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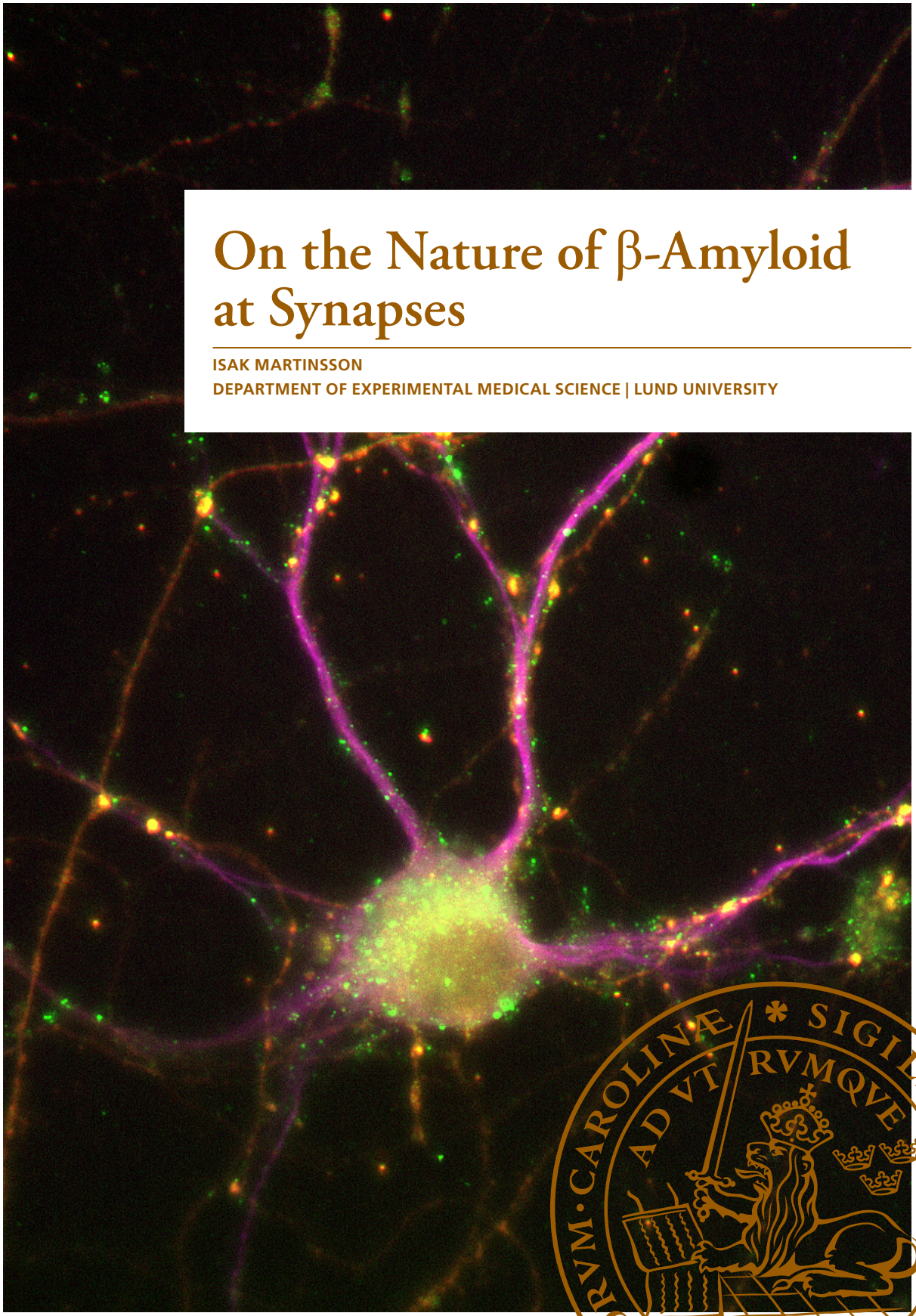

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UNIVERSITY

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Abstract

Alzheimer's disease is an aging related disorder of the mind, accountable for 60-80 percent of all cases of dementia. With its insidious progression and the decline of cognitive abilities, this disease is a major challenge for our society as a whole. This is because of the growing proportion of people living to advanced age and our current lack of capacity to prevent or even give effective symptomatic treatments to the disorder. At the advanced stages of the disease the patient is more or less completely dependent upon help from caregivers and family placing a huge stress on both society and the patient's next of kin. Alzheimer's disease is characterized by two cardinal pathological lesions. First, the senile plaques, consisting of β-amyloid, and second, the neurofibrillary tangles consisting of Tau. While most Alzheimer's disease cases have an unknown etiology there are cases of familial Alzheimer's disease, where a single mutated gene gives rise to the disease, typically with an early onset of symptoms. These disease related mutations all point to a critical role of the peptide β-amyloid and the processing of its precursor protein, the amyloid precursor protein (APP). While β-amyloid and APP have been studied extensively for almost three decades, we still require more knowledge of their normal roles in the brain. Multiple lines of evidence implicates synapses as early and critical sites of β-amyloid and APP function and dysfunction. The loss of synaptic terminals remains the best pathological correlate of the cognitive decline in Alzheimer's disease. In this thesis, on the nature of β-amyloid, we set out to investigate some of the roles β-amyloid and APP play at synapses, both in normal physiology and disease. We found that depletion of APP leads to alterations in synaptic composition and neuronal morphology. We showed that β-amyloid aggregates even before the appearance of plaques and that this concurs with the disappearance of a 20 kD putative tetramer in brains of Alzheimer's disease model transgenic mice. Moreover, we investigated the effects of two familial Alzheimer's disease mutations on synaptic proteins in hippocampal spheroids generated from induced pluripotent stem cells (iPSCs) from patients and healthy gender matched controls. We found that many of the changes between Alzheimer's disease hippocampal spheroids and normal samples were in synaptic proteins. The final study focuses on the effects of β-amyloid on neuronal hyper-excitability, an increasingly highlighted early feature in Alzheimer's disease that is also recapitulated in animal models of the disease. We describe specific effects of β-amyloid on cortical CaMKII positive neurons, increasing both firing frequency and amplitude of these cells, highlighting that β-amyloid targeting of CaMKII positive synapses might contribute to the hyper-excitability that is increasingly viewed as an early feature in Alzheimer's disease. Taken together this work describes and attempts to explain important roles that β-amyloid and APP play at synapses, with implications both for health and disease.

Key words Alzheimer's disease; Synapses; APP knckout, β-amyloid

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To my family

"Until then I had thought each book spoke of the things, human or divine, that lie outside books. Now I realized that not infrequently books speak of books: it is as if they spoke among themselves. In the light of this reflection, the library seemed all the more disturbing to me. It was then the place of a long, centuries-old murmuring, an imperceptible dialogue between one parchment and another, a living thing, a receptacle of powers not to be ruled by a human mind, a treasure of secrets emanated by many minds, surviving the death of those who had produced them or had been their conveyors."

— Umberto Eco, The Name of the Rose

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Original papers

This thesis is composed of the following original papers, referred to in text by their Roman numerals:

I. **Isak Martinsson**, Estibaliz Capetillo-Zarate, Mathilde Faideau, Katarina Willén, Noemi Esteras, Susanne Frykman, Lars O. Tjernberg, Gunnar K. Gouras. **APP depletion alters selective pre- and post-synaptic proteins**. Molecular and Cellular Neuroscience.Volume 95, March 2019, Pages 86-95

II. O. Klementieva, K. Willén, **I. Martinsson**, B. Israelsson, A. Engdahl, J. Cladera, P. Uvdal & G.K. Gouras. Pre-plaque conformational changes in Alzheimer's disease-linked Aβ and APP. NATURE COMMUNICATIONS. Volume 8, January 2017, Article number 14726

III. Yuriy Pomeshchik, Oxana Klementieva, Jeovanis Gil, **Isak Martinsson**, Marita Grønning Hansen, Tessa de Vries, Anna Sancho-Balsells, Kaspar Russ1, Ekaterina Savchenko, Anna Collin, Ana Rita Vaz, Silvia Bagnoli, Benedetta Nacmias, Sandro Sorbi, Dora Brites, György Marko-Varga, Zaal Kokaia, Melinda Rezeli, Gunnar K. Gouras, Laurent Roybon. **Modeling typical and atypical familial Alzheimer's disease in iPSC-derived hippocampal spheroids.** Manuscript submitted to Stem Cell Reports

IV. **Martinsson I**, Quintino L2, Svanbergson A3, Li JY, Lundberg C, Gouras GK. **Neuronal subtype vulnerability of Aβ-induced hyper-excitability**. Manuscript.

Papers outside of thesis

1. Aso E1, **Martinsson I**, Appelhans D, Effenberg C, Benseny-Cases N, Cladera J, Gouras G, Ferrer I, Klementieva O. **Poly(propylene imine) dendrimers with histidine-maltose shell as novel type of nanoparticles for synapse and memory protection.** Nanomedicine. 2019 Apr;17:198-209
2. Isak Martinsson, Oxana Klementieva, Bodil Israelsson, Katarina Willén, Gunnar Gouras. **En ny upptäckt angående β-amyloid**. Neurologi i sverige. 2017. nr 4 - 17

Abbreviations

AD	Alzheimer's disease
ADAM10	A disentigrin and metalloproteinase 10
ADAM17	A disentigrin and metalloproteinase 17
AICD	APP-intracellular domain
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
APP	Amyloid precursor protein
APP KO	Amyloid precursor protein knockout
APLP1	Amyloid precursor like protein 1
APLP2	Amyloid precursor like protein 2
APL-1	Amyloid precursor like 1 (C.elegans)
APPL	Amyloid precursor protein like (D.melanogaster)
ATP	Adenosine-tri-phosphate
BACE1	Beta-site amyloid precursor protein cleaving enzyme 1
BN-PAGE	Blue native polyacrylamide gel electrophoresis
BSA	Bovine serum albumin
Ca2+	Calcium
CNS	Central nervous system
CSF	Cerebrospinal fluid
CTF99	C-terminal fragment 99
CTF83	C-terminal fragment 83
DIV	Days in vitro
DMEM	Dulbecco's modified eagle medium
ECL	Enhanced chemo-luminescence
ER	Endoplasmic reticulum
FBS	Fetal bovine serum
FOV	Field of view
HS	Hippocampal spheroid
iPSC	Induced pluripotent stem cell
KO	Knockout
LTD	Long term depression
LTP	Long term potentiation
MAP2	Microtubule associated protein 2
MCI	Mild cognitive Impairment
mGluR1	Metabotropic glutamate receptor 1
mGluR5	Metabotropic glutamate receptor 5
MOI	Multiplicity of infection
NGS	Normal goat serum

NICD	Notch-intracellular domain
NMDA	N-methyl-D-aspartate
PBS	Phosphate buffered saline
PBS-T	Phosphate buffered saline with 0.1% tween 20
PFA	Paraformaldehyde
PM	Plasma membrane
PSD-95	Post-synaptic-density-protein-95
PS1	Presenilin 1
PS2	Presenilin 2
PVDF	Polyvinylidene fluoride
ROI	Region of interest
SAP97	Synaptosome-associated-protein-97
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
shRNA	Short hairpin RNA
TGN	Trans Golgi network
ThS	Thioflavin S
ThT	Thioflavin T
WT	Wild-type

Summary

Alzheimer's disease is an aging related disorder of the mind, accountable for 60-80 percent of all cases of dementia. With its insidious progression and the decline of cognitive abilities, this disease is a major challenge for our society as a whole. This is because of the growing proportion of people living to advanced age and our current lack of capacity to prevent or even give effective symptomatic treatments to the disorder. At the advanced stages of the disease the patient is more or less completely dependent upon help from caregivers and family placing a huge stress on both society and the patient's next of kin.

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Multiple lines of evidence implicates synapses as early and critical sites of β-amyloid and APP function and dysfunction. The loss of synaptic terminals remains the best pathological correlate of the cognitive decline in Alzheimer's disease.

In this thesis, on the nature of β-amyloid, we set out to investigate some of the roles β-amyloid and APP play at synapses, both in normal physiology and disease. We found that depletion of APP leads to alterations in synaptic composition and neuronal morphology. We showed that β-amyloid aggregates even before the appearance of plaques and that this concurs with the disappearance of a 20 kD putative tetramer in brains of Alzheimer's disease model transgenic mice. Moreover, we investigated the effects of two familial Alzheimer's disease mutations on synaptic proteins in hippocampal spheroids generated from induced pluripotent stem cells (iPSCs) from patients and healthy gender matched controls. We found that many of the changes between Alzheimer's disease hippocampal spheroids and normal samples were in synaptic proteins. The final study focuses on the effects of β-amyloid on neuronal hyper-excitability, an increasingly highlighted early feature in Alzheimer's disease that is also recapitulated in animal models of the disease. We describe specific effects of β-amyloid on cortical CaMKII positive neurons, increasing both firing frequency and amplitude of these cells, highlighting that β-

amyloid targeting of CaMKII positive synapses might contribute to the hyper-excitability that is increasingly viewed as an early feature in Alzheimer's disease.

Taken together this work describes and attempts to explain important roles that β-amyloid and APP play at synapses, with implications both for health and disease.

Populärvetenskaplig sammanfattning

Trots att mer än ett sekel gått sedan Alzheimers sjukdom först beskrevs av Alois Alzheimer saknas fortfarande effektiva behandlingar mot detta växande samhällsproblem. Alzheimers sjukdom drabbar framförallt äldre och det finns ett klart samband mellan ökande ålder och ökad risk för sjukdomen. Därmed står vi inför en enorm utmaning vårdmässigt då vi har en åldrande befolkning. Denna sjukdom leder till en gradvis försämring av kognitiva förmågor samt minne. Vid sjukdomens slutstadier är vårdtagaren nästan helt beroende av andra för att klara vardagen. Det är uppenbart att det därmed finns ett stort behov av nya behandlingar, men nästan alla försök har misslyckats.

När Alois Alzheimer först beskrev sjukdomen 1907 påvisade han två typer av patologiska förändringar i hjärnan på sin patient Auguste Deter. Senila plack bestående av β-amyloid, en peptid med en förmåga att klumpa ihop sig och bilda aggregat, samt neurofibrillära ”nystan”, som består av ett protein som normalt binder till mikrotubuli i nerveller. Dessa kom att bli de karaktäristiska kännetecken för Alzheimers sjukdom som används än idag. Ett annat kännetecken för Alzheimers sjukdom är nedbrytning av synaptiska terminaler. Dessa är de punkter där nervceller kommunicerar med varandra och deras funktion regleras av en stor mängd synaptiska protein.

β-amyloid, som normalt bildas genom klyvning av membranproteinet Amyloidt prekursor protein (APP), vid neuronal aktivitet i hjärnan. Sedan det identifierades som den mest framträdande beståndsdelen i senila plack har β-amyloid studerats av labb runtom i världen i nästan fyra decennier. Trots att både APP och β-amyloid har studerats så intensivt är deras normala fysiologiska funktioner relativt okända. Den här avhandlingen fokuserar på att studera β-amyloid och APP vid synapser, där vi studerat dess funktion i normala och sjukliga tillstånd.

Den första studien karaktäriserar förändringar i synaptiska protein i nervceller från möss där APP har slagits ut med genetiska verktyg. Genom att jämföra med nervceller från normala möss visar vi att APP behövs för en normal sammansättning av synapser och framförallt såg vi att APP hade en stark effekt på en subenhet i AMPA recptorn, GluA1.

I den andra studien visar vi bland annat hur β-amyloid genomgår konformationsförändringar innan plack går att upptäcka med konventionella metoder. Detta genom att använda en typ av absorbtionsspektoskopi, FTIR, med denna metod kunde vi även se ökningar i mängden av dessa konformationsförändringar i nervceller från musmodeller av Alzheimers sjukdom.

Vi beskriver även en möjlig naturlig konformation som β-amyloid antar i hjärnan från friska unga möss som sedan går förlorad när plack uppstår.

Den tredje studien fokuserar på celler från patienter med Alzheimersorsakande mutationer. Dessa programmeras om till stamceller som sedan styrs till att bli nervceller. Mer specifikt nervceller som bär karakteristika för den del av hippocampus som kallas gyrus dentatus. Vi demonstrarar hur dessa celler visar fenotyper som har associerats med Alzheimers sjukdom, framförallt synaptiska förändringar.

I den fjärde och sista artikeln i avhandlingen studerar vi β-amyloids roll i neuronal hyperaktivitet, ett tidigt fenomen i sjukdomsprocessen. Vi beskriver hur nervceller från Alzheimersmöss är mer aktiva samt att liknande ökning i aktivitet kan induceras genom att öka β-amyloid nivåerna i cellodlingsmediet genom farmakologisk manipulation eller genom att tillsätta syntetisk β-amyloid direkt till odlingsmediet.

Dessa studier formar tillsammans en bild av β-amyloid och APP som potentiellt viktiga delar av normala synapser i hjärnan, vars korrekta reglering är av stor vikt för att nervceller ska fungera normalt.

Introduction

Alzheimer's disease

The first described case of Alzheimer's disease, Auguste Deter was a 51 year old woman admitted to the Frankfurt mental hospital after her husband was no longer able to care for her. There she ended up being examined by Doctor Alois Alzheimer. She had started showing signs of dementia already in her forties; displaying memory problems, confusion and sleep problems. After she died in 1906, her brain was investigated by doctor Alzheimer who presented his findings at a conference and the next year published a report (Alzheimer et al., 1995). A few years later, the disease described by doctor Alzheimer was termed Alzheimer's disease (AD).

The prevalence of Alzheimer's disease

Since the first description of the AD patient Auguste Deter, we now know this disease to comprise around 70% of dementia cases across the globe (Prince et al., 2016). AD presents as a gradual decline in cognitive abilities such as memory and executive functioning. The cognitive disturbances interfere with AD patients' normal daily activities and lead to an almost complete dependency on caregivers (Scheltens et al., 2016). The most important risk factor for developing AD is age and the risk of developing AD is doubled every five years after the age of 65 (Corrada et al., 2010). The disease can also strike earlier in life, typically between 45-65 years old, and is then often referred to as early-onset AD in contrast to late-onset AD. Early-onset AD is pathologically and neuro-cognitively highly similar to late-onset AD albeit seemingly accelerated. A recent meta-review of 13 studies on the prevalence of EOAD found that the EOAD form constitutes roughly 5% of cases (Zhu et al., 2015). EOAD has no definitive definition but is frequently defined as dementia before age of 65.The cut-off at 65 years of age has to do with employment and retirement ages in different countries and can therefore be regarded as a sociological partitioning that bears no direct biological significance. Ultimately AD is a fatal disease and, compared with many other diseases of aging, has a particular increased proportion of casualties due to increasing lifespan of global populations.

Mild cognitive impairment

AD is typically preceded by a phase known as mild cognitive impairment (MCI). Patients with MCI experience cognitive decline though it is not yet sufficient to interfere with their daily living or their independence. Although MCI often progresses into AD or other dementias this is not always the case (Petersen et al., 2018). Biomarker studies indicate that the disease progression in AD begins years, even decades before the onset of overt symptoms (Jack et al., 2013). Because of the large number of failing clinical trials for AD, it is suggested that interventions in AD should be given as early as possible. This could also explain why many treatments which have been successful in animal models, fail in human disease.

Hallmarks of AD

Alois Alzheimer and Oscar Fischer described already 1907 the presence of neuropathological lesions in the brains of demented individuals. Today, the most known hallmarks of AD are the senile plaques and neurofibrillary tangles. Senile plaques have been found to consist mostly of a 37-43 amino acid long peptide called β-amyloid. The neurofibrillary tangles were later found to consist of the microtubule-binding protein Tau. The disease is also marked by a profound loss of synapses and a selective loss of neurons in certain areas. There is also a marked increase in micro- and astrogliosis indicating that an inflammatory response plays a role in disease pathology. Further, there are less well-studied lesions in some neurons such as granulovacuolar degeneration and Hirano bodies.

While β-amyloid plaques are a hallmark of AD, they were first discovered already in 1892 by Paul Blocq and Gheorghe Marinescu, who described these plaques in epilepsy patients (Blocq and Marinescu, 1892). Such plaques have been reported in very young epilepsy patients after surgical resection of epileptic foci (Gouras et al., 1997). Interestingly this might be connected to another, emerging hallmark of AD: hyper-excitability. A growing body of literature suggests that early phases of AD include hyper-excitability in neuronal networks. Using intracranial hippocampal electrodes it was shown that AD patients have frequent sub-threshold seizures that might impair the consolidation of memories (Lam et al., 2017). Other clues are that epileptic seizures are more common in Alzheimer's patients and that the hyper-excitability phenomenon appears across multiple animal models of the disease.

