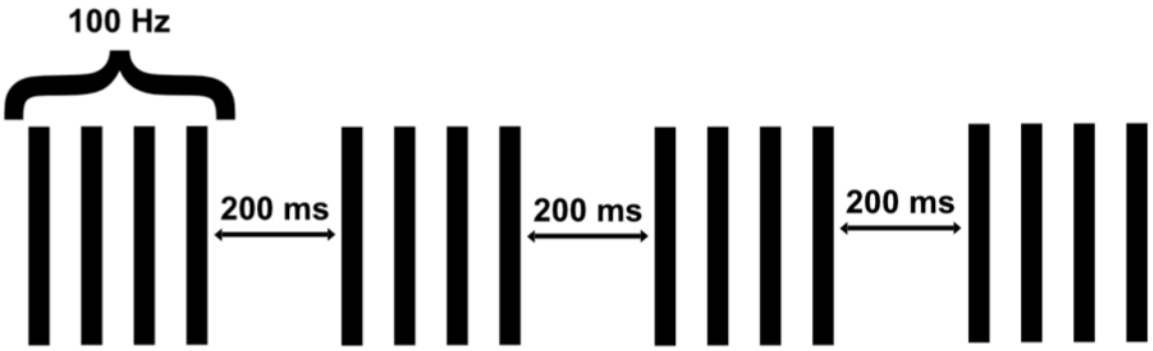

Figure 2. Schematic representation of a theta-burst stimulation protocol
This LTP induction paradigm consists of three trains delivered at 200-milisecond intervals, each train composed of 10 stimulus bursts delivered at 5Hz. Each burst consists of 4 pulses at 100 Hz.

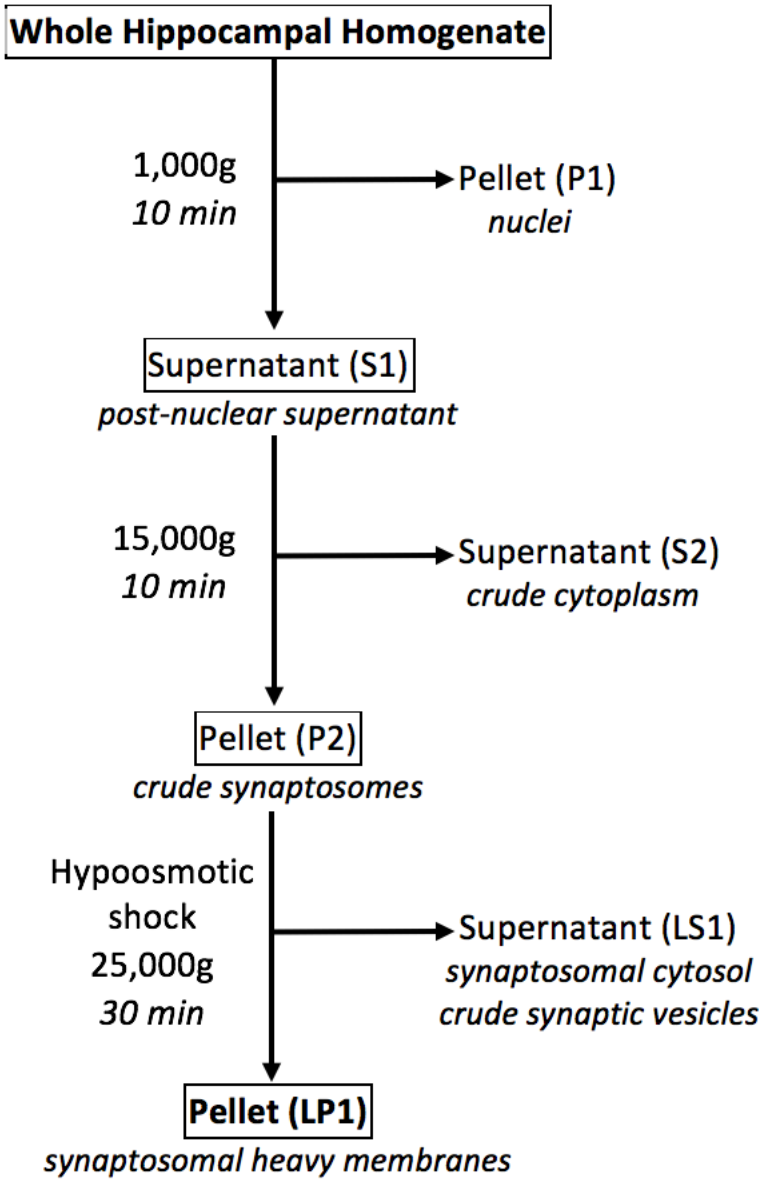

Figure 3. Schematic diagram showing the methodology used to isolate subcellular fractions from hippocampal homogenate