β-amyloid plaques have been extensively studied and can be classified as diffuse- or dense core plaques, based on morphology and staining with dyes or conformation specific antibodies. Dense-core plaques stain positive for Thioflavin S, a dye that

binds to amyloid structures, and are associated with microglia and a corona of dystrophic neurites (Eikelenboom and Stam, 1982). These dystrophic neurites label with APP and BACE (Sadleir et al., 2016), and have been suggested to secrete β-amyloid. Alternatively, dystrophies have been reported to show β-amyloid accumulation. Diffuse plaques on the other hand lack a solid core and are negative for Thioflavin S and Congo Red. These diffuse plaques are less frequently associated with astrogliosis but, electron microscopy of diffuse plaques with β-amyloid antibodies revealed that gold labeled β-amyloid was enriched within swollen, disrupted dendrites with cytoskeletal organelles and that displayed abnormal membrane stacking (Takahashi et al., 2017).

Errors of nature lead the way

While the greatest risk factor for developing AD and other neurodegenerative disorders is aging, there are a number of families world-wide with EOAD caused by mutations on chromosomes 1,14 and 21. The affected genes in these chromosomes were found to be PSEN1, PSEN2 and APP. The PSEN1 and PSEN2 genes code for components of the γ-secretase complex, an enzyme with multiple substrates and multifaceted functions. The APP gene codes for the amyloid precursor protein, the processing of which leads to the generation of β-amyloid, the very peptide fragment found in the core of senile plaques. Interestingly the γ-secretase complex happens to cleave APP C-terminal fragments to generate the β-amyloid peptide. There are also familial mutations in the MAPT gene, which codes for Tau. Interestingly none of the disease causing MAPT mutations have been linked to AD however, but they lead to fronto-temporal dementia. These observations together led to the formulation of the amyloid cascade hypothesis 1992 (Hardy and Higgins, 1992). While it has later been revisited (Karran and De Strooper, 2016) and modified to include oligomeric forms of β-amyloid, it still remains the leading hypothesis describing the aetiology of AD. In the following chapters we will examine the β-amyloid peptide and its precursor APP in more depth.

APP and β-amyloid

To understand the β-amyloid peptide and APP better we will now in higher detail focus on what we currently know about these molecules.

The APP family

APP is a type 1 integral membrane glycoprotein that is part of a larger family of so called APP-like proteins. This family of proteins likely developed from gene duplications of an APP family ancestral gene. Drosophila and C. elegans both carry only a single ortholog of human APP, known as APPL in Drosophila and APL-1 in C. elegans. Now it is known that our branch in evolution has undergone whole genome duplications at least two times. This led to the appearance of four identical APP ancestor genes that then later diverged into the APP and APP-like proteins we carry today. While fish carry four individual APP family genes, mammals carry three; APP, APP-like 1 (APLP1) and APP-like 2 (APLP2); reviewed in (Shariati and De Strooper, 2013). These proteins are similarly processed, although APP is by far the most studied. A property that is common to all the APP family members is that they can homo- and hetero-dimerize both in a cis- and trans- formation. This means that they can function as cell adhesion molecules (Wang et al., 2009; Stahl et al., 2014) but also possibly as cell surface receptors as the C-terminal tail of APP is able to form complexes with heterotrimeric G-proteins (Fogel et al., 2014; Milosch et al., 2014). Interestingly, plants, prokaryotes and yeast lack APP family members, indicating that the appearance of the APP family coincides with the evolution of early nervous systems (Shariati and De Strooper, 2013).

The APP family proteins consist of a short C-terminal tail, an intramembrane region and a long N-terminal domain. In APP the N terminal ectodomain can be subdivided into the E1 and E2 domains. The E1 and E2 domains are extracellular growth hormone like domains, which can bind and interact with extracellular growth signals and interact with extracellular matrix proteins.

Transcription, translation and transport of APP

The APP gene is transcribed in the nucleus; in neurons there are three distinct transcript variants of APP. These are APP770, APP751 and APP695. APP695 is the most common isoform in neurons. It differs from the other transcripts in that it lacks a Kuntitz protease inhibitor domain and lacks the acidic domain. Other cell types express a variety of transcript variants of APP, but remain outside of the scope of this thesis. After transcription and processing APP mRNA is transported to the ER. In the case of neurons, APP mRNA has been found to bind the Fragile X mental retardation protein (FMRP) and is subsequently transported in mRNA form to dendrites and local ER at postsynaptic terminals. Upon certain stimuli, APP mRNA is released from FMRP and can be translated by synaptic polyribosomes (Bramham and Wells, 2007; Westmark, 2013). This localized translation of APP is a feature

shared with many other synaptic proteins. Interestingly there is even some evidence that dysregulation of APP dendritic translation plays a role in Fragile X mental retardation (Westmark and Malter, 2007; Pasciuto et al., 2015; Westmark, 2019). After being translated APP is transported to the Golgi apparatus where it is post-translationally modified; mainly N- and O- glycosylation, phosphorylation and tyrosine sulfation. Like most membrane proteins, mature full length APP is transported via the secretory pathway to the plasma membrane (PM).

Proteolytic processing of APP

Once APP has been transported via the secretory pathway past the Trans Golgi Network (TGN), and arrives at the PM, it can be cleaved by ADAM10 or ADAM17 generating a soluble fragment known as sAPPα and a c-terminal 83 amino acids long fragment known as the C-terminal fragment 83 (CTF83). The CTF83 can then further be processed by γ-secretase to generate a soluble P3 peptide, the role of which is poorly understood, and a short intracellular domain known as APP intracellular domain (AICD). AICD is thought to be transported to the nucleus and regulate transcription in a role similar to that of notch intracellular domain (NICD). The specific targets of the AICD domain in transcription are however still debated, see (Hébert et al., 2006; Slomnicki and Lesniak, 2008; Aydin et al., 2011). The proteolytic cleavage sequence described previously is known as the non-amyloidogenic pathway, one of two canonical APP cleavage pathways; the other being the amyloidogenic pathway. In the amyloidogenic pathway APP is instead internalized by clathrin mediated endocytosis into early endosomes. As these endosomes mature acidified APP can be processed by BACE1, yielding a large N-terminal soluble APP (sAPPβ) and a 99 amino acid long C-terminal fragment (CTF99). This fragment, CTF99, can then be further processed by γ-secretase cleavage to generate β-amyloid fragments of varying lengths. The most common two being β-amyloid$_{1\text{-}40}$ and β-amyloid$_{1\text{-}42}$. Upon cleavage by γ-secretase an AICD fragment is also generated and like the non-amyloidogenic pathway variant believed to take part in transcriptional regulation, although to what extent is still poorly understood. APP can also be processed in non-canonical pathways, these are however outside of the scope of this thesis since it focuses on β-amyloid.

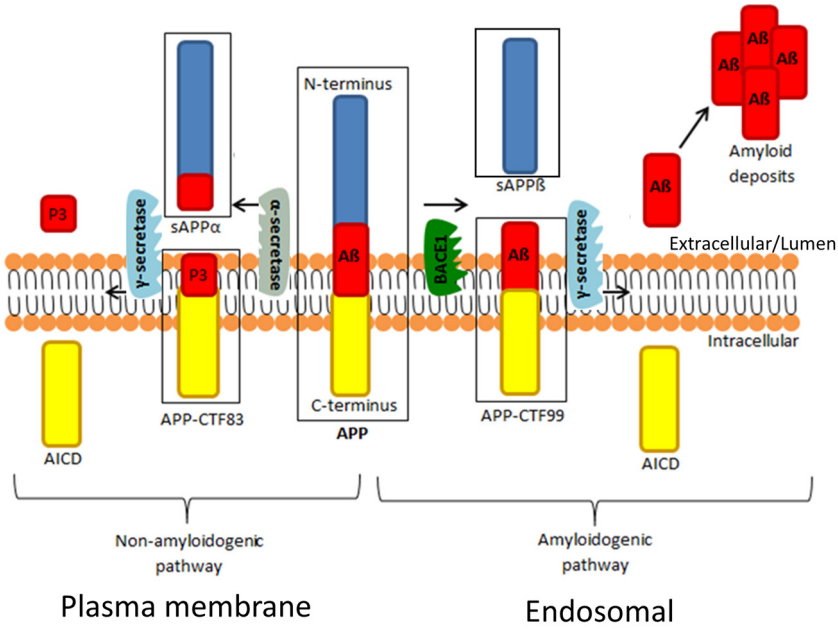

Figure 1. Proteolytic processing of APP. Illustration depicts the two main proteolytic cleavage pathways of APP. Either it is cleaved by α-secretases at the PM, leading to generation of sAPP α and CTF83 and subsequent cleavage of CTF83 by γ-secretase yields P3 and AICD, or, following internalization into early endosomes, BACE1 cleaves APP into sAPPβ and CTF99. Further processing of the C-terminal fragment produces AICD and β-amyloid. The β-amyloid peptide can then aggregate and form oligomers and fibrils of increasing size.

β-amyloid

The β-amyloid peptide itself exists in more than 40 described variants. Variations include N- and C- terminal extensions and truncations, but also post-translational and chemical modifications to the amino acids in the β-amyloid sequence. The most common forms are however the 1-40 and the 1-42 variants. The latter being the disease associated form, constituting 5-10% of total amount that is produced. The two extra amino acids in β-amyloid$_{1\text{-}42}$, an isoleucine and an alanine, makes β-amyloid$_{1\text{-}42}$, more aggregation prone. The 42 amino acid variant is the species found in the core of plaques.

The amyloid cascade hypothesis

The amyloid cascade hypothesis was first proposed in 1992 by Hardy & Higgins (Hardy and Higgins, 1992). Its main proposition was that β-amyloid aggregates and forms neurotoxic plaques which destroy neurons and neuronal networks leading to the cognitive dysfunction observed in AD. The appearance of plaques and the

amount of these however, have a very poor correlation with cognitive decline (Terry et al., 1991). Since the original hypothesis was laid forth there have been some revisions of the original hypothesis leading to a modified amyloid cascade hypothesis in which neurotoxic oligomers cause synaptic dysfunction and hyperphosphorylation of Tau.

Animal models of AD

Since the discovery that familial AD could have a monogenic origin, these fAD causing mutations have been used to generate animal models. In this thesis we have mainly used two specific models; Tg19959 and APP/PS1 transgenic mice.

Tg19959

The Tg19959 mouse was first described in 2004 (Li et al., 2004) and was produced by microinjection of a cosmid insert containing APP695 with the APP Swedish (KM670/671NL) and APP London (V717F) under a hamster PrP promoter, the mice were then backcrossed into C57/B6SJL background. The model is quite aggressive and plaques start forming at 3 months (Li et al., 2004).

APP/PS1

The APP/PS1 mouse model is a commonly used transgenic mouse. First described in Borchelt's lab (Jankowsky et al., 2004). APP/PS1 express two transgenes; the first a chimeric mouse/human APP with the Swedish mutation (KM670/671NL) that was humanized by modifying amino acids at positions 5, 10 and 13 of β-amyloid and second a mutant human PS1 with a deletion in exon 9. These are expressed under a PrP promoter targeting them mainly to neurons. β-amyloid deposits start forming at 6 months and an abundance of plaques is apparent at 9 months (Jankowsky et al., 2004). These mice present with behavioral and synaptic plasticity defects as early as 3 months of age (Volianskis et al., 2010).

Physiological roles of APP family proteins

Although the amyloid cascade hypothesis has been the prevailing line of research in AD for the better part of three decades, the normal physiological roles of APP and its family members remain poorly understood. Studies on APP orthologues in drosophila and C. elegans showed that APL-1 knockout in C. elegans is lethal due to a molting defect (Hornsten et al., 2007). Drosophila in contrast, are viable despite knockout of APPL but display behavioural defects, increased vulnerability to brain injury and have defective neuromuscular synapse junctions (Luo et al., 1992;

Torroja et al., 1999b; Leyssen et al., 2005). Mutations in APPL have further been shown to impair axonal transport (Torroja et al., 1999a). These findings in invertebrates suggest that APP family members might play important roles in axonal transport and neuronal signalling.

The roles of APP family members have also been investigated in mammals through the use of APP knockout (KO) mice (Zheng et al., 1995). The APP KO mice are viable but have distinct and "subtle" defects. These include; reduced bodyweight, reduced brain weight, reduced locomotor activity, reduced grip strength, LTP defects, impaired performance in Morris water maze test, axonal growth defects, and defective axonal transport (Zheng et al., 1995; Seabrook et al., 1999; Senechal et al., 2008).

It has been suggested that the relatively subtle phenotype is due to compensation by other APP family members. Indeed double knockouts of APP and APLP2, APLP1 and APLP2 knockout, and triple knockout of all three proteins are embryonic lethal, seemingly consistent with this view. However, despite potential overlaps in function the possibility remains that APP, APLP1 and APLP2 regulate distinct non-redundant functions, the combined effect of which lead to lethality in the double and triple knockouts. This is also in line with the observation that there is no up-regulation of APLP1 or APLP2 in APP KO mice(Zheng et al., 1995).

APP seems to have important roles during development as neuronal precursor cell migration was impaired when APP is knocked down through in utero electroporation of siRNA (Young-Pearse et al., 2007). These studies also identified FE65, Dab1, DISC1 and heterotrimeric G0 protein interacting with APP that regulate neuronal migration. APP with its ability to bind heparin sulphate proteoglycans and secreted glycoproteins such as Reelin and F-Spondin, highlight important roles in migration during development (Hoe et al., 2005; Hoe et al., 2009).

The expression of APP remains high in the brain even after development suggesting that it also has important roles in the adult brain. APP knockout mice were shown to have reduced spine density in the cortex (Lee et al., 2010; Tyan et al., 2012) and hippocampus (Lee et al., 2010), while mice overexpressing human APP had higher cortical spine density (Lee et al., 2010). Hippocampal neurons from APP knockout mice were shown to have increased post-synaptic excitatory potential and increased synapse formation (Priller et al., 2006); a later study from the same group showed increased spine density in cortical neurons from APP KO mice when compared to WT littermates (Bittner et al., 2009), seemingly in contradiction to findings by Tyan and Lee.

Synapses

Synapses are the loci of communication between neurons. This section will describe these cellular compartments in more detail. Neuronal communication depends on tightly regulated release of neurotransmitter.

Brief anatomy of a neuron

Neurons are cells specialized in communication and signal transduction. Neurons are polarized cells: with one pole, the dendritic tree, a branched network of processes being the receiving end and the other, the axon being the main signalling end. Each neuron receives input from the axons of many other neurons; once this input reaches a threshold value the signal is propagated through the axon onto recipient cells. The axon can branch and contact multiple targets. Neurons can be classified by their morphology, their spiking patterns or the main neurotransmitter they produce. In this thesis we will mainly categorize them.by the neurotransmitter type they produce. Illustrated in (Fig. 2).

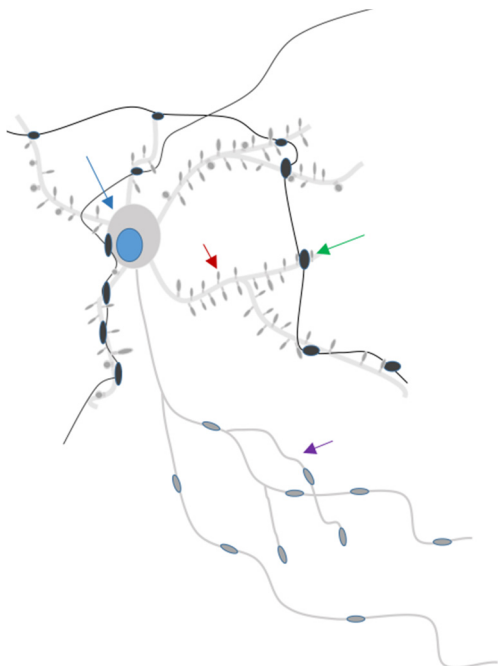

Figure 2. Brief anatomy of a neuron. Blue arrow points to neuronal soma. Red arrow indicas dendritic spines present along the dendrite. The green arrow points to a presynaptic terminal from another neuron making a type 1 synapse onto the neuron. The magenta arrow shows the neurons axon and synaptic terminals along the length of the axon.

Excitatory and inhibitory synapses

In the mammalian brain synapses can be classified by which neurotransmitters they secrete. The main neurotransmitters in the mammalian CNS are glutamate and γ-amino-butyric-acid (GABA). Glutamatergic synapses typically are asymmetric or Gray type I, meaning that a presynaptic compartment contacts a protrusion from the dendrite of a neuron called a spine. Spines are highly specialized structures for receiving signals from other neurons. Inhibitory synapses, also known as Gray type II synapses, which mostly contain GABA, on the other hand mainly localize on the cell body or directly onto the dendrite shaft; reasons for this likely being that the synapse in question can limit and gate the activity from that branch or cell more efficiently.

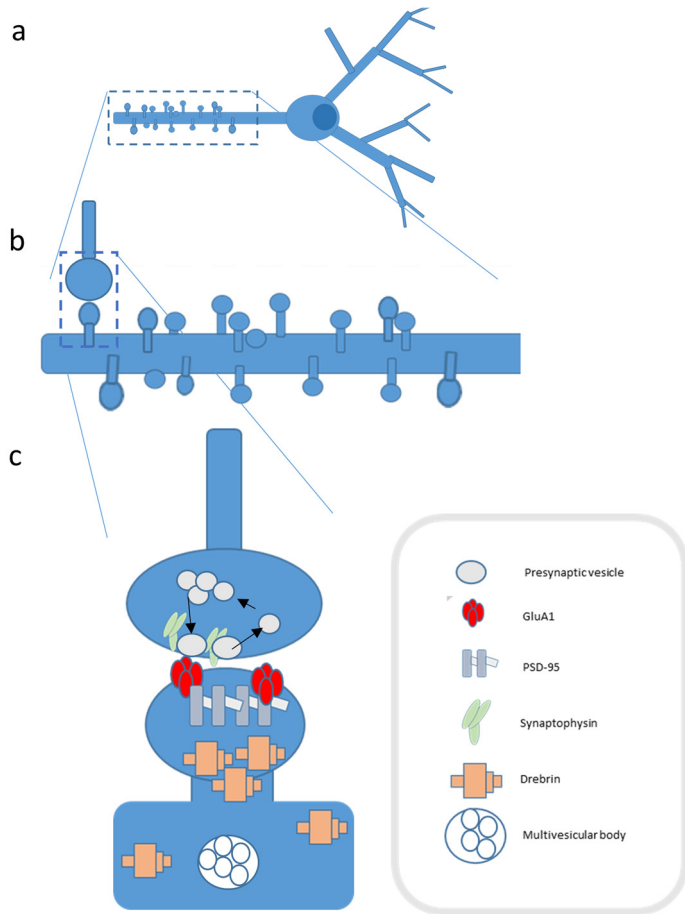

Figure 3. Schematics of pre- and post-synapse. a) shows a schematic neuron. b) insert from a) depicting dendritic spines on a dendrite and an axonal terminal synapsing onto a post-synaptic element. c) magnification of b) illustrating the pre- and post-synapse with depictions of localizations of synaptic proteins studied in Paper I.

Presynaptic compartment

Along the axon there are terminals containing 10 to thousands of tiny vesicles containing neurotransmitters. These are coupled to an intricate release machinery which ensures an energy efficient communication between neurons. The region where vesicles are fusing with the membrane is called the active zone (AZ) and it is seen as an electron dense region in apposition to the post synaptic density (PSD) discussed later. Associated with the AZ there is a sort of matrix called “dense projections”, the role of this grid-like structure is poorly understood. The synaptic vesicles not directly associated with the AZ are known as the reserve pool, these are distinct from the docked vesicles of the AZ which release their neurotransmitters into the synaptic cleft, a ~20nm widening between the pre- and post-synapse. The presynapse consists of many vesicle related proteins such as; synaptophysin, synapsins, SV2, and synaptotagmins. The plasma membrane of the presynapse is filled with adhesion molecules such as neurexins and neuroligins. The membrane is also covered with voltage gated ion channels and autoreceptors for neurotransmitters involved in feedback loops to control release probability.

Postsynaptic compartment

The postsynapse on the other hand is a highly specialized structure for receiving and processing information from the presynaptic neurons. Many of the glutamatergic synapses in the cortex synapse onto dendritic spines illustrated in (Fig. 2 and Fig. 3). These compartments create an environment where signalling proteins and their secondary messengers can reach high concentrations locally before furthering the signal across the dendritic branch. The surface of the spine contains receptors for neurotransmitters, notably the ligand gated ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and n-methyl-d-aspartate (NMDA) receptors. Further out from the center of the synapse there are high concentrations of metabotropic glutamate receptors such as the mGluRs. Apart from receptors, the surface also has many transynaptic adhesion molecules that interact with presynaptic adhesion molecules in order to maintain the stability and localization of the synapse. To keep the different adhesion proteins and receptors in their proper places these are anchored to specific structural proteins such as post-synaptic-density protein 95 (PSD-95) and synapse associated protein 97 (SAP97) in the PSD. These anchoring proteins create an electron dense matrix which is discernible by electron microscopy and is characteristic of type I synapses.

APP and β-amyloid as synaptic proteins

APP family proteins are present in all animals except sponges, which lack a nervous system, and ctenophorans which have a neural system that is different from other animal nervous systems (Moore et al., 2014). Since APP arose evolutionarily in the same time span as the nervous system it is tempting to speculate that the main role of APP is neural. It should be noted however that this also coincides with the appearance of lipoprotein receptors. Consistent with this view, APP has been shown to bind cholesterol (Barrett et al., 2012), and the expression of lipoprotein receptors, and the levels of brain cholesterol is altered in APP knockout mice (Liu et al., 2007). Importantly, cholesterol is required for synaptogenesis and though neurons can produce their own cholesterol during development, neurons in the adult brain are believed to import cholesterol through internalization of lipoprotein receptors.

APP is highly expressed at synapses and multiple growing lines of evidence link it with normal physiological roles at synapses. A growing literature has demonstrated that APP and its family members APLP1 and APLP2 may form trans-synaptic homo- and heterodimers, thus likely participating as synaptic adhesion molecules (Stahl et al., 2014). Notably APP was demonstrated to have synaptogenic properties inducing synapse formation in a co-culture system (Wang et al., 2009). N-terminal APP cleavage products sAPPα and sAPPβ have been implicated as GABAB receptor agonists, possibly regulating synaptic activity (Rice et al., 2019b). The machinery for generating β-amyloid is present both at the pre- and post-synapse, likely indicating roles of the processing of APP. Further APP processing appears to be regulated by synaptic activity, and β-amyloid might by itself exert influence on synaptic activity. Moreover, exogenously added β-amyloid appears to target synapses in cultured neurons (Lacor et al., 2004; Willen et al., 2017)

As mentioned earlier, synapse loss is an early feature of AD which is also the best correlate of cognitive decline among the neuropathological markers (Masliah et al., 1989; Terry et al., 1991; Sze et al., 1997). Oligomeric species of β-amyloid have been found to impair excitatory synaptic function, initiate loss of post-synaptic dendritic spines, and impair long-term potentiation (LTP) (Walsh et al., 2002). However, physiological concentrations of β-amyloid can increase release probability at hippocampal synapses, in an APP dependent manner (Abramov et al., 2009; Fogel et al., 2014) and picomolar levels of β-amyloid were found to facilitate and strengthen LTP (Puzzo et al., 2008).

Neuronal activity

Neuronal function is dependent on the timely communication and firing of synapses. If neurons fire too little we have hypo-activity and conversely overly excitable neurons lead to hyper-excitability and seizures, all of these are features of AD (Vossel et al., 2016).

Membrane potential and ion channels

Neurons are among the most energy consuming cells in the mammalian body. Most of the energy consumed by neurons goes to uphold the membrane potential. This membrane potential is the effect of different ions being sorted to intra and extracellular environments against their gradient by the help of various ATP dependent ion pumps and transporters. The resting membrane potential of a typical neuron lies around -70mV. This is maintained mainly by Na2+ ions and K+ ions being differentially sorted to extra (Na2+) and intra-cellular (K+)-environments. The surface of an excitatory post synapse is covered with receptor proteins for neurotransmitters. One such receptor is the AMPA receptor, which is an ionotropic glutamate receptor. Upon binding glutamate it opens and creates an influx of Na2+ ions which leads to a depolarization of the membrane. If a sufficient number of such events occur in a short time frame, voltage gated sodium channels, which are opened by changes in membrane potential, will be opened creating an even greater influx of Na2+ ions. This is known as the critical threshold level. Once critical threshold depolarization is reached an action potential is initiated. This depolarization spreads across the cell until it reaches the axon hillock region. The axon hillock region contains voltage gated sodium channels and strongly depolarizing stimuli trigger an action potential leading to the firing of the neuron. Voltage gated ion channels along the axon open in sequence until the signal reaches the synaptic terminals where voltage gated Ca2+ channels are opened. Upon increasing the intracellular calcium pre-synaptically docked neurotransmitter containing vesicles fuse with the membrane and release their contents into the synaptic cleft transmitting the signal to the next neuron.

Calcium signalling in neurons and neuronal networks

Calcium is a universal secondary messenger across all eukaryotic cells. The coordination chemistry of Ca2+ is favourable for interactions with the irregular surfaces of proteins whereas Mg2+ ions for instance, require a strict octahedral

coordination. This flexibility of Ca2+ likely led to its multiple roles regulating various aspects of cell signalling and biology. In order to keep a high signal to noise ratio and to maintain calcium homeostasis in the least energy consuming way, cytosolic Ca2+ is kept at low levels with the levels outside cells being approximately 10 000 fold higher. Neurons being highly specialized cells stretching long distances have an intricate Ca2+ signalling biology. In the majority of neurons action potentials are coupled to large and rapid increases in intracellular free Ca2+. When neurons are depolarized by excitatory activity, different channels open up at the PM, once the Ca2+ levels reach a high level, intracellular stores of Ca2+ from endoplasmic reticulum are opened and further release Ca2+ ions, a process known as calcium induced calcium release (CICR).

Ca2+ signalling in neurons control processes over big time scales, from control of neurotransmitter release at the microsecond scale to the regulation of gene transcription, a process that can last for hours (Berridge et al., 2003). Importantly, Ca2+ is implicated in regulating cellular correlates of memory. Once cellular concentrations rise, Ca2+ ions bind proteins and modify their functions. Depending on levels, durations and localizations of Ca2+ transients, the neuron can undergo LTP or long-term depression (LTD) (Zucker, 1999).

Calcium imaging and hyper-excitability in AD

Calcium imaging with molecules that bind Ca2+ have been increasingly used as a window into the machinations of cells and in particular neurons (Grienberger and Konnerth, 2012) Multiple methods have been developed over the years to visualize Ca2+ flux (Peron et al., 2015). One approach to studying Ca2+ signals is to use fluorescent Ca2+ indicators; these are typically hybrids between Ca2+ sensitive chelators such as EGTA or BAPTA and fluorescent dyes. Examples of such are the Oregon green BAPTA and fluo-4 families (Paredes et al., 2008). These dyes are easy to use and provide a good signal-to-noise ratio. These tools are therefore widely used in neuroscience. Recent advances in molecular cloning have yielded various genetically encoded calcium indicators (GECIs), e.g. GCamP6. Although existing GECIs are not sensitive enough to detect activity in certain neuron types, for instance parvalbumin positive interneurons (Chen et al., 2013), GECIs have been used successfully to image calcium changes in the brains of living animals, yielding interesting findings regarding neuronal hyperactivity in AD models (Busche et al., 2019). In-vivo calcium imaging methods demonstrated that neurons in the vicinity of plaques are hyper-active (Busche et al., 2008). Recently a report showed hyper-excitability in an AD transgenic mouse model, possibly through decreased

glutamate reuptake (Zott et al., 2019). Other reports instead point to inhibitory neuron deficits driving hyper-excitability and network hyper-synchrony in AD transgenic mice (Verret et al., 2012). APP has also been reported to drive hyper-excitability in a mouse model of Fragile X Mental Retardation (Westmark et al., 2016).

These findings point to synapses as an important stage were APP and β-amyloid play numerous roles, both good and bad, the purpose of this thesis being to further unravel these.

Aims of the thesis

The overall aim of the thesis was to investigate the roles of β-amyloid and its precursor protein APP at synapses.

More specifically:

Paper I

The aim of this paper was to describe the consequences on synaptic composition from APP depletion. This was investigated studying both KO and shRNA mediated knockdown of APP.

Paper II

The aim of this paper was to study early conformational changes in β-amyloid using non-destructive synchrotron based micro Fourier transform infrared spectroscopy (FTIR) and blue native polyacrylamide gel electrophoresis (BN-PAGE) and to study the effects of early aggregation in brains of AD transgenic mice.

Paper III

The aim of this study was to investigate AD phenotypes in patient derived iPSCs, differentiated into hippocampal spheroids containing dentate gyrus granule-like cells.

Paper IV

The aim of this study was to investigate the role of β-amyloid in driving AD-related hyper-excitability in neuronal cultures from WT and transgenic AD-model mice.

Methodological considerations

Below follows brief descriptions of the main methods used in this thesis, with a focus on methods I have utilized myself; for more details see the attached papers at the end of the thesis.

Animals (Papers I, II and IV)

Mice were kept in 12 h light/dark cycles with free access to water and food. All procedures were approved and performed under the guidelines set by the ethical committee for the use of animals in research at Lund University and Cornell University respectively (part of animal experiments of Paper I were conducted at Cornell).

The mice used in this thesis were APP KO mice (Zheng et al., 1995) (Jackson Labs, Maine, USA, JAX 004133) and B6.Cg-Tg (APPswe, PSEN1dE9)85Dbo/Mmjax mice (APP/PS1) AD transgenic mice and WT C57Bl/6J (Jackson Labs, Maine, USA, JAX 000664).

Primary neuronal cultures (Papers I, II and IV)

Primary embryonic neurons were prepared from the cortices and hippocampi of embryonic day 15-17 mouse embryos. In Paper I these were prepared from heterozygous APP KO (Jackson Labs, Maine, USA, JAX 004133) and in Papers II and IV they were prepared from B6.Cg-Tg (APPswe, PSEN1dE9)85Dbo/Mmjax mice (APP/PS1) AD transgenic and WT mouse embryos.

Briefly, the pregnant mother is sacrificed by cervical dislocation after being put to sleep with isofluorane. The embryos are then removed and washed in 70% ethanol following two rinses in Hank's basal salt solution with 0.45% glucose added. A part of the embryo is collected for genotyping before transfer to individual dishes with Hank's solution. Cortices and hippocampi are dissected out and meninges removed.

The neurons are then dissociated through trypsinization and subsequent trituration in Dulbecco's modified Eagle medium (DMEM) with 10 % fetal bovine serum (FBS) and penicillin/streptomycin. After genotyping of the individual embryos, neurons are plated onto poly-D-lysine coated culturing vessels. After 3-5 hours of incubation in DMEM the media is exchanged for Neurobasal medium supplemented with B27 (Thermo Fisher), L-glutamine (Thermofischer) and penicillin/streptomycin (Thermo Fisher). Neurons are then maintained in Neurobasal media until 12 or 19-21 days in vitro (DIV).

Genotyping (Papers I and IV)

Genotyping was carried out using the PCRbio Rapid Extract PCR kit (Techtum, Sweden). In brief, biopsies were incubated with 70 µl distilled H2O, 20 µl 5x PCRbio buffer A (lysis buffer) and 10 µl 10x PCRbio buffer B (protease containing buffer) per vial at 75 °C for 5 min, followed by heating to 95 °C for 10 min. The vials were placed on ice and allowed to cool before vortexing for 3-4 s and centrifuging at 10,000 rpm for 1 min to pellet the debris. The DNA supernatant was then transferred to a new vial. This was either used directly or stored at -20 °C. For PCR, 1 µl of DNA was incubated with 9.5 µl distilled H2O, 12.5 µl 2x PCRbio rapid PCR mix (containing taq polymerase for DNA amplification), 1 µl primerset F (APP knock out) and 1 µl primerset G (APP WT; both 10 µM.) for 3 min at 95 °C. Temperature was decreased to 55 °C for 15 seconds to allow for annealing of primers. Temperature was then increased to 72 °C for 5 min to allow for extension of DNA. DNA bands were detected using agarose gel electrophoresis.

Constructs (Papers I and IV)

The viral vectors carrying short hairpin RNAs (shRNA) targeting mouse APP (Young-Pearse et al., 2007) were created by restriction digestion and ligation into a modified pENTR plasmid. We then used Gateway recombination to transfer the shRNA and scrambled control RNA into a pLenti acceptor plasmid (Campeau et al., 2009). Lentiviral vectors were then made by a local viral vector core facility at Lund University. Titers were determined by immunofluorescence by the viral vector core facility. Primary neurons were transduced at 10 DIV at a multiplicity of infection (MOI) of 5.

In paper IV we used a lentiviral vector carrying TdTomato under a CaMKII promoter. The red reporter gene TdTomato was inserted via Gene synthesis (Thermo Fisher Scientific) into a Gateway cloning compatible plasmid to be used as an entry clone. Lentivirus production and titration were performed as previously described (Quintino et al., 2013). Primary neurons were transduced at 12-13 DIV at a MOI of 5 and analysed at 19-21 DIV.

Western blot (Paper I, III and IV)

Proteins were extracted from brain homogenates or primary neuronal cultures using radio-immunoprecipitation analysis (RIPA) buffer with protease and phosphatase inhibitors. Total protein content was measured with BCA assay (Thermo Fisher Scientific). Proteins were separated by sodium-dodecyl-sulphate polyacrylamide gel electrophoresis (SDS PAGE) using pre-cast either 10-20% Tricine gels (Invitrogen) or NuPAGE 4-12% BisTris gels (Invitrogen). Separated proteins were blotted onto PVDF membranes (Sigma-Aldrich) using an iBlot semidry transfer machine. Membranes were then blocked in either PBS-tween containing 5 % dry milk or 5% BSA and subsequently incubated with primary antibodies. After incubation with primary antibodies a secondary HRP conjugated antibody was added and blots were developed with ECL (Bio-Rad) solution and visualized in a ChemiDoc XRS+ system from BioRad. Protein intensities were quantified using Image Lab 5.2.1 software from BioRad.

Immunofluorescence (All papers)

Immunofluorescence analysis of cultured neurons offers many advantages despite the cells not being in their natural environment. The neurons are easy to manipulate with various experimental parameters. Importantly, the entire neuron including axon, dendrites and soma can be visualized at once allowing various morphological aspects to be analysed. In brief, cultured neurons grown on coverslips are fixed in either 4% PFA with 4% sucrose for 15 min at room temperature or they are fixed with 99% methanol at -20° C. Neurons are then rinsed with PBS and blocked in PBS with 0.1% saponin (Sigma-Aldrich), 1% bovine serum albumin (BSA; Sigma-Aldrich), and 2% normal goat serum (NGS; Thermo-Fisher Scientific). Primary antibodies are diluted in 2% NGS in PBS-T and added to the neurons overnight. After sufficient incubation neurons are rinsed with PBS-T and the appropriate

secondary antibodies conjugated to fluorescent tags are added. Coverslips contacting neurons are then mounted in SlowFade Gold antifade reagent (Invitrogen).

Microscopy (All papers)

Immunofluorescence labelled coverslips and culture vessels were analysed either at an inverted epifluorescence Olympus IX70 microscope or a Leica SP8 confocal. Channels were imaged sequentially to avoid bleed through.

Live cell calcium imaging (Paper IV)

Cultured neurons at 19-21 were incubated with 2 uM of the calcium dye Fluo-4 AM (Thermo Fisher Scientific, Sweden) in dimethyl sulfoxide (DMSO; Sigma-Aldrich, Sweden) for 30 min before imaging. Cells were imaged under a Nikon microscope at 15 x with 1.4 NA. Cells were imaged every 100 ms for a duration of 2 min, unless otherwise specified.

Image analysis (Paper I, III and IV)

Fluorescence intensity measurements (Paper I)

Quantifications of fluorescence intensities and puncta densities were performed by a blinded experimenter using the integrated morphometric analysis feature in Metamorph (Molecular Devices, California, USA). Thresholds were set so that only puncta with intensities (2x) brighter than that of the neurite shafts were quantified; more than 40 cells from 3 separate cultures were analysed for each genotype.

Spine length and density (Paper I)

Analysis of spine density and length were performed manually by a blinded experimenter using ImageJ. Briefly, confocal images of 100 µm dendritic segments were manually counted and measured for spine number and length. In all analyses blinding was performed before analysis by coding the names of the image files

Sholl analysis (Paper I and Paper III)

Sholl analysis was performed using an ImageJ plugin (Ferreira et al., 2014), briefly, thresholded bitmap images were measured from soma to furthest dendrite in the MAP2 channel. Then concentric circles of gradually increasing (30µm) radii were calculated, and the crossing points with the MAP2 binary neurite skeleton channel automatically counted and assessed by the algorithm.

Neurite length analysis (Paper III)

Morphometric analyses were performed on adherent iPSC derived neurons aged 56 days and labelled with MAP2 antibody. The soma area and dendrites were manually outlined and the length of all the dendrites were counted and summed to obtain the total neurite length.

Calcium imaging analysis

Time-stacks of calcium imaging files were opened in FiJi, individual regions of interest (ROIs) were manually outlined around cell bodies. The ROIs were determined to be CaMKII+ or CaMKII- based on TdTomato labelling. Fluorescence intensity over time was extracted, processed and normalized in the MatLab script PeakCaller (Artimovich et al., 2017). Spike detection threshold was set to 10 % above baseline; for calculation of amplitude heights silent neurons were omitted as these would bias the measurement and underestimate the amplitude heights. Spike frequencies and amplitudes were extracted and raster plots were generated in MatLab.

Statistical analysis (Papers I, III and IV)

Paper I

Statistical analysis was performed in GraphPad Prism 7.2. We denote sample size as n = number of cells analysed, and N = number of animals. Data was tested for normality with D'Agostino-Pearson omnibus K2 normality. To test for determination of statistically appropriate method. Larger sample size data sets were found to fit normal distribution and therefore parametric tests were used. For experiments where $n < 8$, data was assumed to fit a normal distribution without formal testing. All graphs are depicted as mean ± 95% confidence interval. For in between litter comparisons samples were normalized to the wild-type mean of that

litter. Statistical comparisons were made using two-tailed unpaired t tests for comparisons between two groups and paired t-tests for paired tests, and one way ANOVA followed by Tukey's test when comparing multiple groups. Statistical significance was placed at $p < 0.05$ denoted *, $p < 0.005$ denoted ** and $p < 0.001$ denoted *** unless otherwise stated.

Paper III

All data were analysed using GraphPad Prism 7 software and presented as mean ± S.E.M. with 2-5 independent differentiations per cell line (indicated in the figure legend). Unpaired two-tailed t-test or Mann-Whitney U test were used to compare two groups; repeated measures ANOVA was employed for Scholl analysis. A p-value of < 0.05 was considered significant.

Paper IV

All statistical analyses were made with GraphPad Prism 7.2. Sample size was denoted as n = number of cells analysed and N = sets of cultures. Data was first tested for normality using D'Agostino-Pearson omnibus K2 normality test to determine the appropriate statistical test. Data was not normally distributed and therefore non-parametric Mann-Whitney or Kruskal Wallis test was used to compare groups unless otherwise stated. Graphs are expressed as mean ± 95% confidence interval. Differences were considered significant at $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, n.s., not significant.

Results

Summarized in this section are the main results from the four papers included in the thesis. For further details please view the papers in their entirety at the end of this thesis.

APP depletion alters selective pre- and post-synaptic proteins (Paper I)

> "Amyloid precursor protein is a misnomer, it should be called All-purpose protein"
>
> -Proverb

Although Alzheimer's disease has been studied for more than a century, the peptide components of senile plaques were discovered three decades ago. Intensive research around the globe into the production of these peptides from APP, lead to APP being one of the most well studied proteins. The transcription, translation and trafficking of this type 1 integral membrane protein are well known and characterized in multiple cell types. The physiological role of APP and it's cleavage products are however still a mystery, there have been studies indicating that APP could function as a synaptogenic adhesion molecule, the large extracellular part could function as a secreted neurotrophin, and the APP intracellular domain could be involved in transcriptional repression similar to Notch intracellular domain. We had previously in the group characterized alterations in synaptic proteins in neurons from Tg2576 mice that overexpress the APP Swedish FAD variant and shows phenotypes in both brains of mice and cultured neurons. Evidence is mounting suggesting that APP might play important roles at synapses. To investigate the role of APP we used APP knockout mice (KO) that were generated 1995 by Hui Zheng these mice are viable but present some neurological phenotypes such as: impaired memory, increased seizure sensitivity, astrogliosis and long term potentiation defects (Zheng et al., 1995). Studies on synapses in APP KO had yielded seemingly conflicting findings,

e.g. spine density was suggested to be decreased in one study (Lee et al., 2010) while others reported increased spine density (Bittner et al., 2009).

We began by characterizing primary neuronal APP KO cultures to littermate WT cultures; the neurons were grown for 12 or 19 days and were then processed and analysed. Consistent with previous findings (Perez et al., 1997; Tyan et al., 2012), we found decreased dendritic branching and a maturation dependent decrease in phalloidin positive dendritic puncta in APP KO compared to WT neurons at 12 and 19 DIV (Fig. 4).