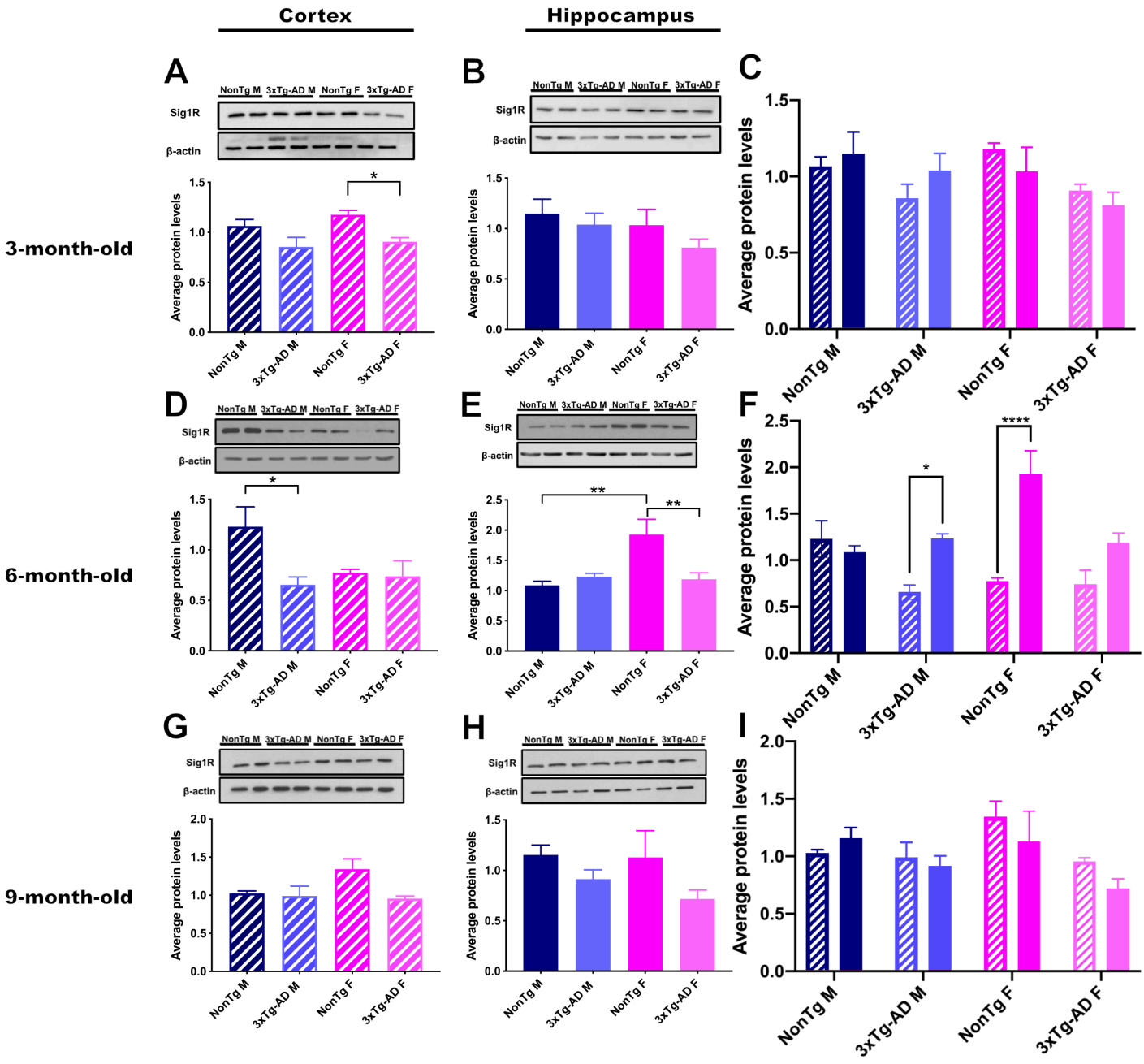

Figure 4. Sig1R expression over time in 3xTg-AD mice in whole-cell fraction
Representative bands and western blot analysis from 3-month-old (**A-C**), 6-month-old (**D-F**) and 9-month-old (**G-I**) male and female NonTg and 3xTg-AD in the cortex (left column) and hippocampus (right column), N=4-8 animals per genotype. β-actin was used as a loading control. Bar graph are mean ± SEM. Results were analyzed using a two-way ANOVA with Tukey's *post hoc* test.