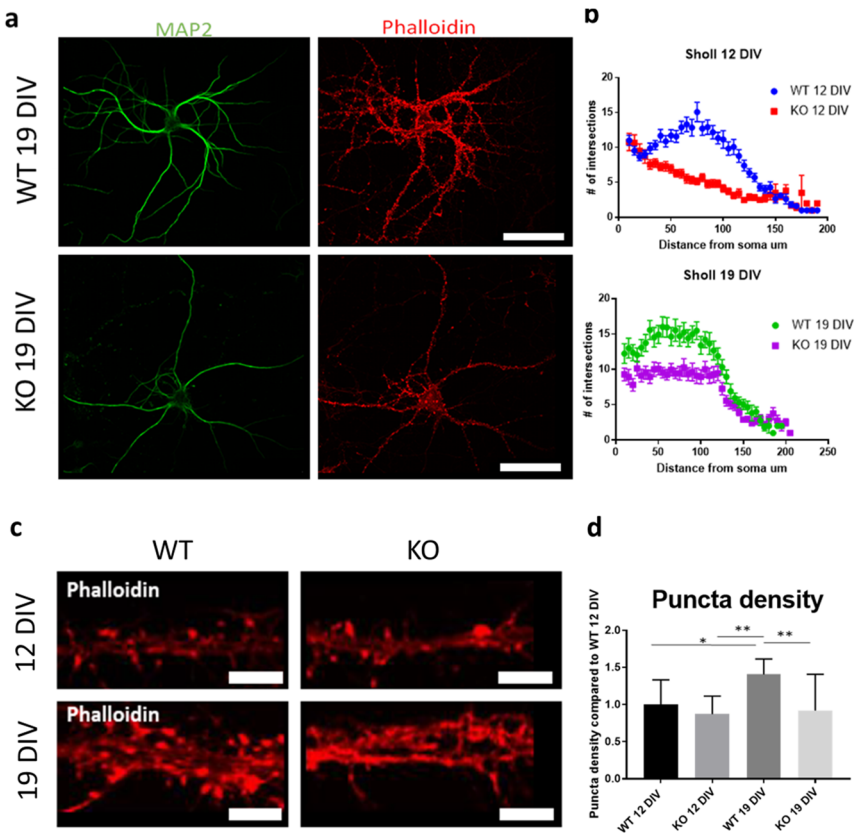

Figure 4. APP knockout neurons display altered neuronal morphology. a) primary neurons from APP KO and WT at 19 DIV labelled with MAP2 and phalloidin. **b**) Sholl analysis revealed reduced dendritic branching in APP KO neurons at 12 and 19 DIV, number of intersections at increasing diameter from soma was counted. Graphs depict mean ± 95% confidence interval. **c**) Representative dedritic segments labelled with phalloidin to visualize putative spines, scalebar = 3 microns. **d**) Quantification of relative puncta density at 12 and 19 DIV, density of phalloid positive puncta is decreased in 19 DIV APP KO neurons as compared to WT. Graphs show mean ± 95%confidence interval. **(Figure adapted from Paper I)**

As phalloidin binds to filamentous actin, one way to visualize putative spines, this indicated that the APP KO neurons either did not generate as many spines or that they were not as stable over time. We were interested to look at the relative amounts of select pre- and post-synaptic protein levels in these neurons. Interestingly, at 12 DIV we found a substantial almost four fold increase in the AMPA receptor subunit 1 (GluA1) by western blot (Fig. 5a-b).

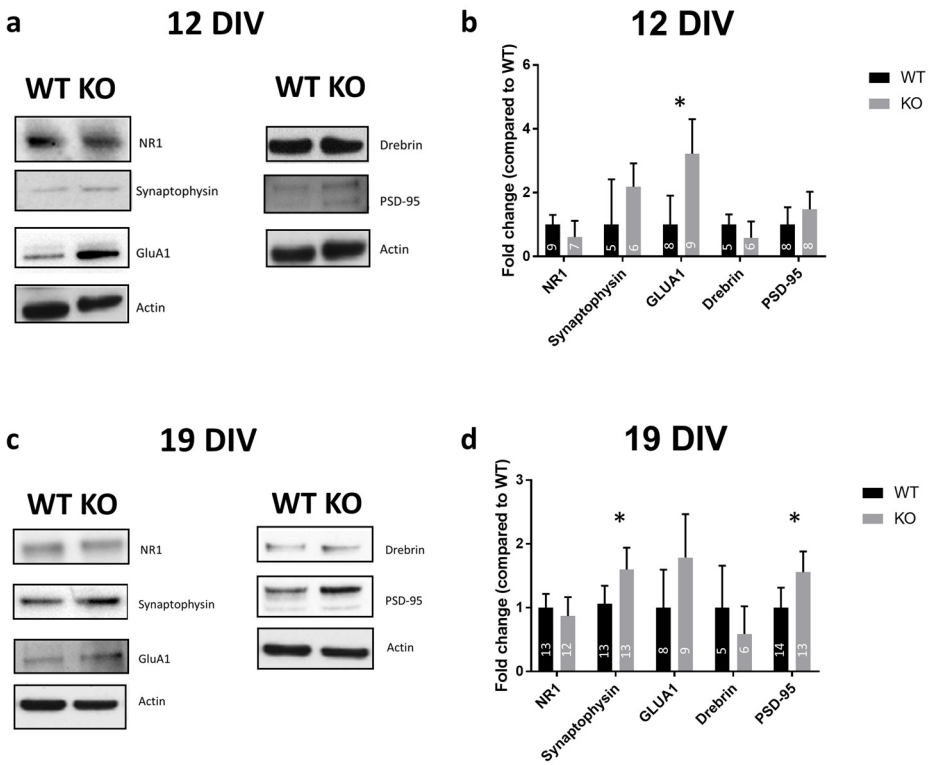

Figure 5. Alterations in pre- and post-synaptic protein levels in APP KO neurons. a) Representative western blot of 12 DIV APP KO compared to WT neurons. b) Graph showing quantifications of optical density from a), GluA1 was found to be increased almost 4 fold. c) Representative western blots of 19 DIV APP KO and WT neurons. d) Graph depicts quantifications of optical density from c). Both levels of synaptophysin and PSD-95 were increased in APP KO compared to WT neurons. Graphs show mean ± 95%confidence interval (Figure adapted from Paper I)

We also detected local increases of synaptophysin and GluA1 by confocal microscopy of neurite segments of APP KO compared to WT neurons (Fig. 6.). At 19 DIV we showed increases in the post-synaptic protein PSD-95 and synaptophysin by western blot (Fig. 5c-d), whereas locally there was a decrease in the actin binding protein drebrin, consistent with decreased phalloidin staining at this time-point (Fig. 4 and Fig. 6).

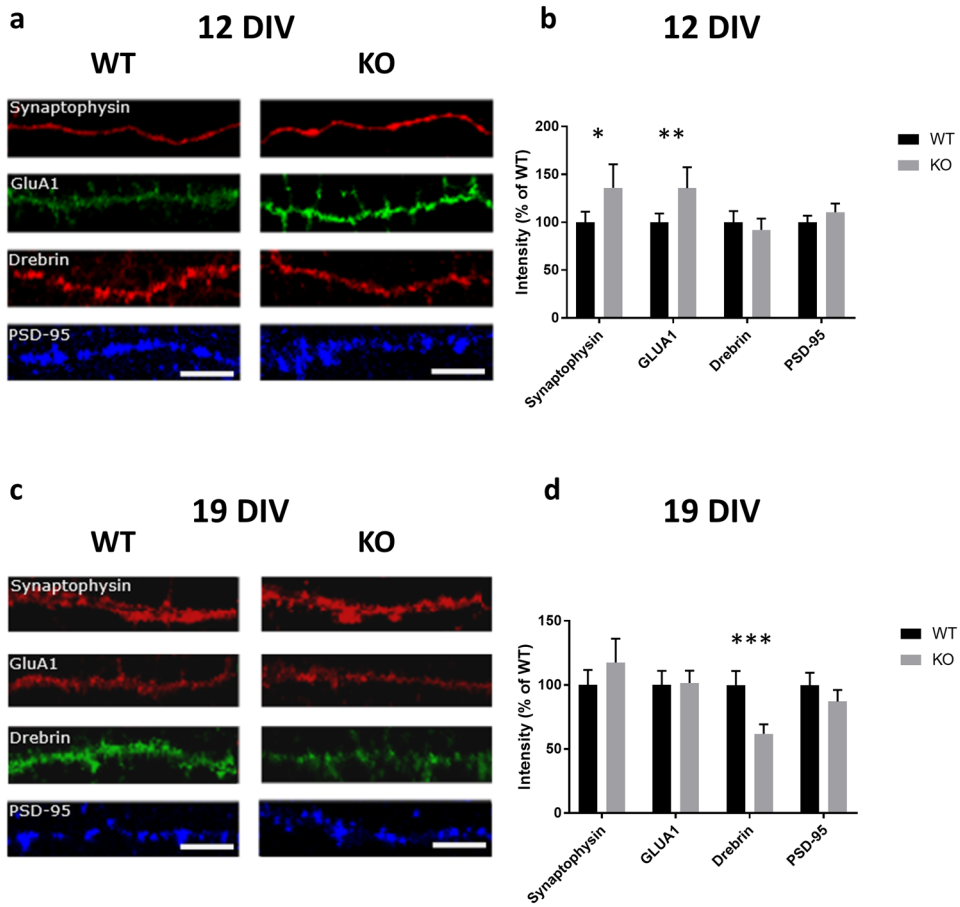

Figure 6. Local alterations in synaptic proteins in APP KO neurons. a) Representative micrographs of 12 DIV WT and APP KO neurite segments label for synaptophysin, Glua1, Drebrin and PSD-95, scalebar 5µm. b) quantifications of the levels of synaptic proteins in neurite segments from WT and APP KO neurons at 12 DIV, both the levels of synaptophysin and of GluA1 were increased in APP KO neurons compared to WT. Intensity in WT are taken as 100%. c) Representative micrographs of neurite segments at 19 DIV in WT and APP KO labelled for synaptophysin, GluA1 drebrin and PSD-95. Scalebar 5µm. d) Quantification of synaptic protein levels at 19 DIV in neurite segments from (c). WT intensity is taken as 100% . Graphs depict mean±95% confidence interval.(Figure adapted from Paper I)

Further we used western blot to investigate the levels of these proteins in brains of APP KO and WT mice at 3 and 12 months of age. Interestingly, the alterations seen in immature 12 DIV cultures and mature 19 DIV cultures were almost mirrored in brain at young 3 compared to older 12 months old APP KO mice. At 3 months of age, APP KO neurons had increased levels of synaptophysin and GluA1, while at 12 months they showed increased levels of PSD-95 and decreased levels of drebrin. These findings indicated that neurons lacking APP had significant alterations in their synaptic composition. However, since APP has been implicated in neuronal

development we developed a viral vector carrying a short hairpin RNA targeting APP. Using this we knocked down APP after the formation of synapses at 10 DIV and investigated the neurons at 14 DIV. First we validated the knockdown of APP by western blot against the C-terminal fragment of APP. By western blot we discovered that GluA1 was increased in 4 separate experiments. Not only in western blot but also locally by confocal microscopy at dendritic arbours was a decrease in GluA1 evident. To our surprise 4 days of APP knockdown was not enough to significantly alter spine density or length (Fig. 7).

Together our findings suggest that APP and/or its proteolytic cleavage fragments are required for the normal composition of synapses.

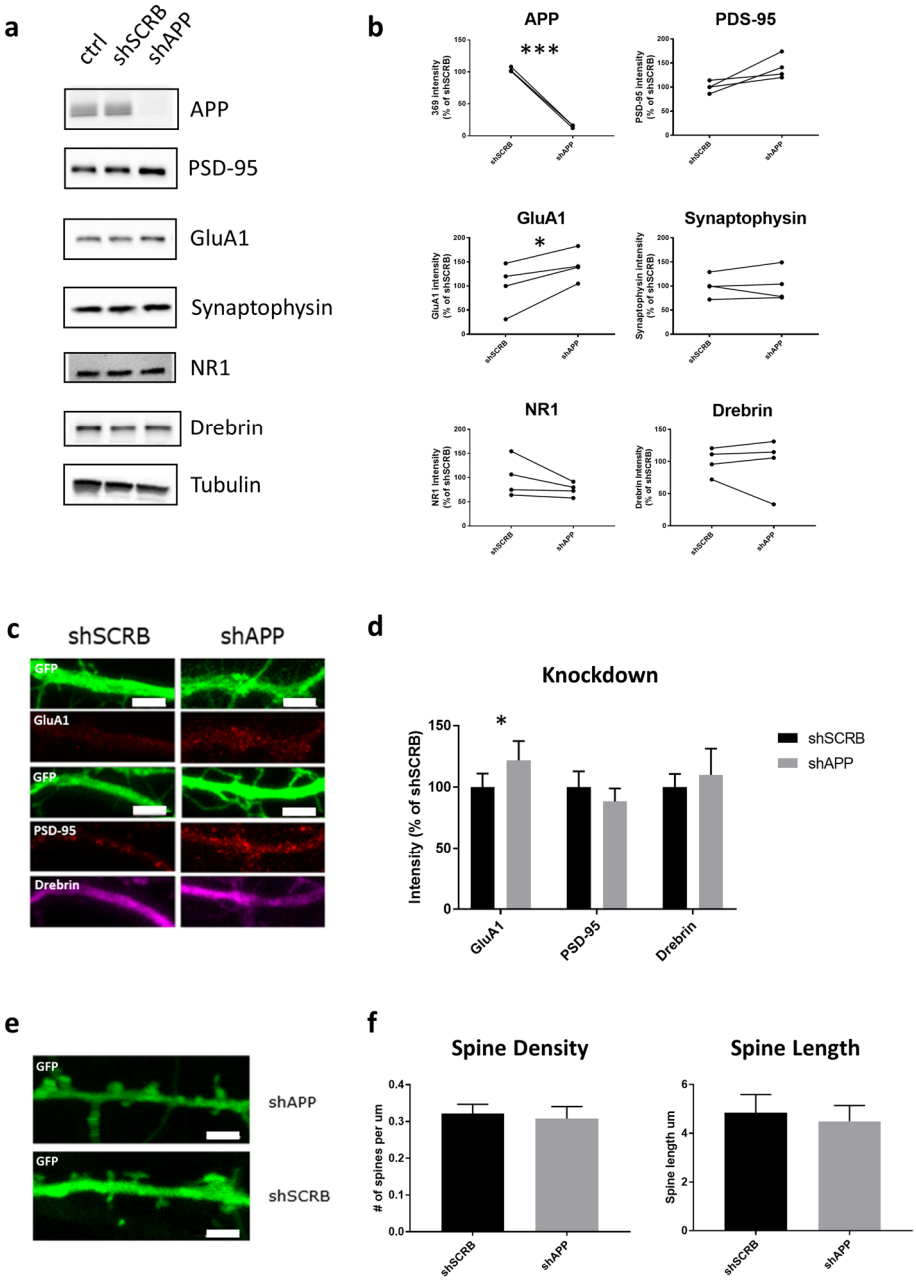

Figure 7. Knockdown of APP alters GluA1 levels. a) Representative western blots of synaptic proteins. b) quantifications of western blots in (a). APP was decreased to almost 10 % of that of a scrambled control shRNA. GluA1 was significantly increased in the shAPP treated cultures. c) Representavive neurite segments . d) Quantification of fluorescence intensity along neurite segments reveal increased intensity of GluA1 in shAPP treated neurons. e) Micrographs depicting GFP labeled processes from shSCRB and shAPP neurons. f) Quantifications of spine density and spine length did not show any differences after 4 days of knockdown Graphs depict mean±95% confidence interval. (Figure adapted from Paper I)

Pre-plaque conformational changes in Alzheimer's disease-linked beta-amyloid and APP (Paper II)

> "There is nothing more difficult to take in hand, more perilous to conduct, or more uncertain in its success, than to take the lead in the introduction of a new order of things"
>
> -Niccolo Macchiavelli

One of the main hallmarks of AD is the senile plaque. This lesion consists of β-amyloid fibrils. One problem with studies on the brains of transgenic animals and also AD patients is that the tissue has to be chemically processed in order to detect β-amyloid. Amyloid fibrils can be tricky to detect in early stages, partly because antibodies and dyes used to study them can be structure sensitive or insensitive; chemical processing and fixation might also cause alterations in structure. Together these limitations in detection have made investigation into early aggregation and deposition challenging. In this study we used non-destructive methods to assess the conformational state of beta-amyloid at different stages in brains of AD transgenic mice. First we studied brains of Tg19959 mice, which typically develop plaques by three months of age. By using Fourier transform infrared micro-spectroscopy (FTIR) imaging, a method which studies absorption of infrared radiation to provide insights into the secondary structure of proteins, we found a marked increase of beta-sheet structures in the brains of Tg19959 mice which was not present in WT mice of the same age. Surprisingly the beta-sheet signal appeared at 2 months, even before plaques were detectable by conventional methods in Tg19959 mice, as demonstrated by ThS staining and fibrillar oligomer antibody OC labelling of adjacent sections (Fig. 8).

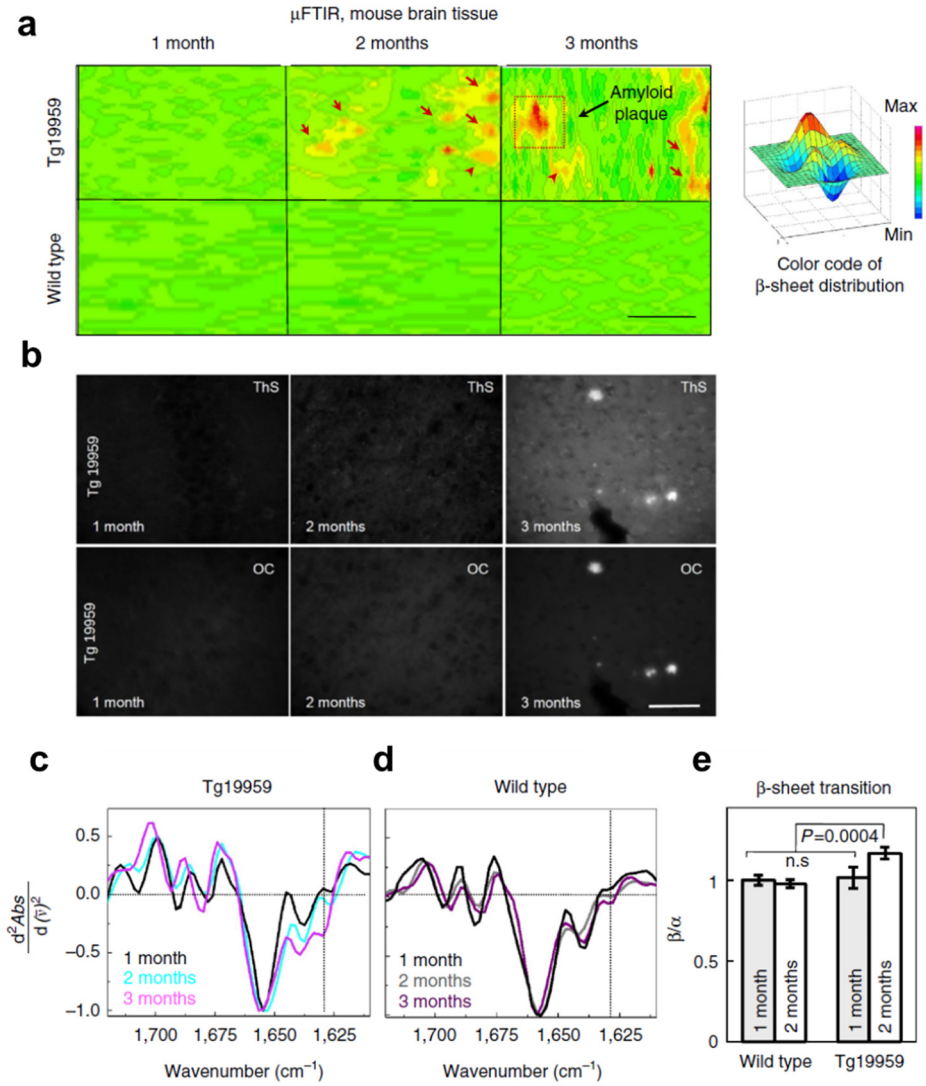

Figure 8. microFTIR reveals alterations in conformation before the appearance of plaques. a) heatmaps of FTIR signal in brain slices from transgenic and WT mice at 1,2 and 3 months of age. Integrated for visualization of the β-sheet spectral region at 1635-1620 cm-1. b) Histology on adjecent sections with ThS and OC antibody (scalebar 50 μm), note the appearance of ThS and OC positive plaques at 3 but not 2 months of age in TG19959. c) Second derivative spectra of IR absorbtion in Tg19959 with animals of different ages overlapped shows increased β-sheet content at 2 and 3 months d) second derivative sprectra of WT overlapped. e) Quantification of β/α ratio demostrating higher β-sheet transition in 2 month Tg19959. Protein aggregation is normalized to WT- β-sheet content. ANOVA ($p = 0.0004$), n = 9, Data represented as mean ± s.d. (Figure adapted from Paper II)

We speculated that this increase in beta-sheet folding could be due to low amounts of fibrils or that the content of these pre-plaques had a non-fibrillar nature. We modelled aggregation of β-amyloid $_{1\text{-}40}$ bound with copper to produce non-fibrillar β-amyloid oligomers with beta-sheet structures, and verified by small-angle-X-ray-scattering (SAXS), Thioflavin T (ThT) aggregation assays, transmission electron microscopy and FTIR that such non-fibrillar β-amyloid $_{1\text{-}40}$ could give rise to the same increased β-sheet signals we observed pre-plaque formation in brains of Tg19959. Further, we demonstrated that increased β-sheet signal also was present in cultured primary neurons from APP/PS1 mice at 19 but not at 12 days in vitro or in WT neurons. This increase in β-sheet signal concurred with an increase in endogenously expressed β-amyloid in these cultured neurons (Fig. 9).