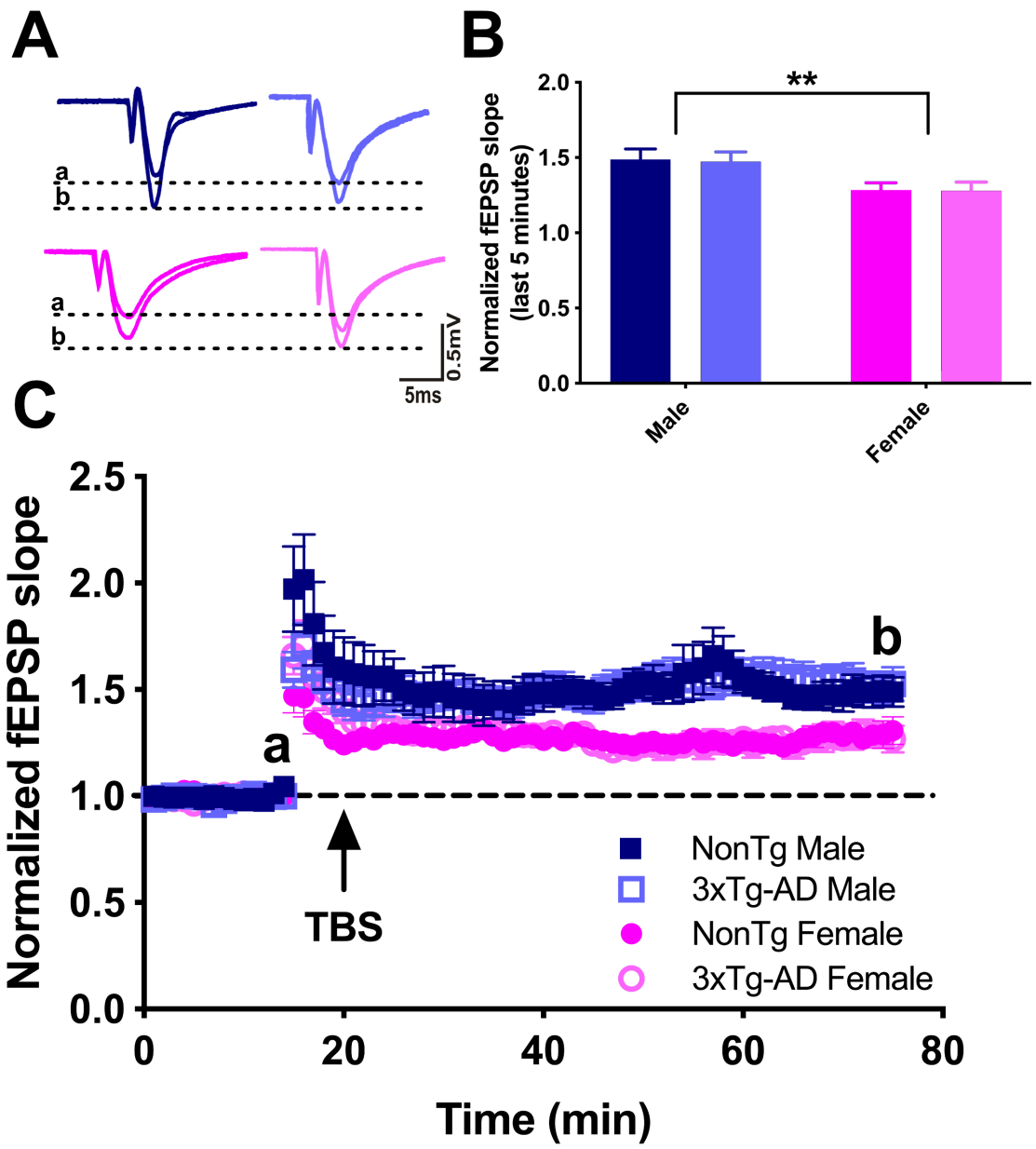

Figure 5. Male mice exhibit a larger magnitude of long-term potentiation
(**A**) Representative field excitatory post-synaptic potentials (fEPSPs) from 6-month-old male and female NonTg and 3xTg mice during baseline recording (a) and following theta-burst stimulation (b). (**B**) Bar graph showing the amount of potentiation between 55 and 60 minutes after TBS. Long-term potentiation is impaired in female mice compared to their male counterparts (average last 5 min), NonTg M $n = 5$, 3xTg-AD M $n = 8$, NonTg F $n = 7$, 3xTg-AD F $n = 6$. Results are presented as mean ± SEM and were analyzed using a two-way ANOVA with Tukey's *post hoc* test. (**C**) Summary plot shows the average normalized slope of fEPSPs recorded in the CA1 region of the hippocampus from male and female NonTg and 3xTg-AD over time (minutes). Theta-burst stimulation (arrow) consisting of three trains of stimuli at 100Hz, each train composed of ten stimulus bursts, was given after 15-min baseline recording. Results are presented as mean ± SEM and were analyzed using a two-way ANOVA with Tukey's *post hoc* test.