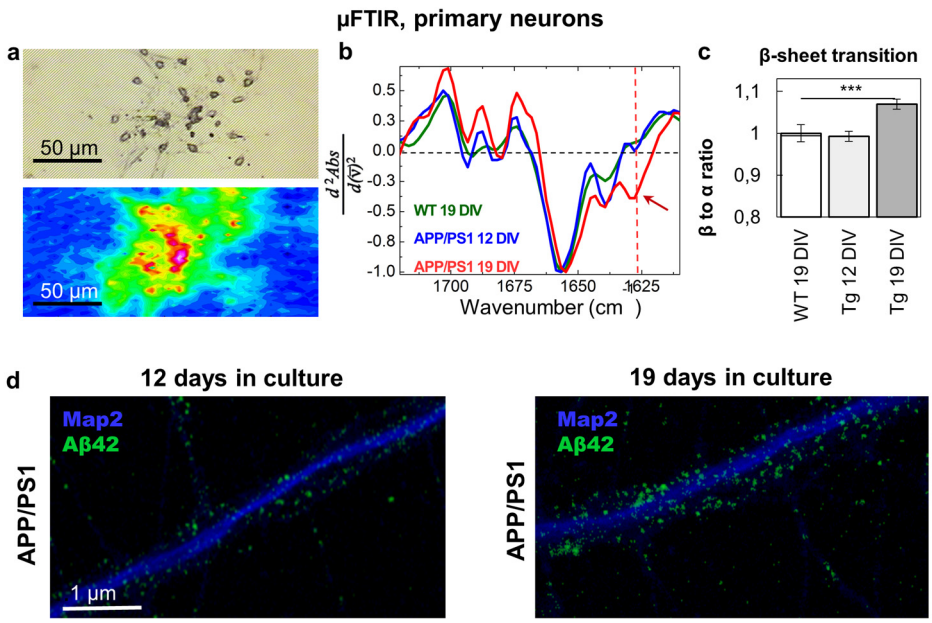

Figure 9. Increased aggregation in primary neurons from transgenic AD model mice. a) Bright field image (upper panel) depicts cultured AD transgenic neurons from APP/PS1 cultured 19 DIV, FTIR heatmap (lower panel) of same neurons, scalebar 50μm. b) averaged and normalized second derivative spectra of taken from APP/PS1 and WT neurons at 12 and 19 DIV. Arrow indicates the peak corresponding to β-sheet content present only in APP/PS1 neurons at 19 DIV. c) Quantification of β/α ratio normalized to WT shows an increase in β-sheet content at 19 DIV ($p < 0.01$), ANOVA followed by Bonferroni correction for multiple testing, n = 50 neurons per genotype/age, Data are shown as mean ± s.d. d) High resolution confocal micrographs shown immunolabeled β-amyloid$_{1\text{-}42}$(green) and MAP2(blue) a dendritic protein in APP/PS1 neurons of 12 and 19 DIV. Note increased β-amyloid$_{1\text{-}42}$ labelling in 19 DIV cultures, scalebar = 1μm. (Figure adapted from Paper II)

We next investigated brain extracts of Tg19959 mice using non-denaturing blue native PAGE followed by western blot and various APP and β-amyloid antibodies to study aggregation at different ages. As expected with aging and the appearance

of plaques, a high molecular weight smear was detected on membranes blotted for β-amyloid. What was surprising though was the presence of a protein band at approximately 20 kD in WT and 1 month Tg19959 brains which was then decreased or absent in 2 months and 3 months Tg19959. This band did not appear when probed with antibodies raised against the N- or C-terminus of APP, indicating that it consisted of β-amyloid and was not the C-terminal fragment of APP. Of note, we also saw that synthetic β-amyloid was mostly 20 kd on native PAGE. The disappearance of the 20 kD band in Tg19959 mouse brains was coincident with the appearance of a smear of putative higher molecular weight oligomers at 2 months of age, possibly indicating that this β-amyloid form becomes unstable and begins to aggregate into higher molecular weight oligomers and fibrils.

To further investigate the initial pre-plaque aggregation in brains of mice we labelled Tg19959 mouse brain slices with β-amyloid $_{1-42}$ specific antibodies and detected an accumulation of β-amyloid at synaptic terminals at 2 – 3 months of age. Furthermore, we noted that labelling of pre-and post-synaptic proteins showed increased co-localization at these time-points, which might indicate a breakdown of the synaptic cyto-architecture.

Together our findings provide evidence that β-amyloid undergoes pre-fibrillar aggregation states where the peptide is ThS and OC negative but still displays increased β-sheet structures. We also showed that this aggregation occurs in neurons cultured in vitro and is correlated with an increased labelling of β-amyloid. Moreover, we investigated the disappearance of a 20 kDa form of β-amyloid, the disappearance of which coincides with formation of higher molecular weight oligomers. Then we demonstrated a local accumulation of β-amyloid in synaptic compartments of Tg19959 mice concomitant with an apparent breakdown of their synaptic structures.

Modelling typical and atypical familial Alzheimer's disease in iPSC-derived hippocampal spheroids (Paper III)

"If we knew what it was we were doing, it would not be called research, would it?"

-Albert Einstein

A major pitfall in Alzheimer's disease research has been the translation of findings in animal models to therapies which work in the clinic. Although much has been learned and can be learned from animal models overexpressing APP and presenilin mutations, at some point human disease models are necessary to drive research forward. One such source of human cells are induced pluripotent stem cell (iPSC) derived neurons. These cells can be harvested from patients carrying disease linked mutations or who present with idiopathic late onset disease; the cells are then reprogrammed to a pluripotent state through viral introduction of the Yamanaka factors (Takahashi et al., 2007). Such reprogramming yields a cell type referred to as an iPSC, although taken from elderly patients these cells undergo a rejuvenation when expressing the Yamanaka factors. These pluripotent stem cells can then be tricked into developing into certain lineages by mimicking important developmental stages.

In this study we investigated two such cell lines derived from patients in the clinic, one with a typical AD phenotype carrying one of the most common fAD causing mutations (APP London mutation), and one with an atypical AD associated PS1 mutation (Assini et al., 2003). These were then compared to iPSCs derived neurons from gender matched non-demented controls.

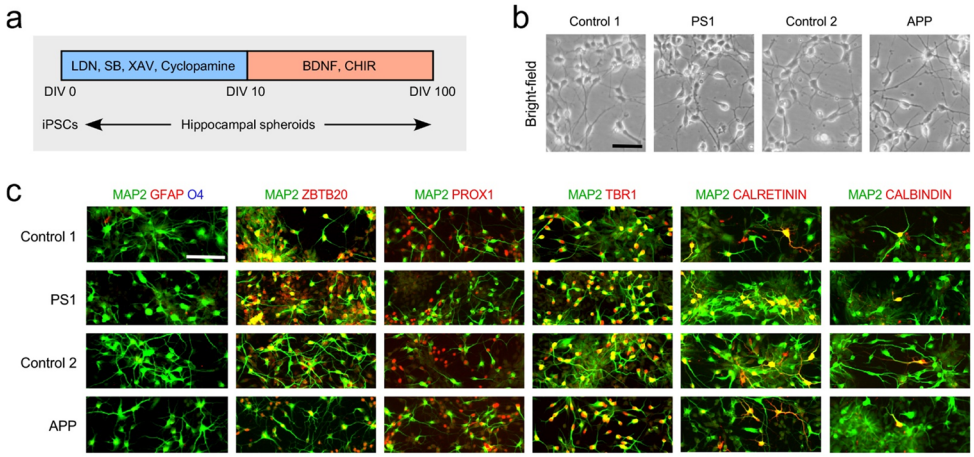

Figure 10. Characterization of hippocampal spheroids from iPSC derived neurons. a) Shows schematic model of differentiation protocol for generation of HSs from iPSCs. b) Bright field micrographs showing neuronal morphalogy in 2d cultures at 56 DIV, scalebar = 100μm. c) micrographs showing characterization of Astrocytic(GFAP), Oligodendrycytic (O4) and Neuronal (MAP2/ZBTB20/PROX1/TBR1/CALRETININ/CALBINDIN) markers in cultures from HSs at 56DIV scale bar 200μm. (Figure adapted from Paper III)

These iPSC neurons were then differentiated using a novel protocol into what we refer to as hippocampal spheroids (HSs); these harbour many of the characteristics of the hippocampus, an area associated with early pathology in AD (Mueller et al., 2010), which is particularly interesting in AD due to its role in memory formation and consolidation. These hippocampal spheroids were characterized and found to express many of the markers of cells present in the hippocampal formation. In particular a large population of PROX1 positive dentate granule-like cells were seen. We demonstrated that the hippocampal spheroids express neuronal markers and even form putative synapses (Fig.10 and Fig. 11).

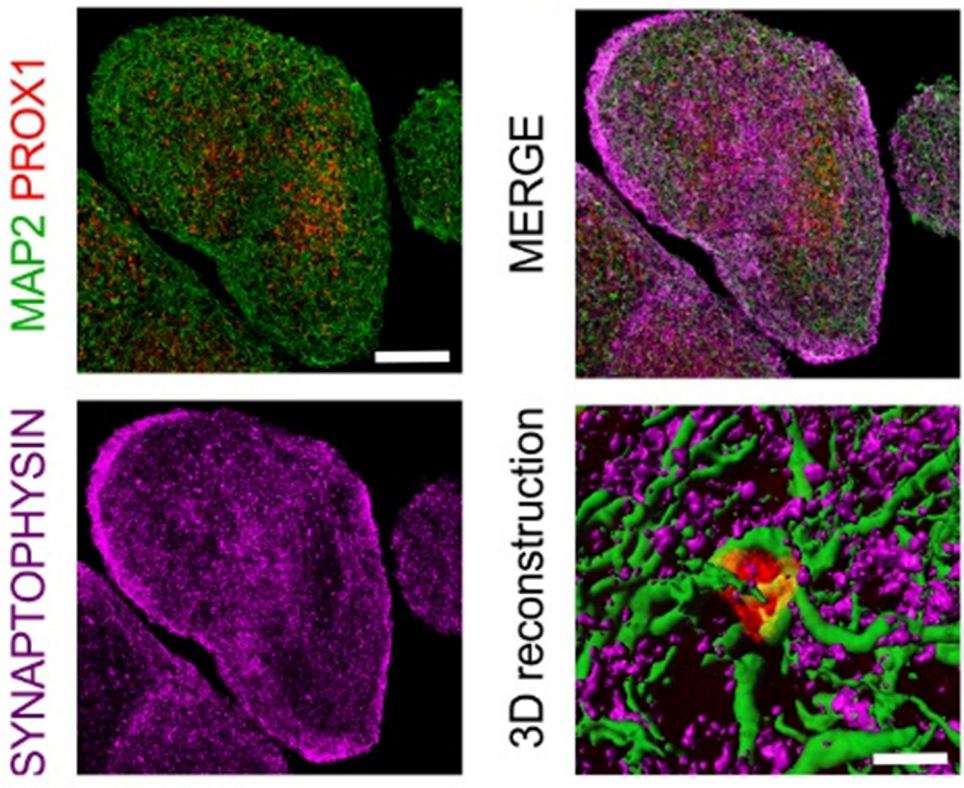

Figure 11. Hippocampal spheroid with 3d reconstructed Map2 positive dendrites and synaptophysin puncta. Figure shows immunostaining of 100 DIV HS labeled with dendritic marker MAP2(green), hippocampal granule neuron marker PROX1 (red) and presynaptic marker Synaptophysin (magenta) scalebar is 100µm for HS hippocampal confocal images. Lower right panel shows a 3d reconstruction of a PROX1/MAP2/Synaptophyin positive granule neuron in a HS, scalebar 10µm (Figure adapted from Paper III)

Encouraged by these findings we analysed the cells for pathological phenotypes, and found increases in the β-amyloid42/40 ratio in both APP London and PS1 mutants, we also saw alterations in Tau phosphorylation with AT8 antibody (Fig. 12c and d). Intriguingly we found altered levels of the synaptic proteins synaptophysin and drebrin, also known to be reduced in human AD brains (Fig. 12f and g). These findings indicated that these hippocampal spheroids showed disease phenotypes at 100 days in vitro (DIV). Interestingly, the APP London mutant showed increased dendritic branching and overall dendrite length (Fig. 13)

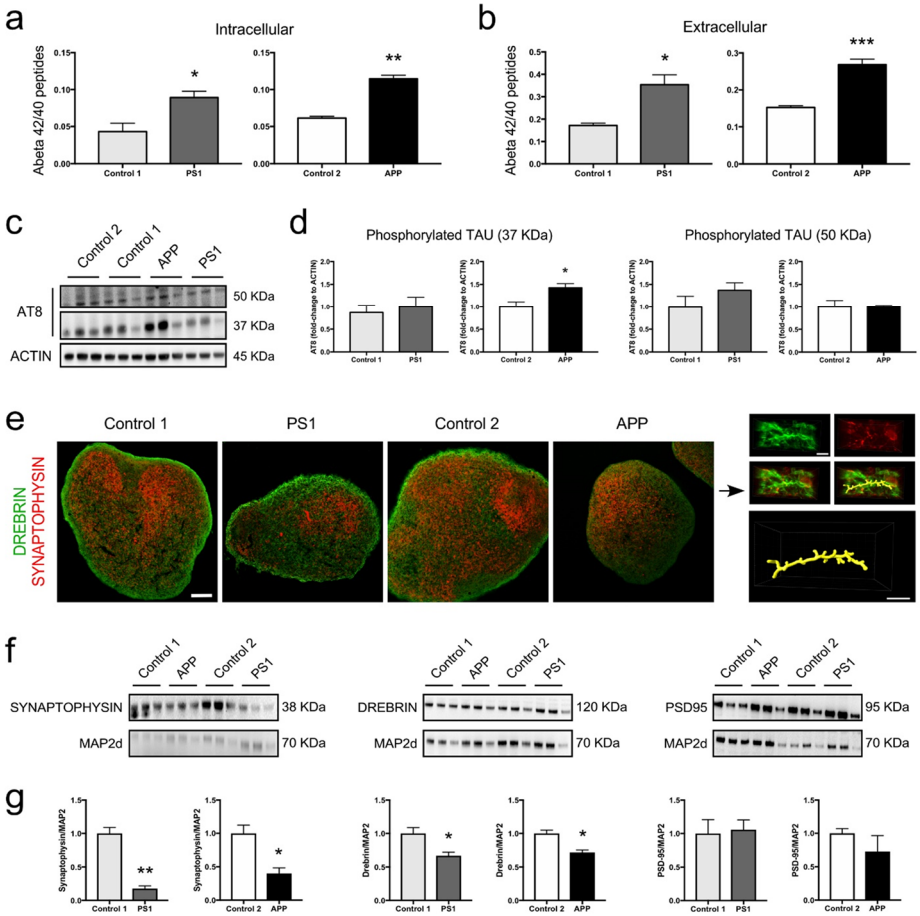

Figure 12. HSs generated from AD patients display AD related pathology. a) Intracellular β-amyloid 42/40 ratio is increased in APP and PS1 mutant compared to their gender matched controls. b) secreted β-amyloid 42/40 ratio is increased in APP and PS1 mutant compared to their gender matched controls. results presented as mean ±S.E.M. n = independent differentiations. Analyzed by two tailed T-test. c) Immunoblot of phospho Tau marker AT8 in hippocampal spheroids at 100 DIV normalized to β-actin. d) quantification of optical density of two AT8 bands at 37 and 50 kDa. APP mutant was found to show increased Tau 37 kDa in comparison to igender matched control. e) Representative immunolabeling of HSs from all variants and a 3d reconstruction of a dendritic segment with putative dendritic spines as delineated by drebrin labeling in proximity to synaptophysin puncta. scalebars 100μm (HSs) and 5μm (reconstruction) f) Immunoblots of synaptic markers synaptophysin, drebrin and PSD-95. g) Densitometric quantification of synaptic protein levels normalized to the mature neuronal marker MAP2d graphs depict mean ± S.E:M. n= 3 independent differentiations. P <0.05 *, P<0.001**, p<0.0001***. Two tailed T-test. (Figure adapted from Paper III)

To further investigate the increased levels of β-amyloid we used Fourier transform infrared spectroscopy (FTIR) to investigate the amount of aggregation and phosphorylation in our samples. Interestingly, a marked increase of β-sheet structures was seen in the APP London but not in the PS1 mutant lines, a finding which was surprising given that β-amyloid 42/40 ratio was increased in both

mutants, although this is an unusual PS1 mutation with atypical features (Assini et al., 2003). We also found increases in overall protein phosphorylation in the APP London but not the PS1 mutant line, which was consistent with increased AT8 immunoreactivity in the APP London but not the PS1 mutant line.

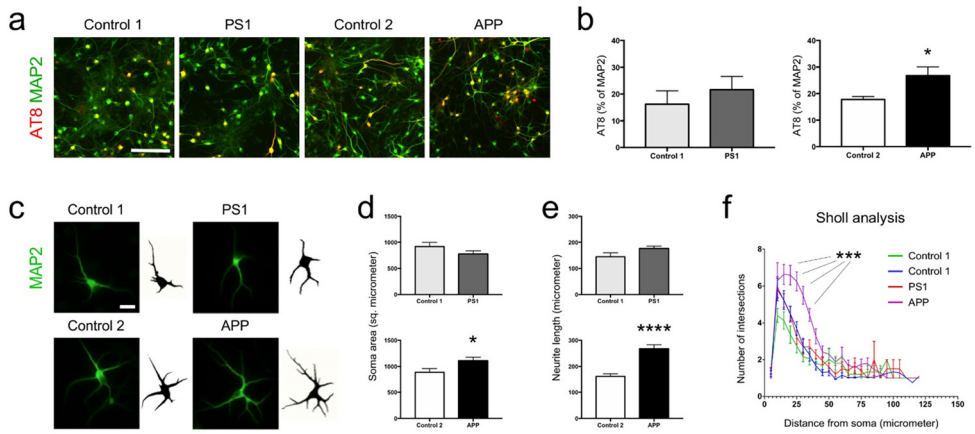

Figure 13. APP variant neurons exhibit morphological alterations. a) Phospho Tau labeling by AT8 antibody in 2d cultured neurons, scalebar 200 µm. b) Quantification of AT8 intensity normalizad to MAP2. Mean ± S.E.M., n = 3 independent differentiations. c) representative micrographs of neurons labeled with MAP2. Scalebar 20 µm. d Quantification of soma area sq. microns, and e) neurite length in microns, Two tailed T-test, n = 16-23 neurons from 3 independent differentiations. f) Sholl analysis of dendrite complexity. Y-axis shows number of intersections a circle with growing diameter has with a binary value dendritic tree. Graph shows mean ± S.E.M. P values are * = p<0.05, ** = p<0.01, *** = p<0.001 and **** p<0.0001. Repeated measures ANOVA (Figure adapted from Paper III)

In the final part of the study hippocampal spheroids were analysed through mass spectrometry. The four lines separated in two principal components. Interestingly, principal component 1, which was gender, showed a higher effect than principal component 2, which was genotype. This highlights the importance of gender-matched controls when analysing neurodegenerative diseases. Gene ontology analysis of the differentially expressed proteins revealed that APP London displays decreased proteins in pathways related to endosomal, lysosomal and phagosomal function. Further, both genotypes displayed alterations in 19 shared synaptic proteins associated with synaptic vesicles and neurotransmitter release.

Together our findings in this study highlight that APP/β-amyloid strongly influences synaptic parameters also in human induced neurons.