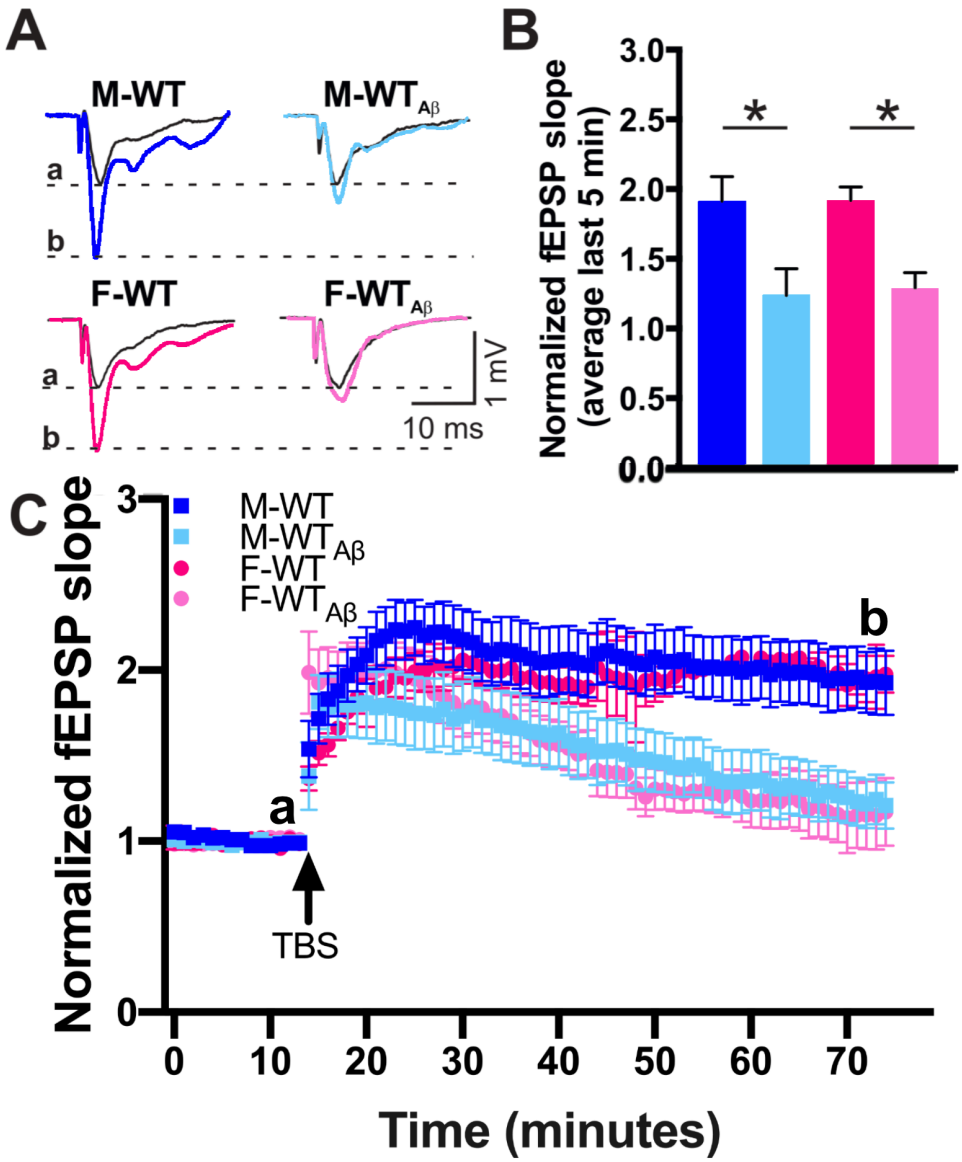

Figure 6. Aβ infusion reduces long-term potentiation in male and female WT mice
(A) Representative field excitatory post-synaptic potentials (fEPSPs) from 6-month-old male and female wild-type (WT) animals infused with or without Aβ during baseline recording (a) and following theta-burst stimulation (b). (B) Summary graph indicates that long-term potentiation is reduced in both male and female WT mice infused with Aβ compared to WT mice (average last 5 min), M-WT n = 12 slices, M-WT$_{A\beta}$ n = 8 slices, F-WT n = 7, F-WT$_{A\beta}$ n = 10. Errors bars ± SEM, asterisk indicates $p < 0.05$ (C) Summary plot shows the average normalized slope of fEPSPs recorded in the CA1 region of the hippocampus from M-WT, M-WT$_{A\beta}$, F-WT and F-WT$_{A\beta}$ over time (minutes). Theta-burst stimulation consisting of three trains of stimuli at 100Hz, each train composed of ten stimulus bursts, was given after 15-min baseline recording (Melissa Snyder).

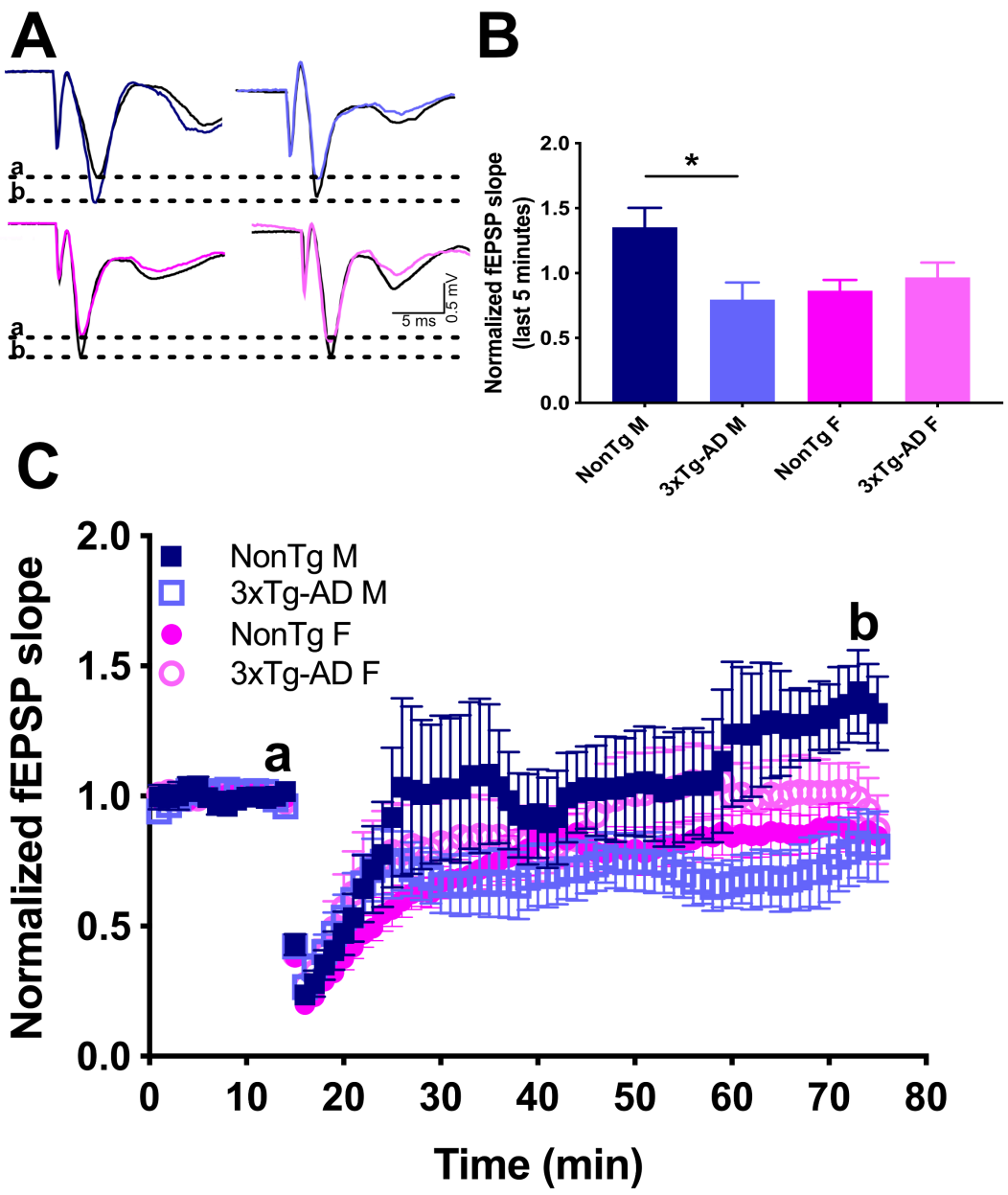

Figure 7. LTD is facilitated in male 3xTg-AD mice

(**A**) Representative field excitatory post-synaptic potentials (fEPSPs) from 6-month-old male and female NonTg and 3xTg-AD mice during baseline recording (a) and following paired-pulse low-frequency stimulation (b). (**B**) Summary graph indicates that synaptic plasticity is reduced in both male and female WT mice infused with Aβ compared to WT mice (average last 5 min), NonTg M n = 4 slices, 3xTg-AD M n = 5 slices, NonTg F n = 6, 3xTg-AD F n = 5. Errors bars ± SEM, asterisk indicates $p < 0.05$ (**C**) Summary plot shows the average normalized slope of fEPSPs recorded in the CA1 region of the hippocampus from NonTg M, 3xTg-AD M, NonTg F and 3xTg-AD F over time (minutes). Paired-pulse low-frequency stimulation consisting 900 pairs of stimuli with a 50 ms paired-pulse interval (PPI), was given after 15-min baseline recording. Results are presented as mean ± SEM and were analyzed using a two-way ANOVA with Tukey's *post hoc* test.

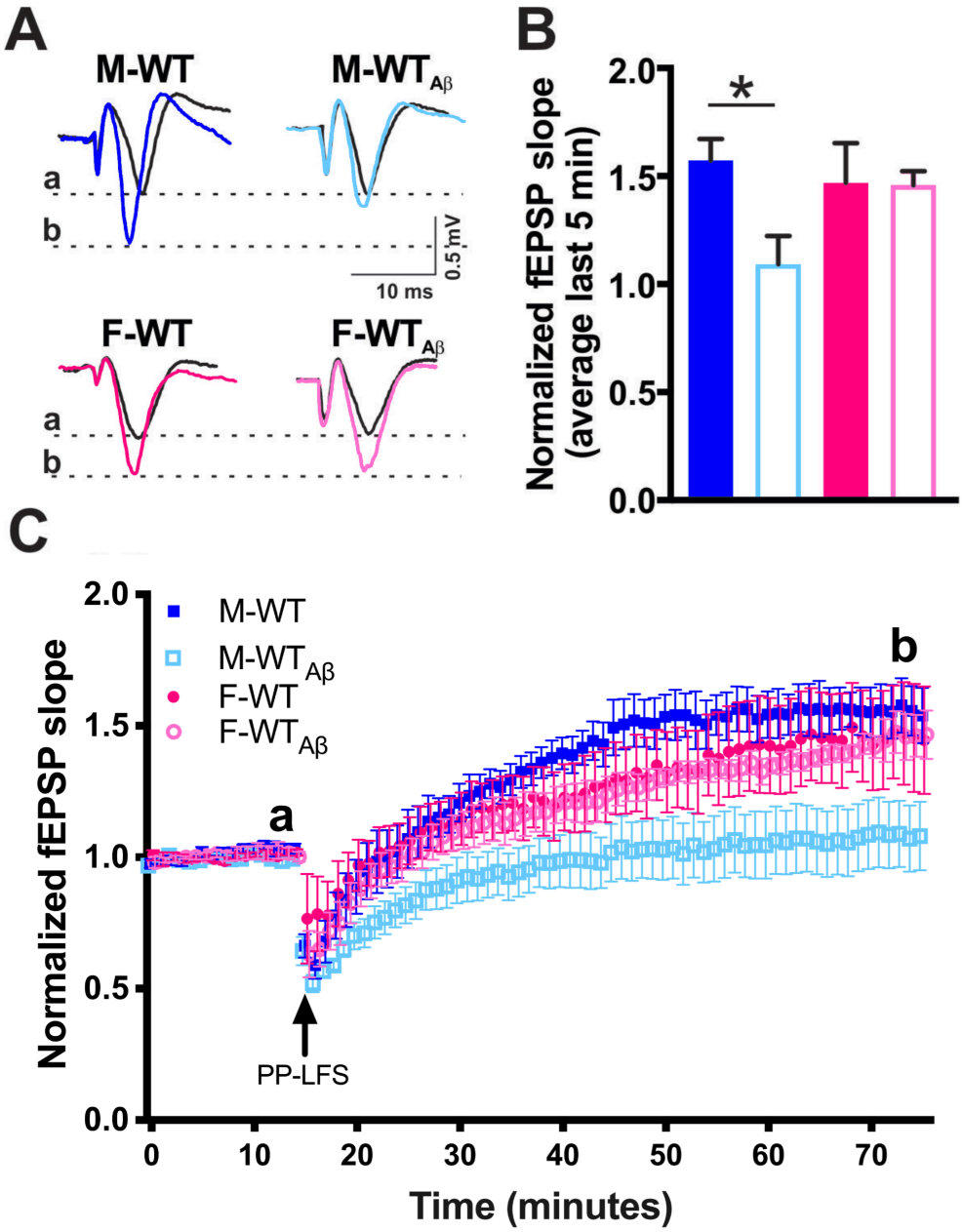

Figure 8. Aβ infusion facilitates LTD in male WT mice

(**A**) Representative field excitatory post-synaptic potentials (fEPSPs) from 6-month-old male and female wild-type (WT) animals infused with or without Aβ during baseline recording (a) and following paired-pulse low-frequency stimulation (b). (**B**) Summary graph indicates that synaptic plasticity is reduced in both male and female WT mice infused with Aβ compared to WT mice (average last 5 min), M-WT n = 6 slices, M-WT$_{Aβ}$ n = 6 slices, F-WT n = 5, F-WT$_{Aβ}$ n = 5. Errors bars ± SEM, asterisk indicates $p < 0.05$ (**C**) Summary plot shows the average normalized slope of fEPSPs recorded in the CA1 region of the hippocampus from M-WT, M-WT$_{Aβ}$, F-WT and F-WT$_{Aβ}$ over time (minutes). Paired-pulse low-frequency stimulation (arrow) consisting 900 pairs of stimuli with a 50 ms paired-pulse interval (PPI), was given after 15-min baseline recording (Melissa Snyder).