Neuronal subtype vulnerability of β-amyloid induced hyper-excitability (Paper IV)

> "The devil is not as black as he is painted"
>
> -Dante Alighieri

Since β-amyloid was discovered as the main constituent of the amyloid plaques characteristic of AD this peptide has been the centre of numerous studies. These initial studies led to the formulation of the amyloid cascade hypothesis which posited that β-amyloid produced by neurons accumulates and causes hyper-phosphorylation of tau and downstream of that leads to neuronal dysfunction and death. The hypothesis has been revised a few times, and alternative interpretations have been suggested.

Increasing evidence shows that hyper-excitability is an early feature in prodromal AD (Palop and Mucke, 2010) and hyper-excitability has been demonstrated in a number of transgenic mouse models (Warner et al., 2015; Busche et al., 2019). It was recently shown by hippocampal EEG that AD patients have subthreshold seizure-like activity which impact memory consolidation in the hippocampus (Lam et al., 2017). In-vivo two photon calcium imaging showed hyper-activity of neurons in close proximity to plaques (Busche et al., 2008). Further, APP/PS1 mutant transgenic AD mice were demonstrated to have increased sensitivity to chemically induced seizures (Warner et al., 2015). The mechanism of hyper-excitability in AD and AD models is not well understood. Some studies have implicated that PS1 mutations could cause such a hyper-excitability (Lerdkrai et al., 2018). However studies in mouse brain have shown that perfusion with β-amyloid can cause a similar hyper-excitability (Zott et al., 2019). In this study we set out to investigate AD related hyper-excitability in cortico-hippocampal neurons from APP/PS1 transgenic mice.

Calcium imaging can be used as a window into the internal activity of neurons. Calcium is a ubiquitous second messenger and by studying its rise and fall we can learn a lot about neuronal function. We started by comparing the activity levels of neurons from APP/PS1 mutant to those of WT littermates. Interestingly, we found that neurons cultured from the APP/PS1 mice displayed increased firing rates and amplitudes of their calcium transients (Fig. 14)

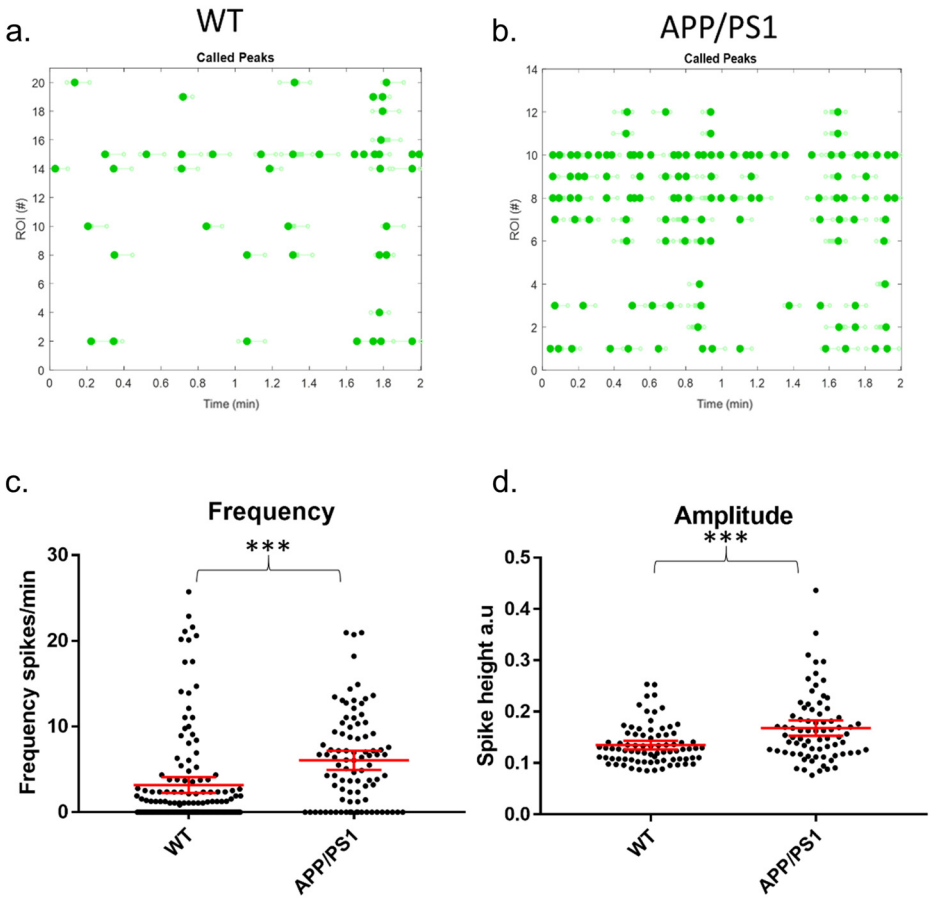

Figure 14. Spontaneous firing frequency and amplitude of calcium spikes increased in APP/PS1 neurons. a) raster plot of representative WT neuron field of view during 2 min of imaging. b) raster plot of activity in a AD transgenic neuron field of view. c) Quantification of frequency defined as spikes per minute, APP/PS1 neurons show higher frequency than WT neurons. d) Spike amplitude higher in APP/PS1 neurons. Mann whitney U test, *** = p<0.001. (Figure adapted from Paper IV)

Since GABA can be excitatory during early developmental stages, and because it is not known whether APP or PS1 mutations alter neuronal maturation in culture, we treated our 19 DIV primary neuron cultures with the GABA antagonists bicuculline or picrotoxin; these treatments led to marked increases in neuronal activity (Fig. 15a-c) suggesting that at this time point in culture GABA is acting in an inhibitory manner. Knowing this we set out to further investigate the spontaneous activity in APP/PS1 mutant compared to littermate WT control neurons.

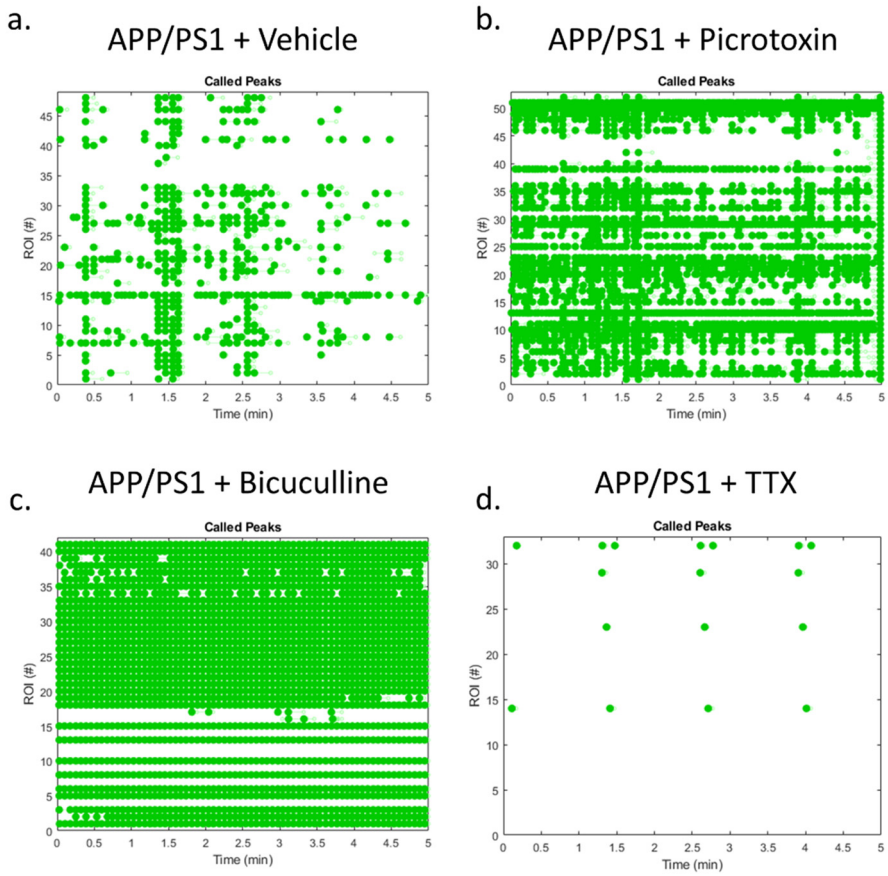

Figure 15. APP/PS1 neuron hyper-activity is modifiable by GABA inhibition and is sodium channel dependent. a) Representative raster plot of 5 minutes of activity in a field of view in APP/PS1 neuron culture treated with vehicle. b) 5 minutes of activity in the presence of XXuM picrotoxin. c) Raster plot of APP/PS1 in the presence of XXum bicuculline. d) Representative raster plot of APP/PS1 in the presence of 1 µm TTX. X axis = time in minutes. Y axis ROI#. (Figure adapted from Paper IV)

We treated APP/PS1 neurons with tetrodotoxin (TTX) which led to an almost complete silencing of activity, indicating that the increased activity in these neurons requires network activity and is not cell intrinsic (Fig. 15d). Then we next set out to investigate whether the increased activity was mainly in excitatory or inhibitory neurons. To do this we used a lentiviral vector carrying the fluorescent cell marker TdTomato under a CaMKII promoter. This promoter drives expression of TdTomato specifically in excitatory neurons allowing identification of neurons as either CaMKII positive or CaMKII negative. Using this strategy we discovered that the baseline activity and calcium spike amplitude were increased mainly in the excitatory neurons (Fig. 16).

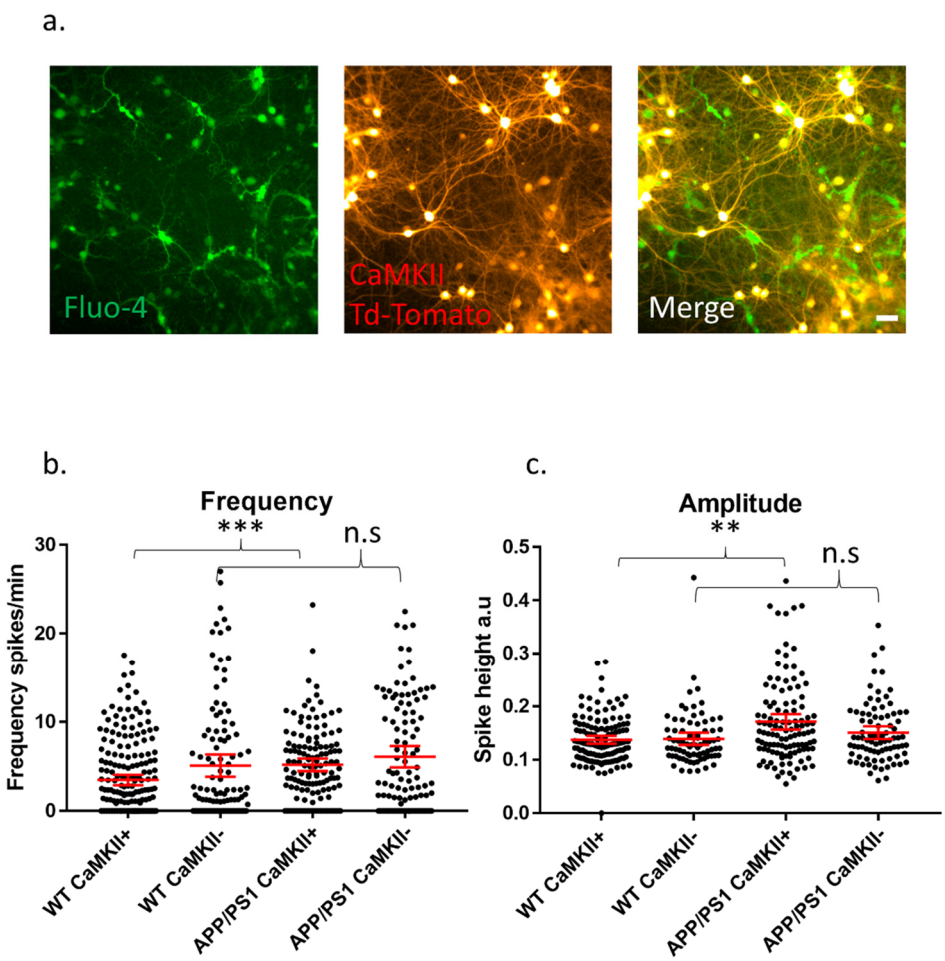

Figure 16. Frequency and amplitude of calcium transients specifically increased in excitatory CaMKII expressing neurons. a) micrograph showing Fluo4 labeled neurons overlayed with CaMKII TdTomato expressing neurons. b) Firing frequency increased specifically in APP/PS1 CaMKII+ neurons. c) Amplitude of calcium transients higher in CaMKII + APP/PS1 neurons. (Figure adapted from Paper IV)

To further investigate the role of β-amyloid in this hyper-excitation we treated neurons with thiorphan, an inhibitor of neprilysin, the main β-amyloid degrading enzyme in the brain (Iwata et al., 2004). One hour of treatment was enough to see an increase in both firing rate and amplitude in cultured WT neurons compared to vehicle treated controls (Fig. 17a-b). However, as neprilysin additionally can cleave other neuropeptides such as substance P, we also increased β-amyloid levels through the direct addition of 1 uM β-amyloid$_{1\text{-}42}$ to cell culture media. Interestingly, this led

to a strong decrease in activity but did not significantly alter the amplitude of calcium spikes (Fig. 17c-d).

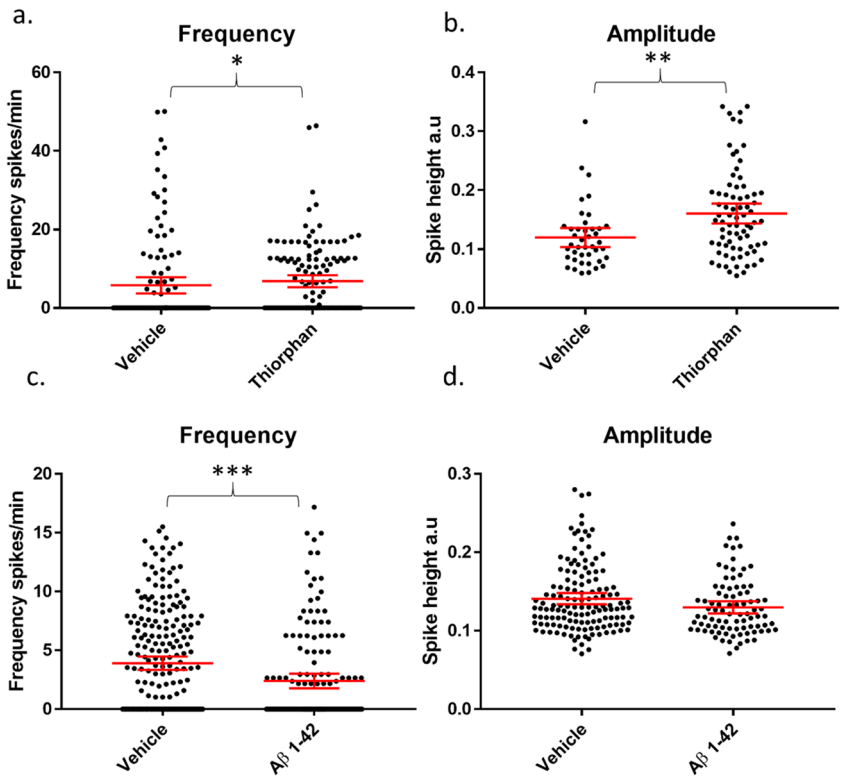

Figure 17. Thiorphan induces increased firing frequency and amplitude while 1μM of β-amyloid decreases firing frequency. a) Quantification of firing frequency shows increase in thiorphan treated cultures, b) quantification of amplitude in thiorphan treated WT neurons.c) 1 μM β-amyloid$_{1-42}$ added to WT neurons led to decreased firing frequency but d) no change in amplitude (Figure adapted from Paper IV)

Although frequently used across numerous studies, 1 uM concentration of β-amyloid$_{1-42}$ is very high and likely not physiologically relevant in the extracellular milieu. Therefore we added β-amyloid in the picomolar range to neurons, a concentration range more similar to that in the brain parenchyma (Cirrito et al., 2003). 200 pm of β-amyloid conversely led to an increase in spiking both in CaMKII positive and negative neurons, and an increase in the amplitude of CaMKII positive but not CaMKII negative neurons (Fig. 18a-b). Interestingly, labelling for exogenously added β-amyloid showed enrichment at dendritic spines of CaMKII positive neurons (Fig. 18c-d) as seen with human specific 6E10 antibody and the conformation specific OC antibody which labels amyloid fibrils.

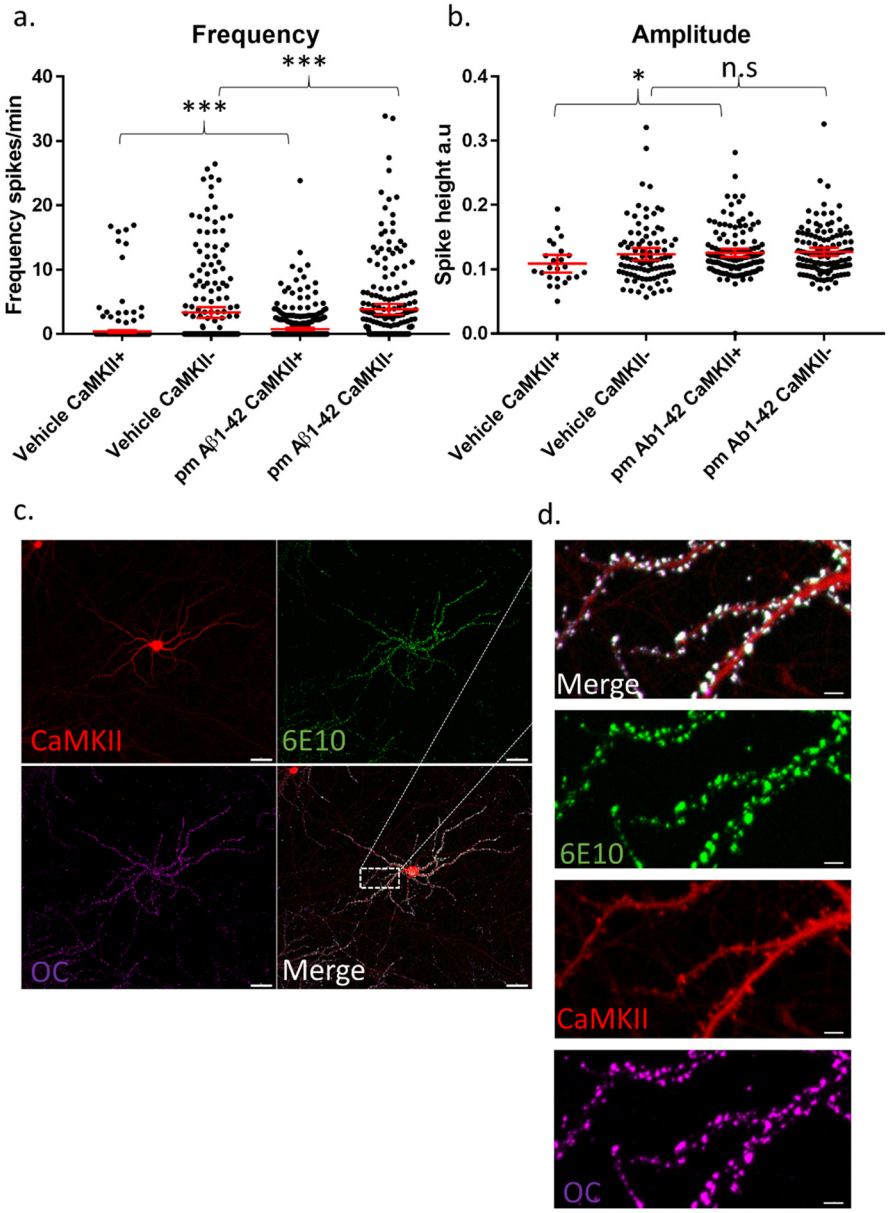

Figure 18. Specific targeting of CaMKII positive neuronal terminals. a) 200pM of synthetic β-amyloid was added to WT neurons firing rate was significatly increased in both CaMII+ and CaMKII- neurons. b) 200 pM of β-amyloid led to increased amplitude in CaMII+ neurons but not CaMKII- neurons. c) Micrograph of CaMKII+ neuron with strong specific labelling with human Specific anti APP/ β-amyloid antibody 6E10 and anti-amyloid antibody OC, scalebar = 50μm. d) insert from c demonstrating β-amyloid binding to dendritic spines on CaMKII+ neurons. Scalebar 5 μm. **(Figure adapted from Paper IV)**

Taken together our results indicate important effects of β-amyloid in modulating neuronal activity, especially in excitatory neuronal populations, possibly by directly binding and influencing synapses.