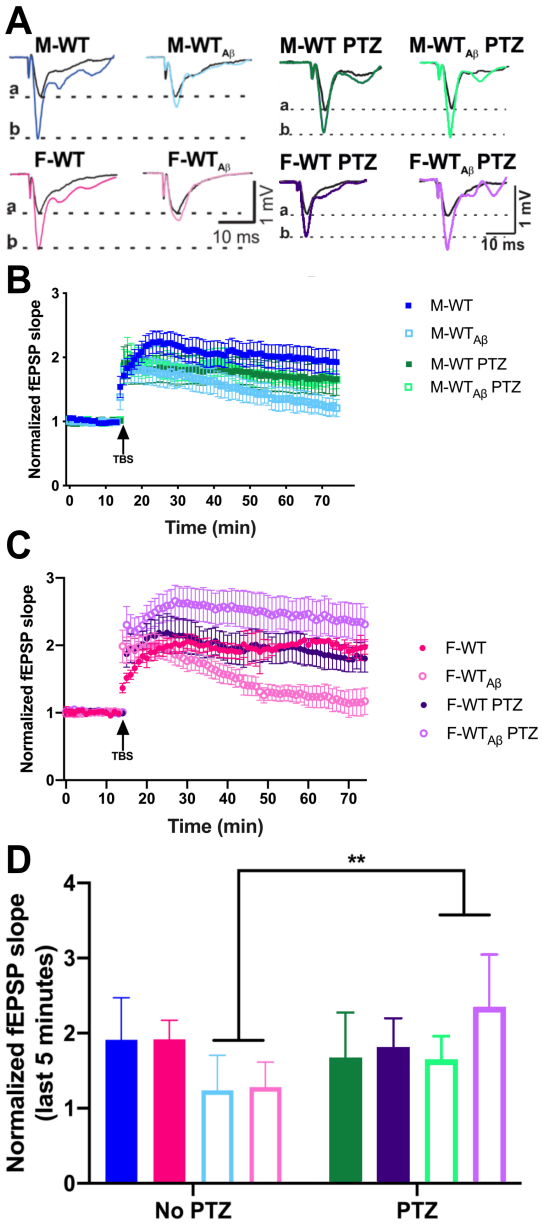

Figure 9. PTZ rescues LTP following Aβ infusion in male and female WT mice
(**A**) Representative traces of M-WT, M-WT$_{A\beta}$, M-WT PTZ, M-WT$_{A\beta}$ PTZ, F-WT, F-WT$_{A\beta}$, F-WT PTZ and F-WT$_{A\beta}$ PTZ over time (minutes). (**B**) Summary plot shows the average normalized slope of fEPSPs recorded in the CA1 region of the hippocampus from M-WT, M-WT$_{A\beta}$, M-WT PTZ and M-WT$_{A\beta}$ PTZ over time (minutes). Theta-burst stimulation (arrow) consisting of three trains of stimuli at 100Hz, each train composed of ten stimulus bursts, was given after 15-min baseline recording. (**C**) Summary plot shows the average normalized slope of fEPSPs recorded in the CA1 region of the hippocampus from 6-month-old F-WT, F-WT$_{A\beta}$, F-WT PTZ and F-WT$_{A\beta}$ PTZ over time (minutes). Theta-burst stimulation (arrow) consisting of three trains of stimuli at 100Hz, each train composed of ten stimulus bursts, was given after 15-min baseline recording. (**D**) Summary graph indicated that long term potentiation is rescued by PTZ following Aβ infusion in male and female WT mice. (Melissa Snyder).

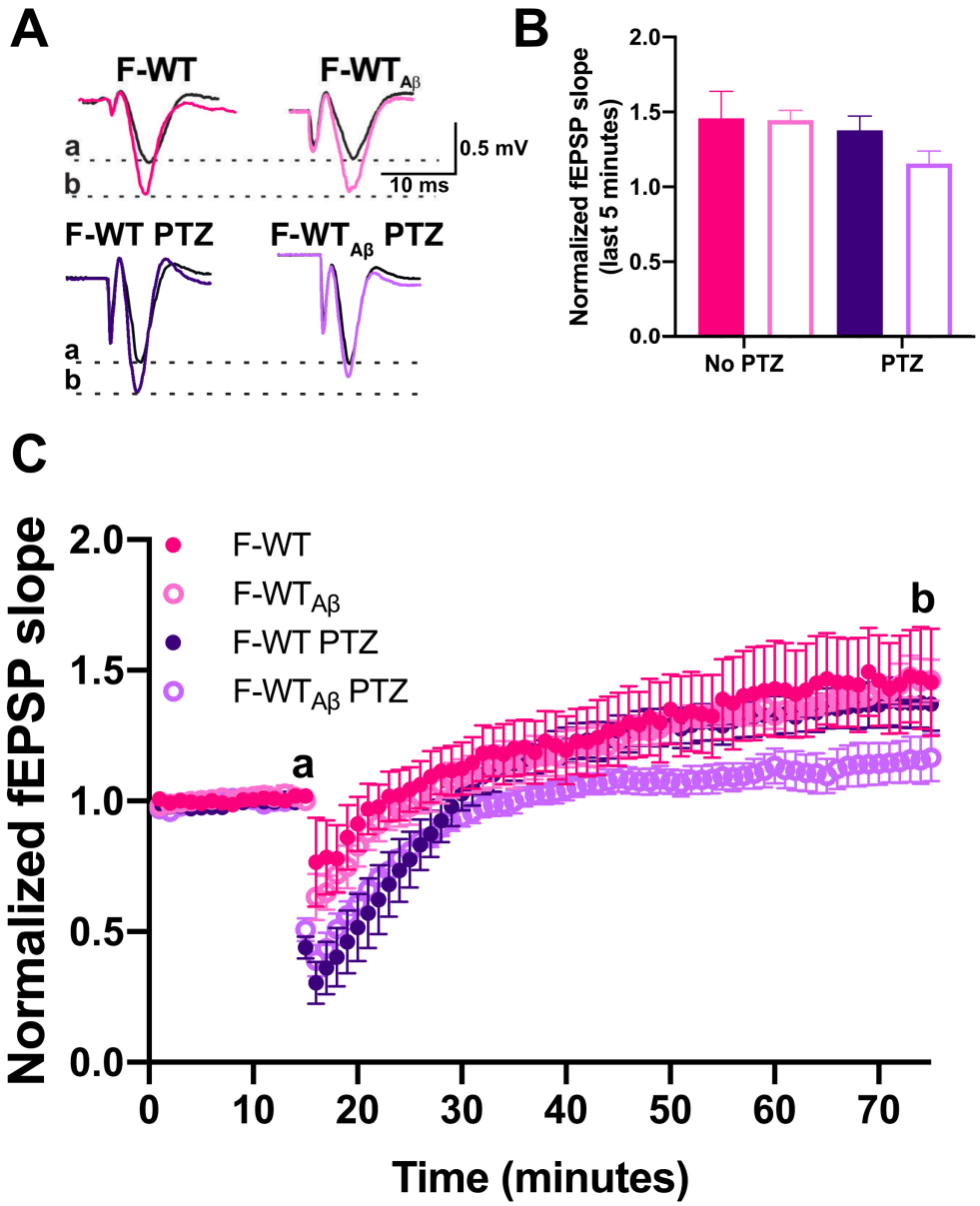

Figure 10. PTZ does not affect LTD magnitude in female WT mice following Aβ infusion
(**A**) Representative field excitatory post-synaptic potentials (fEPSPs) from 6-month-old female wild-type (WT) animals infused with or without Aβ during baseline recording (a) and following paired-pulse low-frequency stimulation (b). (**B**) The amount of potentiation between 55 and 60 minutes after TBS was 1.46 ± 0.18 in F-WT and was not significantly different from F-WT PTZ (1.38± 0.09). In F-WT$_{A\beta}$ (1.45 ± 0.06), the amount of potentiation wasn't significantly different from F-WT$_{A\beta}$ PTZ (1.15± 0.08), F-WT n = 5, F-WT$_{A\beta}$ n = 5, F-WT PTZ n = 6, F-WT$_{A\beta}$ PTZ n = 6. Results are presented as mean ± SEM and were analyzed using a two-way ANOVA with Tukey's *post hoc* test.

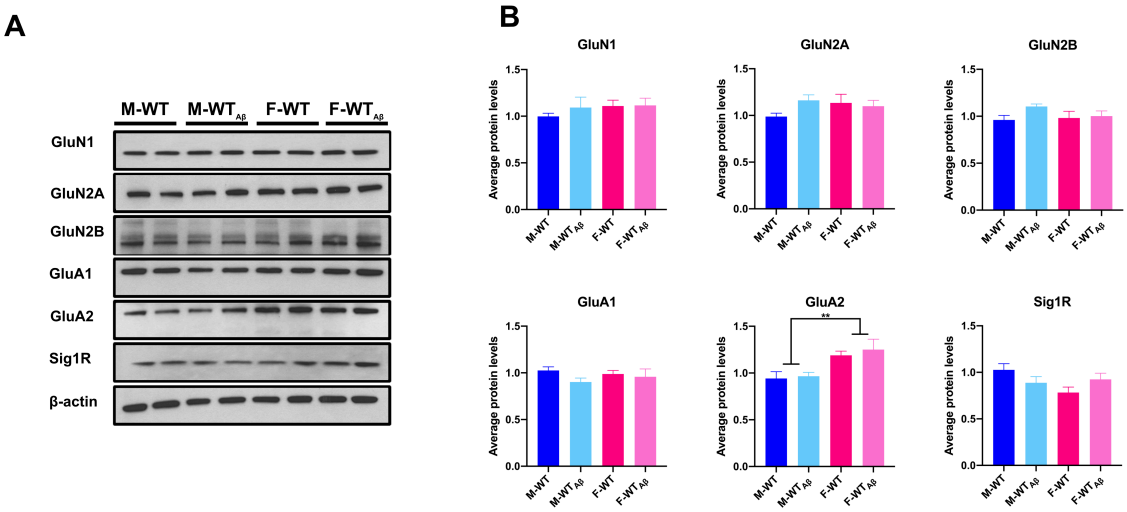

Figure 11. GluA2 protein levels in LP1 fraction are differently altered in male and female WT mice following Aβ infusion

Representative bands (**A**) and western blot analysis (**B**) from 6-month-old male and female WT mice infused with Aβ, M-WT $n = 4$, M-WT$_{Aβ}$ $n = 4$, F-WT $n = 8$ and F-WT$_{Aβ}$ $n = 8$. β-actin was used as a loading control. Bar graph are mean ± SEM. A significant increase of GluA2 expression was observed in female WT mice compared to male WT mice. Two-way ANOVA showed a significant effect of sex (p= 0.0086), but not for Aβ treatment (p= 0.6480) and interaction (p= 0.8427). ** p<0.01

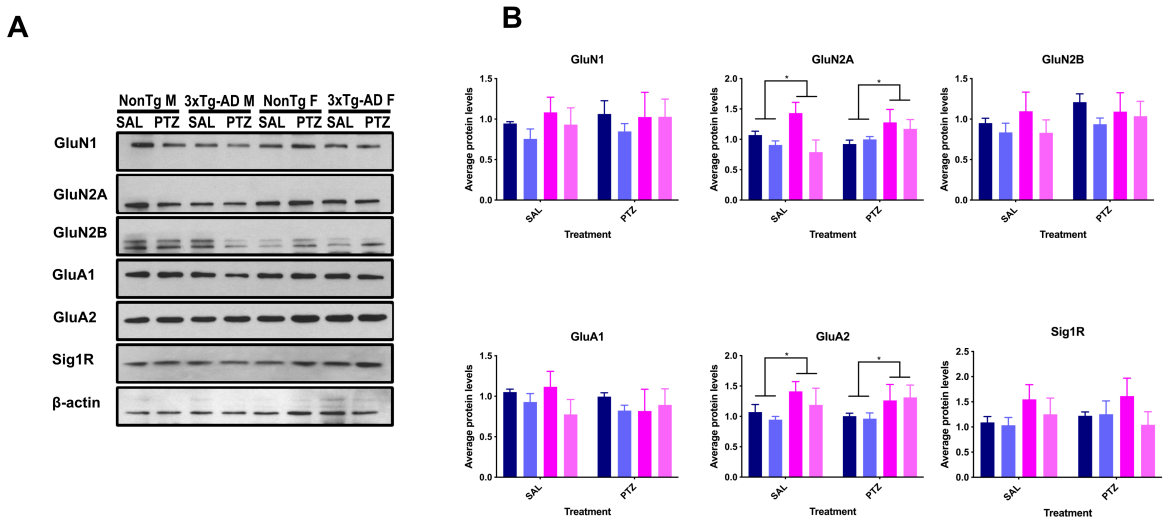

Figure 12. GluN2A and GluA2 protein levels in LP1 fraction are altered in male and female NonTg and 3xTg-AD mice