Discussion and Concluding Remarks

The focus of this thesis was to investigate synaptic roles of β-amyloid and APP. While frequently viewed as a toxic by-product, β-amyloid might play important roles at the synapse. Several findings point to the importance of APP and β-amyloid at synapses, both in normal physiology and in disease: exogenously added β-amyloid binds synapses (Lacor et al., 2004; Willen et al., 2017); APP and APP family members interact trans-synaptically and have synaptogenic properties (Wang et al., 2009; Stahl et al., 2014); β-amyloid when added to brain slices and neurons impairs and disrupts LTP (Walsh et al., 2002); APP mRNA is locally translated at synapses (Westmark and Malter, 2007); β-amyloid accumulates and aggregates in multivesicular bodies in synapses in early AD (Takahashi et al., 2002); APP is transported down axons and dendrites where BACE and γ-secretase are present (Koo et al., 1990; Lundgren et al., 2015; Schedin-Weiss et al., 2016); and the generation of β-amyloid from APP is regulated by synaptic activity (Kamenetz et al., 2003; Cirrito et al., 2005; Tampellini et al., 2009). Moreover, accumulation of oligomeric β-amyloid species in synaptosomes could distinguish cognitively normal individuals with high AD pathology from dementia cases and correlated with the severity of dementia (Bilousova et al., 2016). These reports highlight the importance of understanding synapses in the context of AD.

In paper I we demonstrate that lack of APP greatly affects the molecular composition of synapses. In particular on the AMPA receptor subunit GluA1 which was altered both in APP KO neurons, brains of APP KO mice and in cultures treated with a shRNA targeting APP. Interestingly, GluA1 was found to be decreased in Tg2576 neurons, a mouse model that over-expresses human APP with the Swedish mutation (Almeida et al., 2005). Interestingly the APP KO neurons also showed increased levels of PSD-95, a membrane associated guanylate kinase that is implicated in recruitment and anchoring of glutamate receptors (Ehrlich and Malinow, 2004) even though APP KO neurons had decreased spine density, which has also been described by other groups (Tyan et al., 2012). The findings were not always consistent between western blot and immunofluorescence microscopy although it is important to note that, while western blot assays the total amount of protein in a given sample, immunofluorescence microscopy gives an indication of

the local protein levels, thus discrepancies between the methods could indicate altered transport or localization of a given protein. Indeed there are findings by other groups which suggest that APP KO neurons have altered axonal transport (Smith et al., 2010).

Further, in paper II we demonstrated that β-amyloid aggregation can be studied even before the appearance of Thioflavin S or OC positive plaques. This pre-plaque aggregated β-amyloid coincides with the disappearance of a 20 kDa band present in non-denaturing blue native PAGE. We demonstrated that it is present in WT and one month old transgenic mice and is not a C-terminal fragment. Further we demonstrated that the pre-plaque aggregation also occurs in primary neuronal cultures from APP/PS1 mice with time in culture. This is interesting and fits with earlier data from our lab demonstrating progressive accumulation and aggregation of intracellular β-amyloid with time in culture and in the synapses of aging Tg2756 mice (Takahashi et al., 2004). This is consistent with pre-plaque β-amyloid in the brain having an intraneuronal origin and being the origin of neuritic plaques (Gouras et al., 2005; Pensalfini et al., 2014).

In paper III, our studies in human iPSC derived neurons revealed that the APP London mutation which increases the ratio of β-amyloid1-42 over β-amyloid1-40, lead to significant changes in synaptic proteins. Interestingly these neurons had longer neurites and more branches at early time-points. We also demonstrated disease associated phenotypes such as increased Tau phosphorylation. A potential caveat in this finding is that neurons also express hyper-phosphorylated Tau during developmental stages.

Lastly, in paper IV, we demonstrate that β-amyloid has concentration dependent effects on synaptic activity. This regulation of synaptic activity could be part of a normal function that gets dysregulated in disease leading to hyper-activity. The observations that β-amyloid binds glutamatergic synapses coupled with the particular effect on CaMKII expressing neurons suggest these are sensitive to the pathological effects of β-amyloid. Recent clinical findings demonstrated that up to 42.4% of AD patients had sub-clinical epileptic seizures and furthermore this was correlated with steeper progression of global cognitive decline (Vossel et al., 2016). Therefore understanding this early pathological feature is of great interest.

To summarize, in the studies that constitute this thesis, we have explored synaptic roles for APP and β-amyloid. We demonstrated that APP and/or its cleavage products are necessary for the normal composition of synapses. We showed that APP/PS1 neurons in culture have increased β-sheet signals. Further we showed that the APP London mutation led to changes in the levels of synaptic proteins in iPSC

derived human neurons. Finally we demonstrated concentration dependent regulation of neuronal activity by β-amyloid peptides in cultured neurons from WT and APP/PS1 mice. The complex biology of APP and its cleavage products remains to be fully understood. We believe that increasing our knowledge on the subject will help us understand the initial stages of AD and could be of great value for the development of novel therapies.

Future perspectives

In this thesis, we have explored various aspects of the synaptic and neuronal biology of APP and β-amyloid.

In paper IV we showed that β-amyloid had distinct effects on CaMKII positive neurons. However, in future studies it will be important to investigate the potential effects on for instance GABAergic interneuron subpopulations. It would be interesting to continue this line of work, using different strategies for visualizing other subgroups of neurons and potentially in the future also combining these strategies with ever more available single cell- transcriptomics and genomics approaches. Another aspect to investigate in the future is the effect on synaptic activity if β-amyloid is removed, although these experiments inherently are filled with caveats. Some ways to investigate the role of β-amyloid would be to use antibodies to remove it from the cell media, replace conditioned WT media with conditioned media from APP KO neurons, pharmacological manipulation of APP processing (e.g. BACE inhibition or γ-secretase inhibition) or studying APP mutants that generate less or no β-amyloid.

Another angle to investigate is whether there are homeostatic plasticity adaptations in the APP/PS1 cultured neurons, since they appear to have higher firing frequency than WT neurons and that activity tends to regulate itself to a given set point. It is conceivable that the higher baseline activity in AD transgenic neurons leads to an increased inhibitory tone in order to dampen the excitatory firing. Indeed, observations have been made that show that neurons in the CA1 and CA3 regions of hippocampus have increased inhibitory input from parvalbumin positive neuron populations in young APP/PS1 mice (Hollnagel et al., 2019). Such alterations and adaptations in neuronal networks might buffer deficits caused by increased β-amyloid levels.

The hyper-activity in AD and AD models could have in essence three causes; 1. Increased excitation which could be due to increased glutamate or sensitivity to glutamate (Zott et al., 2019), 2. Decreased inhibition, such as dysfunction in inhibitory interneuron populations (Verret et al., 2012) or 3. Alterations in the intrinsic excitability of neurons, e.g. alterations to neuronal chloride and potassium

channels or by changes in dendritic morphology (Siskova et al., 2014). Such perturbations would increase the activity level in a given neuronal network. A growing literature suggests that mean firing rate as reflected by the average spontaneous activity in a neural circuit is under homeostatic control (Hengen et al., 2013; Slomowitz et al., 2015). It was recently proposed by Boaz Styr and Inna Slutsky that and imbalance between firing rate homeostasis and synaptic plasticity could underlie early AD (Styr and Slutsky, 2018), especially the transition from "silent" aberrant network activity to clinical memory and behavioral impairments. Interestingly, β-amyloid was recently demonstrated to "overshoot" synaptic scaling in response to chronic inactivity both in vitro and in vivo (Gilbert et al., 2016). The observation that APP influences the AMPA receptor subunit numbers both when overexpressed (Almeida et al., 2005) and when knocked out (paper I); indicates that APP and/or its proteolytic fragments are important in regulating excitatory activity in neurons. Indeed, sAPP fragments were shown to interact with GABAB receptors (Rice et al., 2019a) which are implicated in homeostatic synaptic plasticity mechanisms (Vertkin et al., 2015). Further, it has been shown that the PS1 mutation M146V and PS1 KO hippocampal neurons have defective post-synaptic scaling (Pratt et al., 2011). The APP holo-protein might play an important role as a homeostatic regulator of activity at synapses, fine tuning and keeping activity within physiological levels. APP KO mice have increased susceptibility to seizures (Steinbach et al., 1998). In line with this, a report on a human child born with a truncating mutation in APP mirrored many of the phenotypes observed in APP KO mice. Of note the child had frequent seizures and severe mental retardation (Klein et al., 2016).

The β-amyloid induced hyper-activity described by us and others could also have a more exotic implication. Bruce Yankner's group recently published that downregulation of genes involved in excitatory synaptic transmission was correlated with longevity in both human populations and experiments in C. elegans. Through a number of experiments they demonstrated that increased neuronal activity decreased the lifespan of the nematodes. Given that β-amyloid can cause hyper-excitability, instead of AD being regarded as an age-related disorder maybe we could also regard it as a disorder that accelerates aging.

Although Ca2+ imaging is a versatile method for studying large scale neuronal activity, it is not without its potential weaknesses. For instance, in order to detect Ca2+, either with dyes or GECIs, the Ca2+ sensor not only detects but also buffers the Ca2+ which could have subtle effects on neuronal activity that slightly bias the estimations (McMahon and Jackson, 2018). Further, Ca2+ sensors are still slow when compared to electrophysiology where spike-trains can be detected with

precision. Recent advances in microelectrode array (MEA) technology might provide a complement to Ca2+ imaging since MEAs with their high electrode density can give local field potential information with high spatial resolution in cultured neurons (Obien et al., 2015).

With β-amyloid immunotherapies, BACE inhibitors and γ-secretase inhibitors failing in the clinic it is becoming clear that the amyloid cascade hypothesis might need to be further revised. Our studies and others propose that APP and β-amyloid might play important roles at synapses. Disrupting these might cause considerable harm to neurons and neuronal networks. Supporting this view are the observations of psychiatric side effects with BACE inhibitors and increased seizures in BACE KO mice (Hitt et al., 2010),

The future holds many directions to take and many interesting avenues to study. The most important thing is that we keep trying to improve the lives of those afflicted with Alzheimer's and other neurodegenerative disorders.

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References

Abramov, E., Dolev, I., Fogel, H., Ciccotosto, G.D., Ruff, E., and Slutsky, I. (2009). Amyloid-β as a positive endogenous regulator of release probability at hippocampal synapses. *Nature Neuroscience* 12**,** 1567. doi: 10.1038/nn.2433 https://www.nature.com/articles/nn.2433#supplementary-information.

Almeida, C.G., Tampellini, D., Takahashi, R.H., Greengard, P., Lin, M.T., Snyder, E.M., et al. (2005). Beta-amyloid accumulation in APP mutant neurons reduces PSD-95 and GluR1 in synapses. *Neurobiol Dis* 20(2)**,** 187-198. doi: 10.1016/j.nbd.2005.02.008.

Alzheimer, A., Stelzmann, R.A., Schnitzlein, H.N., and Murtagh, F.R. (1995). An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde". *Clin Anat* 8(6)**,** 429-431. doi: 10.1002/ca.980080612.

Artimovich, E., Jackson, R.K., Kilander, M.B.C., Lin, Y.C., and Nestor, M.W. (2017). PeakCaller: an automated graphical interface for the quantification of intracellular calcium obtained by high-content screening. 18(1)**,** 72. doi: 10.1186/s12868-017-0391-y.

Assini, A., Terreni, L., Borghi, R., Giliberto, L., Piccini, A., Loqui, D., et al. (2003). Pure spastic paraparesis associated with a novel presenilin 1 R278K mutation. *Neurology* 60(1)**,** 150. doi: 10.1212/01.wnl.0000040252.43269.83.

Aydin, D., Filippov, M.A., Tschape, J.A., Gretz, N., Prinz, M., Eils, R., et al. (2011). Comparative transcriptome profiling of amyloid precursor protein family members in the adult cortex. *BMC Genomics* 12**,** 160. doi: 10.1186/1471-2164-12-160.

Barrett, P.J., Song, Y., Van Horn, W.D., Hustedt, E.J., Schafer, J.M., Hadziselimovic, A., et al. (2012). The Amyloid Precursor Protein Has a Flexible Transmembrane Domain and Binds Cholesterol. *Science* 336(6085)**,** 1168. doi: 10.1126/science.1219988.

Berridge, M.J., Bootman, M.D., and Roderick, H.L. (2003). Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol* 4(7)**,** 517-529. doi: 10.1038/nrm1155.

Bilousova, T., Miller, C.A., Poon, W.W., Vinters, H.V., Corrada, M., Kawas, C., et al. (2016). Synaptic Amyloid-beta Oligomers Precede p-Tau and Differentiate High Pathology Control Cases. *Am J Pathol* 186(1)**,** 185-198. doi: 10.1016/j.ajpath.2015.09.018.

Bittner, T., Fuhrmann, M., Burgold, S., Jung, C.K., Volbracht, C., Steiner, H., et al. (2009). Gamma-secretase inhibition reduces spine density in vivo via an amyloid precursor protein-dependent pathway. *J Neurosci* 29(33)**,** 10405-10409. doi: 10.1523/JNEUROSCI.2288-09.2009.

Blocq, P., and Marinescu, G. (1892). *Sur les lésions et la pathogénie de l'épilepsie dite essentielle.* S.L.: s.n.

Bramham, C.R., and Wells, D.G. (2007). Dendritic mRNA: transport, translation and function. *Nature Reviews Neuroscience* 8(10)**,** 776-789. doi: 10.1038/nrn2150.

Busche, M.A., Eichhoff, G., Adelsberger, H., Abramowski, D., Wiederhold, K.H., Haass, C., et al. (2008). Clusters of hyperactive neurons near amyloid plaques in a mouse model of Alzheimer's disease. *Science* 321(5896)**,** 1686-1689. doi: 10.1126/science.1162844.

Busche, M.A., Wegmann, S., and Dujardin, S. (2019). Tau impairs neural circuits, dominating amyloid-beta effects, in Alzheimer models in vivo. 22(1)**,** 57-64. doi: 10.1038/s41593-018-0289-8.

Campeau, E., Ruhl, V.E., Rodier, F., Smith, C.L., Rahmberg, B.L., Fuss, J.O., et al. (2009). A Versatile Viral System for Expression and Depletion of Proteins in Mammalian Cells. *PLoS ONE* 4(8)**,** e6529. doi: 10.1371/journal.pone.0006529.

Chen, T.-W., Wardill, T.J., Sun, Y., Pulver, S.R., Renninger, S.L., Baohan, A., et al. (2013). Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* 499**,** 295. doi: 10.1038/nature12354 https://www.nature.com/articles/nature12354#supplementary-information.

Cirrito, J.R., May, P.C., O'Dell, M.A., Taylor, J.W., Parsadanian, M., Cramer, J.W., et al. (2003). In vivo assessment of brain interstitial fluid with microdialysis reveals plaque-associated changes in amyloid-beta metabolism and half-life. *J Neurosci* 23(26)**,** 8844-8853.

Cirrito, J.R., Yamada, K.A., Finn, M.B., Sloviter, R.S., Bales, K.R., May, P.C., et al. (2005). Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. *Neuron* 48(6)**,** 913-922. doi: 10.1016/j.neuron.2005.10.028.

Corrada, M.M., Brookmeyer, R., Paganini-Hill, A., Berlau, D., and Kawas, C.H. (2010). Dementia incidence continues to increase with age in the oldest old: the 90+ study. *Annals of neurology* 67(1)**,** 114-121. doi: 10.1002/ana.21915.

Ehrlich, I., and Malinow, R. (2004). Postsynaptic density 95 controls AMPA receptor incorporation during long-term potentiation and experience-driven synaptic plasticity. *J Neurosci* 24(4)**,** 916-927. doi: 10.1523/jneurosci.4733-03.2004.

Eikelenboom, P., and Stam, F.C. (1982). Immunoglobulins and complement factors in senile plaques. An immunoperoxidase study. *Acta Neuropathol* 57(2-3)**,** 239-242. doi: 10.1007/bf00685397.

Ferreira, T.A., Blackman, A.V., Oyrer, J., Jayabal, S., Chung, A.J., Watt, A.J., et al. (2014). Neuronal morphometry directly from bitmap images. *Nature Methods* 11**,** 982. doi: 10.1038/nmeth.3125.

Fogel, H., Frere, S., Segev, O., Bharill, S., Shapira, I., Gazit, N., et al. (2014). APP homodimers transduce an amyloid-beta-mediated increase in release probability at excitatory synapses. *Cell Rep* 7(5)**,** 1560-1576. doi: 10.1016/j.celrep.2014.04.024.

Gilbert, J., Shu, S., Yang, X., Lu, Y., Zhu, L.Q., and Man, H.Y. (2016). beta-Amyloid triggers aberrant over-scaling of homeostatic synaptic plasticity. 4(1)**,** 131. doi: 10.1016/j.bmc.2016.12.027.

Gouras, G.K., Almeida, C.G., and Takahashi, R.H. (2005). Intraneuronal Abeta accumulation and origin of plaques in Alzheimer's disease. *Neurobiol Aging* 26(9), 1235-1244. doi: 10.1016/j.neurobiolaging.2005.05.022.

Gouras, G.K., Relkin, N.R., Sweeney, D., Munoz, D.G., Mackenzie, I.R., and Gandy, S. (1997). Increased apolipoprotein E epsilon 4 in epilepsy with senile plaques. *Ann Neurol* 41(3), 402-404. doi: 10.1002/ana.410410317.

Grienberger, C., and Konnerth, A. (2012). Imaging calcium in neurons. *Neuron* 73(5), 862-885. doi: 10.1016/j.neuron.2012.02.011.

Hardy, J.A., and Higgins, G.A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256(5054), 184-185. doi: 10.1126/science.1566067.

Hébert, S.S., Serneels, L., Tolia, A., Craessaerts, K., Derks, C., Filippov, M.A., et al. (2006). Regulated intramembrane proteolysis of amyloid precursor protein and regulation of expression of putative target genes. *EMBO reports* 7(7), 739-745. doi: 10.1038/sj.embor.7400704.

Hengen, K.B., Lambo, M.E., Van Hooser, S.D., Katz, D.B., and Turrigiano, G.G. (2013). Firing rate homeostasis in visual cortex of freely behaving rodents. *Neuron* 80(2), 335-342. doi: 10.1016/j.neuron.2013.08.038.

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

Hoe, H.S., Lee, K.J., Carney, R.S., Lee, J., Markova, A., Lee, J.Y., et al. (2009). Interaction of reelin with amyloid precursor protein promotes neurite outgrowth. *J Neurosci* 29(23), 7459-7473. doi: 10.1523/jneurosci.4872-08.2009.

Hoe, H.S., Wessner, D., Beffert, U., Becker, A.G., Matsuoka, Y., and Rebeck, G.W. (2005). F-spondin interaction with the apolipoprotein E receptor ApoEr2 affects processing of amyloid precursor protein. *Mol Cell Biol* 25(21), 9259-9268. doi: 10.1128/MCB.25.21.9259-9268.2005.

Hollnagel, J.-O., Elzoheiry, S., Gorgas, K., Kins, S., Beretta, C.A., Kirsch, J., et al. (2019). Early alterations in hippocampal perisomatic GABAergic synapses and network oscillations in a mouse model of Alzheimer's disease amyloidosis. *PloS one* 14(1), e0209228-e0209228. doi: 10.1371/journal.pone.0209228.

Hornsten, A., Lieberthal, J., Fadia, S., Malins, R., Ha, L., Xu, X., et al. (2007). APL-1, a Caenorhabditis elegans protein related to the human beta-amyloid precursor protein, is essential for viability. *Proc Natl Acad Sci U S A* 104(6), 1971-1976. doi: 10.1073/pnas.0603997104.

Iwata, N., Mizukami, H., Shirotani, K., Takaki, Y., Muramatsu, S.-i., Lu, B., et al. (2004). Presynaptic Localization of Neprilysin Contributes to Efficient Clearance of Amyloid-β Peptide in Mouse Brain. *The Journal of Neuroscience* 24(4), 991-998. doi: 10.1523/jneurosci.4792-03.2004.

Jack, C.R., Jr., Knopman, D.S., Jagust, W.J., Petersen, R.C., Weiner, M.W., Aisen, P.S., et al. (2013). Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol* 12(2), 207-216. doi: 10.1016/s1474-4422(12)70291-0.

Jankowsky, J.L., Fadale, D.J., Anderson, J., Xu, G.M., Gonzales, V., Jenkins, N.A., et al. (2004). Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. *Hum Mol Genet* 13(2)**,** 159-170. doi: 10.1093/hmg/ddh019.

Kamenetz, F., Tomita, T., Hsieh, H., Seabrook, G., Borchelt, D., Iwatsubo, T., et al. (2003). APP processing and synaptic function. *Neuron* 37(6)**,** 925-937.

Karran, E., and De Strooper, B. (2016). The amyloid cascade hypothesis: are we poised for success or failure? *J Neurochem* 139 Suppl 2**,** 237-252. doi: 10.1111/jnc.13632.