Representative bands (**A**) and western blot analysis (**B**) from 6-month-old male and female NonTg and 3xTg-AD, N=5 animals per genotype. β-actin was used as a loading control. Bar graph are mean ± SEM. A significant increase of GluN2A and GluA2 expression was observed in female mice compared to male mice. Three-way ANOVA revealed a significant effect of sex for GluN2A ($p=0.0195$) and GluA2 ($p=0.0326$). Interestingly, for GluN2A, the interaction of sex and triple transgenic mutation was significant (p=0,0397), * $p<0.05$.

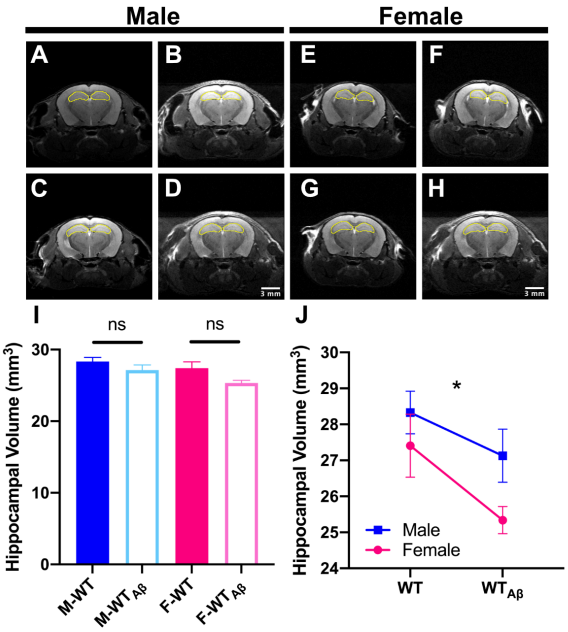

Figure 13. Aβ infusion reduces hippocampal volume in male and female WT mice
Representative coronal T2- weighted magnetic resonance (MR) images from 6-month-old M-WT one week before surgery (**A**), 2 weeks after (**B**); M-WT$_{A\beta}$ one week before surgery (**C**), 2 weeks after (**D**); F-WT one week before surgery (**E**), 2 weeks after (**F**); F-WT$_{A\beta}$ one week before surgery (**G**), 2 weeks after (**H**). (**I**) Hippocampal volumes of M-WT, M-WT$_{A\beta}$, F-WT and F-WT$_{A\beta}$. (**J**) Decreased hippocampal volume following Aβ infusion. Results are expressed as mean ± SEM and were analyzed using a two-way ANOVA with Tukey's *post hoc* test.

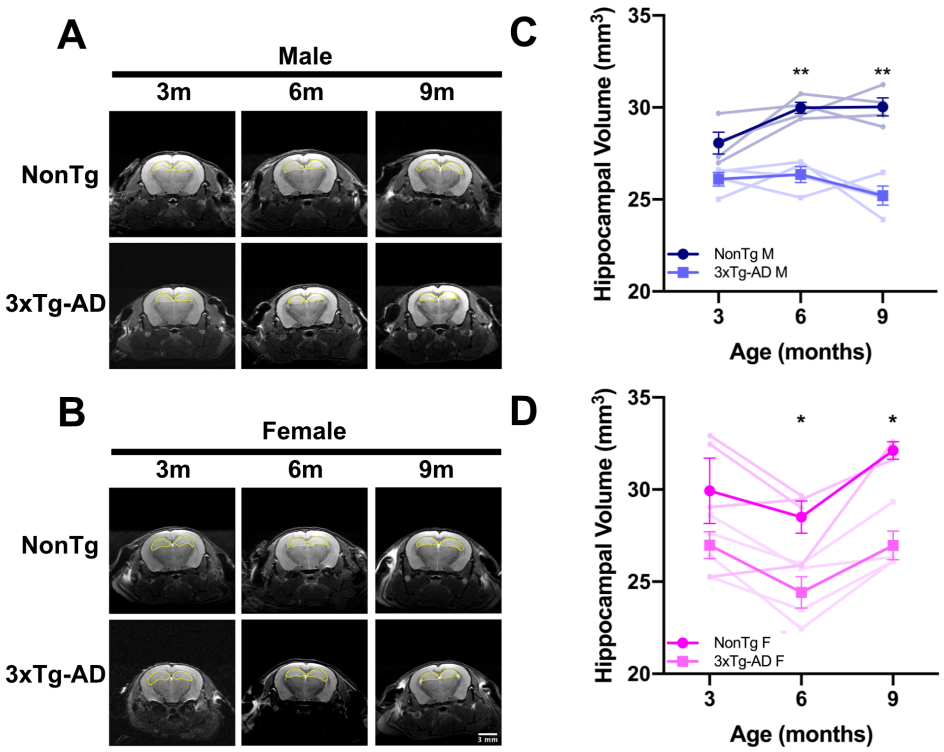

Figure 14. The structural integrity of the hippocampus is impaired in aged 3xTg-AD mice
Representative coronal T_2- weighted magnetic resonance (MR) images from 6-month-old male (**A**) and female (**B**) 3xTg-AD mice at different time points. Hippocampal volumes of male (**C**) and female (**D**) NonTg and 3xTg-AD.

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