Klein, S., Goldman, A., Lee, H., Ghahremani, S., Bhakta, V., Center, U.C.G., et al. (2016). Truncating mutations in APP cause a distinct neurological phenotype. *Annals of Neurology* 80(3)**,** 456-460. doi: 10.1002/ana.24727.

Koo, E.H., Sisodia, S.S., Archer, D.R., Martin, L.J., Weidemann, A., Beyreuther, K., et al. (1990). Precursor of amyloid protein in Alzheimer disease undergoes fast anterograde axonal transport. *Proc Natl Acad Sci U S A* 87(4)**,** 1561-1565.

Lacor, P.N., Buniel, M.C., Chang, L., Fernandez, S.J., Gong, Y., Viola, K.L., et al. (2004). Synaptic targeting by Alzheimer's-related amyloid beta oligomers. *J Neurosci* 24(45)**,** 10191-10200. doi: 10.1523/jneurosci.3432-04.2004.

Lam, A.D., Deck, G., Goldman, A., Eskandar, E.N., Noebels, J., and Cole, A.J. (2017). Silent hippocampal seizures and spikes identified by foramen ovale electrodes in Alzheimer's disease. *Nat Med* 23(6)**,** 678-680. doi: 10.1038/nm.4330.

Lee, K.J., Moussa, C.E., Lee, Y., Sung, Y., Howell, B.W., Turner, R.S., et al. (2010). Beta amyloid-independent role of amyloid precursor protein in generation and maintenance of dendritic spines. *Neuroscience* 169(1)**,** 344-356. doi: 10.1016/j.neuroscience.2010.04.078.

Lerdkrai, C., Asavapanumas, N., Brawek, B., Kovalchuk, Y., Mojtahedi, N., Olmedillas Del Moral, M., et al. (2018). Intracellular Ca(2+) stores control in vivo neuronal hyperactivity in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* 115(6)**,** E1279-e1288. doi: 10.1073/pnas.1714409115.

Leyssen, M., Ayaz, D., Hebert, S.S., Reeve, S., De Strooper, B., and Hassan, B.A. (2005). Amyloid precursor protein promotes post-developmental neurite arborization in the Drosophila brain. *Embo j* 24(16)**,** 2944-2955. doi: 10.1038/sj.emboj.7600757.

Li, F., Calingasan, N.Y., Yu, F., Mauck, W.M., Toidze, M., Almeida, C.G., et al. (2004). Increased plaque burden in brains of APP mutant MnSOD heterozygous knockout mice. *J Neurochem* 89(5)**,** 1308-1312. doi: 10.1111/j.1471-4159.2004.02455.x.

Liu, Q., Zerbinatti, C.V., Zhang, J., Hoe, H.-S., Wang, B., Cole, S.L., et al. (2007). Amyloid precursor protein regulates brain apolipoprotein E and cholesterol metabolism through lipoprotein receptor LRP1. *Neuron* 56(1)**,** 66-78. doi: 10.1016/j.neuron.2007.08.008.

Lundgren, J.L., Ahmed, S., Schedin-Weiss, S., Gouras, G.K., Winblad, B., Tjernberg, L.O., et al. (2015). ADAM10 and BACE1 are localized to synaptic vesicles. *J Neurochem* 135(3)**,** 606-615. doi: 10.1111/jnc.13287.

Luo, L., Tully, T., and White, K. (1992). Human amyloid precursor protein ameliorates behavioral deficit of flies deleted for appl gene. *Neuron* 9(4)**,** 595-605. doi: https://doi.org/10.1016/0896-6273(92)90024-8.

Masliah, E., Terry, R.D., DeTeresa, R.M., and Hansen, L.A. (1989). Immunohistochemical quantification of the synapse-related protein synaptophysin in Alzheimer disease. *Neurosci Lett* 103(2)**,** 234-239.

McMahon, S.M., and Jackson, M.B. (2018). An Inconvenient Truth: Calcium Sensors Are Calcium Buffers. *Trends Neurosci* 41(12)**,** 880-884. doi: 10.1016/j.tins.2018.09.005.

Milosch, N., Tanriover, G., Kundu, A., Rami, A., Francois, J.C., Baumkotter, F., et al. (2014). Holo-APP and G-protein-mediated signaling are required for sAPPalpha-induced activation of the Akt survival pathway. *Cell Death Dis* 5**,** e1391. doi: 10.1038/cddis.2014.352.

Moore, D.B., Gillentine, M.A., Botezatu, N.M., Wilson, K.A., Benson, A.E., and Langeland, J.A. (2014). Asynchronous evolutionary origins of Abeta and BACE1. *Mol Biol Evol* 31(3)**,** 696-702. doi: 10.1093/molbev/mst262.

Mueller, S.G., Schuff, N., Yaffe, K., Madison, C., Miller, B., and Weiner, M.W. (2010). Hippocampal atrophy patterns in mild cognitive impairment and Alzheimer's disease. *Hum Brain Mapp* 31(9)**,** 1339-1347. doi: 10.1002/hbm.20934.

Obien, M.E.J., Deligkaris, K., Bullmann, T., Bakkum, D.J., and Frey, U. (2015). Revealing neuronal function through microelectrode array recordings. *Frontiers in neuroscience* 8**,** 423-423. doi: 10.3389/fnins.2014.00423.

Palop, J.J., and Mucke, L. (2010). Synaptic depression and aberrant excitatory network activity in Alzheimer's disease: two faces of the same coin? *Neuromolecular Med* 12(1)**,** 48-55. doi: 10.1007/s12017-009-8097-7.

Paredes, R.M., Etzler, J.C., Watts, L.T., Zheng, W., and Lechleiter, J.D. (2008). Chemical calcium indicators. *Methods* 46(3)**,** 143-151. doi: 10.1016/j.ymeth.2008.09.025.

Pasciuto, E., Ahmed, T., Wahle, T., Gardoni, F., D'Andrea, L., Pacini, L., et al. (2015). Dysregulated ADAM10-Mediated Processing of APP during a Critical Time Window Leads to Synaptic Deficits in Fragile X Syndrome. *Neuron* 87(2)**,** 382-398. doi: 10.1016/j.neuron.2015.06.032.

Pensalfini, A., Albay, R., 3rd, Rasool, S., Wu, J.W., Hatami, A., Arai, H., et al. (2014). Intracellular amyloid and the neuronal origin of Alzheimer neuritic plaques. *Neurobiol Dis* 71**,** 53-61. doi: 10.1016/j.nbd.2014.07.011.

Perez, R.G., Zheng, H., Van der Ploeg, L.H., and Koo, E.H. (1997). The beta-amyloid precursor protein of Alzheimer's disease enhances neuron viability and modulates neuronal polarity. *J Neurosci* 17(24)**,** 9407-9414.

Peron, S., Chen, T.-W., and Svoboda, K. (2015). Comprehensive imaging of cortical networks. *Current Opinion in Neurobiology* 32**,** 115-123. doi: https://doi.org/10.1016/j.conb.2015.03.016.

Petersen, R.C., Lopez, O., Armstrong, M.J., Getchius, T.S.D., Ganguli, M., Gloss, D., et al. (2018). Practice guideline update summary: Mild cognitive impairment: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology. *Neurology* 90(3)**,** 126-135. doi: 10.1212/wnl.0000000000004826.

Pratt, K.G., Zimmerman, E.C., Cook, D.G., and Sullivan, J.M. (2011). Presenilin 1 regulates homeostatic synaptic scaling through Akt signaling. *Nature neuroscience* 14(9)**,** 1112-1114. doi: 10.1038/nn.2893.

Priller, C., Bauer, T., Mitteregger, G., Krebs, B., Kretzschmar, H.A., and Herms, J. (2006). Synapse formation and function is modulated by the amyloid precursor protein. *J Neurosci* 26(27)**,** 7212-7221. doi: 10.1523/JNEUROSCI.1450-06.2006.

Prince, M., Ali, G.-C., Guerchet, M., Prina, A.M., Albanese, E., and Wu, Y.-T. (2016). Recent global trends in the prevalence and incidence of dementia, and survival with dementia. *Alzheimer's research & therapy* 8(1)**,** 23-23. doi: 10.1186/s13195-016-0188-8.

Puzzo, D., Privitera, L., Leznik, E., Fa, M., Staniszewski, A., Palmeri, A., et al. (2008). Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. *J Neurosci* 28(53)**,** 14537-14545. doi: 10.1523/jneurosci.2692-08.2008.

Quintino, L., Manfre, G., Wettergren, E.E., Namislo, A., Isaksson, C., and Lundberg, C. (2013). Functional neuroprotection and efficient regulation of GDNF using destabilizing domains in a rodent model of Parkinson's disease. *Mol Ther* 21(12)**,** 2169-2180. doi: 10.1038/mt.2013.169.

Rice, H.C., de Malmazet, D., and Schreurs, A. (2019a). Secreted amyloid-beta precursor protein functions as a GABABR1a ligand to modulate synaptic transmission. 363(6423). doi: 10.1126/science.aao4827.

Rice, H.C., de Malmazet, D., Schreurs, A., Frere, S., Van Molle, I., Volkov, A.N., et al. (2019b). Secreted amyloid-β precursor protein functions as a GABA_BR1a ligand to modulate synaptic transmission. *Science* 363(6423)**,** eaao4827. doi: 10.1126/science.aao4827.

Sadleir, K.R., Kandalepas, P.C., Buggia-Prévot, V., Nicholson, D.A., Thinakaran, G., and Vassar, R. (2016). Presynaptic dystrophic neurites surrounding amyloid plaques are sites of microtubule disruption, BACE1 elevation, and increased Aβ generation in Alzheimer's disease. *Acta neuropathologica* 132(2)**,** 235-256. doi: 10.1007/s00401-016-1558-9.

Schedin-Weiss, S., Caesar, I., Winblad, B., Blom, H., and Tjernberg, L.O. (2016). Super-resolution microscopy reveals gamma-secretase at both sides of the neuronal synapse. *Acta Neuropathol Commun* 4**,** 29. doi: 10.1186/s40478-016-0296-5.

Scheltens, P., Blennow, K., Breteler, M.M., de Strooper, B., Frisoni, G.B., Salloway, S., et al. (2016). Alzheimer's disease. *Lancet* 388(10043)**,** 505-517. doi: 10.1016/s0140-6736(15)01124-1.

Seabrook, G.R., Smith, D.W., Bowery, B.J., Easter, A., Reynolds, T., Fitzjohn, S.M., et al. (1999). Mechanisms contributing to the deficits in hippocampal synaptic plasticity in mice lacking amyloid precursor protein. *Neuropharmacology* 38(3)**,** 349-359.

Senechal, Y., Kelly, P.H., and Dev, K.K. (2008). Amyloid precursor protein knockout mice show age-dependent deficits in passive avoidance learning. *Behav Brain Res* 186(1)**,** 126-132. doi: 10.1016/j.bbr.2007.08.003.

Shariati, S.A., and De Strooper, B. (2013). Redundancy and divergence in the amyloid precursor protein family. *FEBS Lett* 587(13)**,** 2036-2045. doi: 10.1016/j.febslet.2013.05.026.

Siskova, Z., Justus, D., Kaneko, H., Friedrichs, D., Henneberg, N., Beutel, T., et al. (2014). Dendritic structural degeneration is functionally linked to cellular hyperexcitability in

a mouse model of Alzheimer's disease. *Neuron* 84(5)**,** 1023-1033. doi: 10.1016/j.neuron.2014.10.024.

Slomnicki, L.P., and Lesniak, W. (2008). A putative role of the Amyloid Precursor Protein Intracellular Domain (AICD) in transcription. *Acta Neurobiol Exp (Wars)* 68(2)**,** 219-228.

Slomowitz, E., Styr, B., Vertkin, I., Milshtein-Parush, H., Nelken, I., Slutsky, M., et al. (2015). Interplay between population firing stability and single neuron dynamics in hippocampal networks. *Elife* 4. doi: 10.7554/eLife.04378.

Smith, K.D.B., Peethumnongsin, E., Lin, H., Zheng, H., and Pautler, R.G. (2010). Increased Human Wildtype Tau Attenuates Axonal Transport Deficits Caused by Loss of APP in Mouse Models. *Magnetic resonance insights* 4**,** 11-18. doi: 10.4137/mri.s5237.

Stahl, R., Schilling, S., Soba, P., Rupp, C., Hartmann, T., Wagner, K., et al. (2014). Shedding of APP limits its synaptogenic activity and cell adhesion properties. *Front Cell Neurosci* 8**,** 410. doi: 10.3389/fncel.2014.00410.

Steinbach, J.P., Muller, U., Leist, M., Li, Z.W., Nicotera, P., and Aguzzi, A. (1998). Hypersensitivity to seizures in beta-amyloid precursor protein deficient mice. *Cell Death Differ* 5(10)**,** 858-866. doi: 10.1038/sj.cdd.4400391.

Styr, B., and Slutsky, I. (2018). Imbalance between firing homeostasis and synaptic plasticity drives early-phase Alzheimer's disease. 21(4)**,** 463-473. doi: 10.1038/s41593-018-0080-x.

Sze, C.I., Troncoso, J.C., Kawas, C., Mouton, P., Price, D.L., and Martin, L.J. (1997). Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. *J Neuropathol Exp Neurol* 56(8)**,** 933-944. doi: 10.1097/00005072-199708000-00011.

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., et al. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131(5)**,** 861-872. doi: 10.1016/j.cell.2007.11.019.

Takahashi, R.H., Almeida, C.G., Kearney, P.F., Yu, F., Lin, M.T., Milner, T.A., et al. (2004). Oligomerization of Alzheimer's beta-amyloid within processes and synapses of cultured neurons and brain. *J Neurosci* 24(14)**,** 3592-3599. doi: 10.1523/jneurosci.5167-03.2004.

Takahashi, R.H., Milner, T.A., Li, F., Nam, E.E., Edgar, M.A., Yamaguchi, H., et al. (2002). Intraneuronal Alzheimer abeta42 accumulates in multivesicular bodies and is associated with synaptic pathology. *Am J Pathol* 161(5)**,** 1869-1879.

Takahashi, R.H., Nagao, T., and Gouras, G.K. (2017). Plaque formation and the intraneuronal accumulation of β-amyloid in Alzheimer's disease. *Pathology International* 67(4)**,** 185-193. doi: 10.1111/pin.12520.

Tampellini, D., Rahman, N., Gallo, E.F., Huang, Z., Dumont, M., Capetillo-Zarate, E., et al. (2009). Synaptic activity reduces intraneuronal Abeta, promotes APP transport to synapses, and protects against Abeta-related synaptic alterations. *J Neurosci* 29(31)**,** 9704-9713. doi: 10.1523/jneurosci.2292-09.2009.

Terry, R.D., Masliah, E., Salmon, D.P., Butters, N., DeTeresa, R., Hill, R., et al. (1991). Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the

major correlate of cognitive impairment. *Ann Neurol* 30(4)**,** 572-580. doi: 10.1002/ana.410300410.

Torroja, L., Chu, H., Kotovsky, I., and White, K. (1999a). Neuronal overexpression of APPL, the Drosophila homologue of the amyloid precursor protein (APP), disrupts axonal transport. *Curr Biol* 9(9)**,** 489-492. doi: 10.1016/s0960-9822(99)80215-2.

Torroja, L., Packard, M., Gorczyca, M., White, K., and Budnik, V. (1999b). The Drosophila beta-amyloid precursor protein homolog promotes synapse differentiation at the neuromuscular junction. *J Neurosci* 19(18)**,** 7793-7803.

Tyan, S.H., Shih, A.Y., Walsh, J.J., Maruyama, H., Sarsoza, F., Ku, L., et al. (2012). Amyloid precursor protein (APP) regulates synaptic structure and function. *Mol Cell Neurosci* 51(1-2)**,** 43-52. doi: 10.1016/j.mcn.2012.07.009.

Walsh, D.M., Klyubin, I., Fadeeva, J.V., Cullen, W.K., Anwyl, R., Wolfe, M.S., et al. (2002). Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 416(6880)**,** 535-539. doi: 10.1038/416535a.

Wang, Z., Wang, B., Yang, L., Guo, Q., Aithmitti, N., Songyang, Z., et al. (2009). Presynaptic and postsynaptic interaction of the amyloid precursor protein promotes peripheral and central synaptogenesis. *J Neurosci* 29(35)**,** 10788-10801. doi: 10.1523/JNEUROSCI.2132-09.2009.

Warner, T.A., Kang, J.-Q., Kennard, J.A., and Harrison, F.E. (2015). Low brain ascorbic acid increases susceptibility to seizures in mouse models of decreased brain ascorbic acid transport and Alzheimer's disease. *Epilepsy research* 110**,** 20-25. doi: 10.1016/j.eplepsyres.2014.11.017.

Verret, L., Mann, E.O., Hang, G.B., Barth, A.M., Cobos, I., Ho, K., et al. (2012). Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. *Cell* 149(3)**,** 708-721. doi: 10.1016/j.cell.2012.02.046.

Vertkin, I., Styr, B., Slomowitz, E., Ofir, N., Shapira, I., Berner, D., et al. (2015). GABAB receptor deficiency causes failure of neuronal homeostasis in hippocampal networks. *Proc Natl Acad Sci U S A* 112(25)**,** E3291-3299. doi: 10.1073/pnas.1424810112.

Westmark, C.J. (2013). What's hAPPening at synapses? The role of amyloid beta-protein precursor and beta-amyloid in neurological disorders. *Mol Psychiatry* 18(4)**,** 425-434. doi: 10.1038/mp.2012.122.

Westmark, C.J. (2019). Fragile X and APP: a Decade in Review, a Vision for the Future. *Mol Neurobiol* 56(6)**,** 3904-3921. doi: 10.1007/s12035-018-1344-x.

Westmark, C.J., Chuang, S.-C., Hays, S.A., Filon, M.J., Ray, B.C., Westmark, P.R., et al. (2016). APP Causes Hyperexcitability in Fragile X Mice. *Frontiers in molecular neuroscience* 9**,** 147-147. doi: 10.3389/fnmol.2016.00147.

Westmark, C.J., and Malter, J.S. (2007). FMRP mediates mGluR5-dependent translation of amyloid precursor protein. *PLoS Biol* 5(3)**,** e52. doi: 10.1371/journal.pbio.0050052.

Willen, K., Sroka, A., Takahashi, R.H., and Gouras, G.K. (2017). Heterogeneous Association of Alzheimer's Disease-Linked Amyloid-beta and Amyloid-beta Protein Precursor with Synapses. *J Alzheimers Dis* 60(2)**,** 511-524. doi: 10.3233/jad-170262.

Volianskis, A., Kostner, R., Molgaard, M., Hass, S., and Jensen, M.S. (2010). Episodic memory deficits are not related to altered glutamatergic synaptic transmission and

plasticity in the CA1 hippocampus of the APPswe/PS1deltaE9-deleted transgenic mice model of ss-amyloidosis. *Neurobiol Aging* 31(7)**,** 1173-1187. doi: 10.1016/j.neurobiolaging.2008.08.005.

Vossel, K.A., Ranasinghe, K.G., Beagle, A.J., Mizuiri, D., Honma, S.M., Dowling, A.F., et al. (2016). Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. *Ann Neurol* 80(6)**,** 858-870. doi: 10.1002/ana.24794.

Young-Pearse, T.L., Bai, J., Chang, R., Zheng, J.B., LoTurco, J.J., and Selkoe, D.J. (2007). A critical function for beta-amyloid precursor protein in neuronal migration revealed by in utero RNA interference. *J Neurosci* 27(52)**,** 14459-14469. doi: 10.1523/JNEUROSCI.4701-07.2007.

Zheng, H., Jiang, M., Trumbauer, M.E., Sirinathsinghji, D.J., Hopkins, R., Smith, D.W., et al. (1995). beta-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. *Cell* 81(4)**,** 525-531. doi: 10.1016/0092-8674(95)90073-x.

Zhu, X.-C., Tan, L., Wang, H.-F., Jiang, T., Cao, L., Wang, C., et al. (2015). Rate of early onset Alzheimer's disease: a systematic review and meta-analysis. *Annals of translational medicine* 3(3)**,** 38-38. doi: 10.3978/j.issn.2305-5839.2015.01.19.

Zott, B., Simon, M.M., Hong, W., Unger, F., Chen-Engerer, H.-J., Frosch, M.P., et al. (2019). A vicious cycle of β amyloid–dependent neuronal hyperactivation. *Science* 365(6453)**,** 559-565. doi: 10.1126/science.aay0198.

Zucker, R.S. (1999). Calcium- and activity-dependent synaptic plasticity. *Current Opinion in Neurobiology* 9(3)**,** 305-313. doi: https://doi.org/10.1016/S0959-4388(99)80045-2.

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