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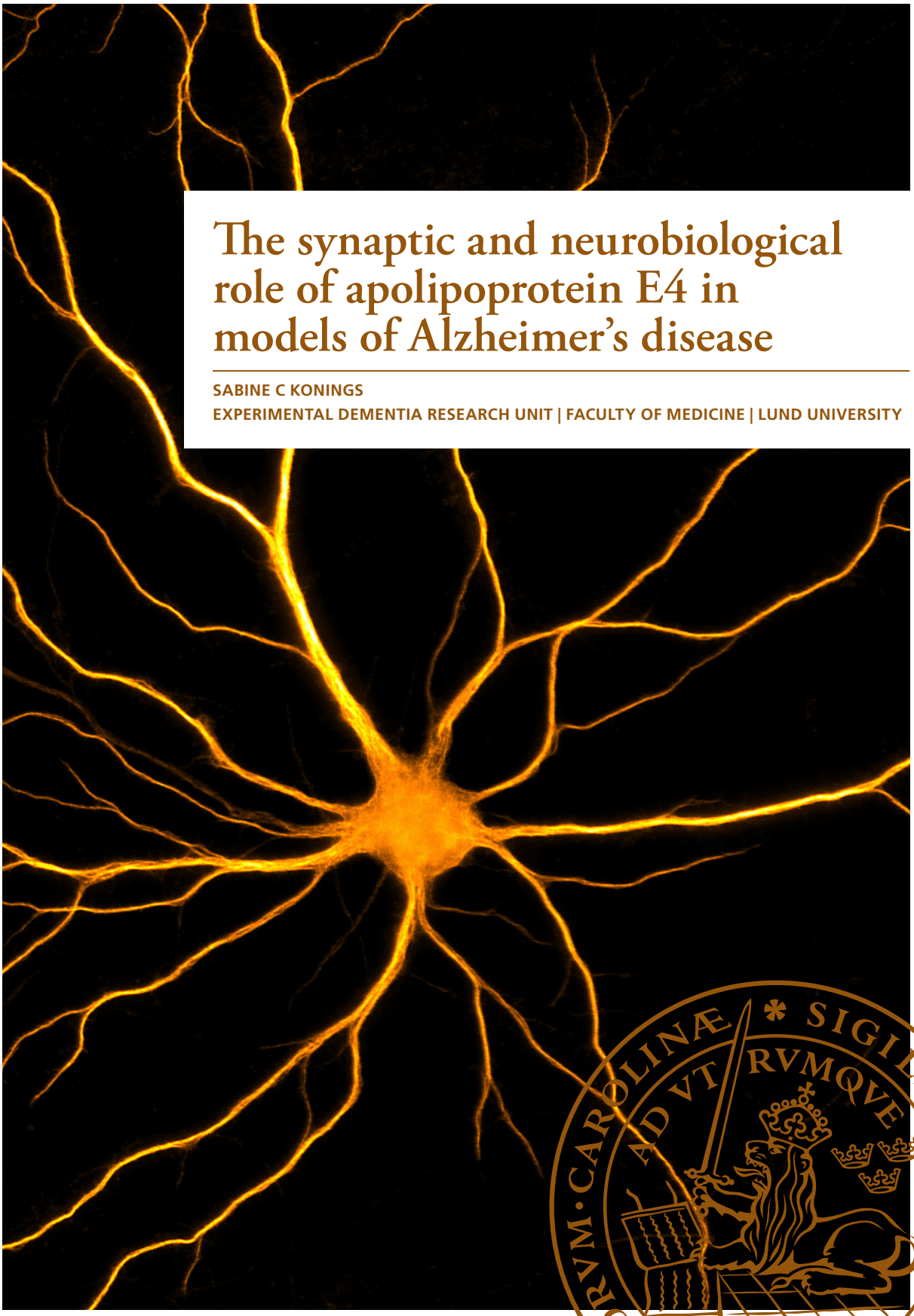

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LUND
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Abstract

Alzheimer's disease (AD) is a progressive, age-related neurodegenerative disorder that is clinically characterized by memory problems. On a neuropathological level, AD is characterized by amyloid plaques consisting of aggregated β-amyloid (Aβ) and neurofibrillary tangles containing Tau. Aging, genes, and lifestyle factors can all influence AD risk. Apolipoprotein E4 (ApoE4) is the major genetic risk factor for AD. The ApoE gene has three major isoforms: ApoE2, ApoE3 and ApoE4, and while ApoE4 increases AD risk, ApoE2, on the other hand, is known to be protective against AD. At early AD stages, prior to clinical onset and the appearance of plaques and tangles, there are already cellular changes, including endosomal and synaptic alterations and intraneuronal Aβ accumulation. ApoE4 has been shown to affect synaptic and endosomal function and intraneuronal Aβ levels. However, the exact link and mechanism(s) involved between ApoE4 and AD remain poorly understood. The overall aim of this thesis was to investigate the role of different ApoE isoforms, in particular the AD-risk variant ApoE4, in early neuronal changes associated with AD.

We found that ApoE is localized at cellular sites that are impacted in early AD, including synaptic terminals and the endosome-lysosome system. ApoE differentially influences neuronal activity depending on the ApoE isoform and on the origin, either astrocytic or neuronal. Additionally, we demonstrated that ApoE4 impairs protein translation, a process needed for synaptic plasticity. Similarly, amyloid precursor protein (APP) and Aβ impact neuronal activity and induce hyperactivity in AD-transgenic neurons overexpressing APP and Aβ in culture, which may be caused by dysregulated homeostatic synaptic plasticity. We also showed that adding astrocyte-derived ApoE3, but not ApoE4, can ameliorate neuronal hyperactivity in AD transgenic neurons. Moreover, we found that astrocyte-derived ApoE predominantly localizes to late endosomes/lysosomes and, interestingly, intracellularly coincides with Aβ..

Altogether, this thesis shows a cellular localization and effect of ApoE in sites altered in early AD stages, including synapses and endosomes. This thesis work reinforces the literature on the involvement of ApoE in cellular changes observed in early AD, including endosomal impairment, synaptic dysfunction and the presence of intracellular Aβ. Our research contributes to a better understanding of the role of ApoE4 in early AD and may help in discovering new therapies for AD.

Key words: Apolipoprotein E4; Alzheimer's disease; synapses; endosomes; β-amyloid; neurons; astrocytes

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Cover photo by Sabine C Konings

The cover photo shows an ApoE4 primary neuron labeled for neuronal marker MAP2.

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To my family

‘Learn from yesterday, live for today, hope for tomorrow.
The important thing is not to stop questioning’

– Albert Einstein

Table of Contents

Original articles

Original papers included in the thesis

The thesis is based on the following published articles and manuscripts:

I. **Sabine C Konings**, Laura Torres-Garcia, Isak Martinsson and Gunnar K Gouras (2021). Astrocytic and Neuronal Apolipoprotein E Isoforms Differentially Affect Neuronal Excitability. Frontiers in Neuroscience, 15, 734001.

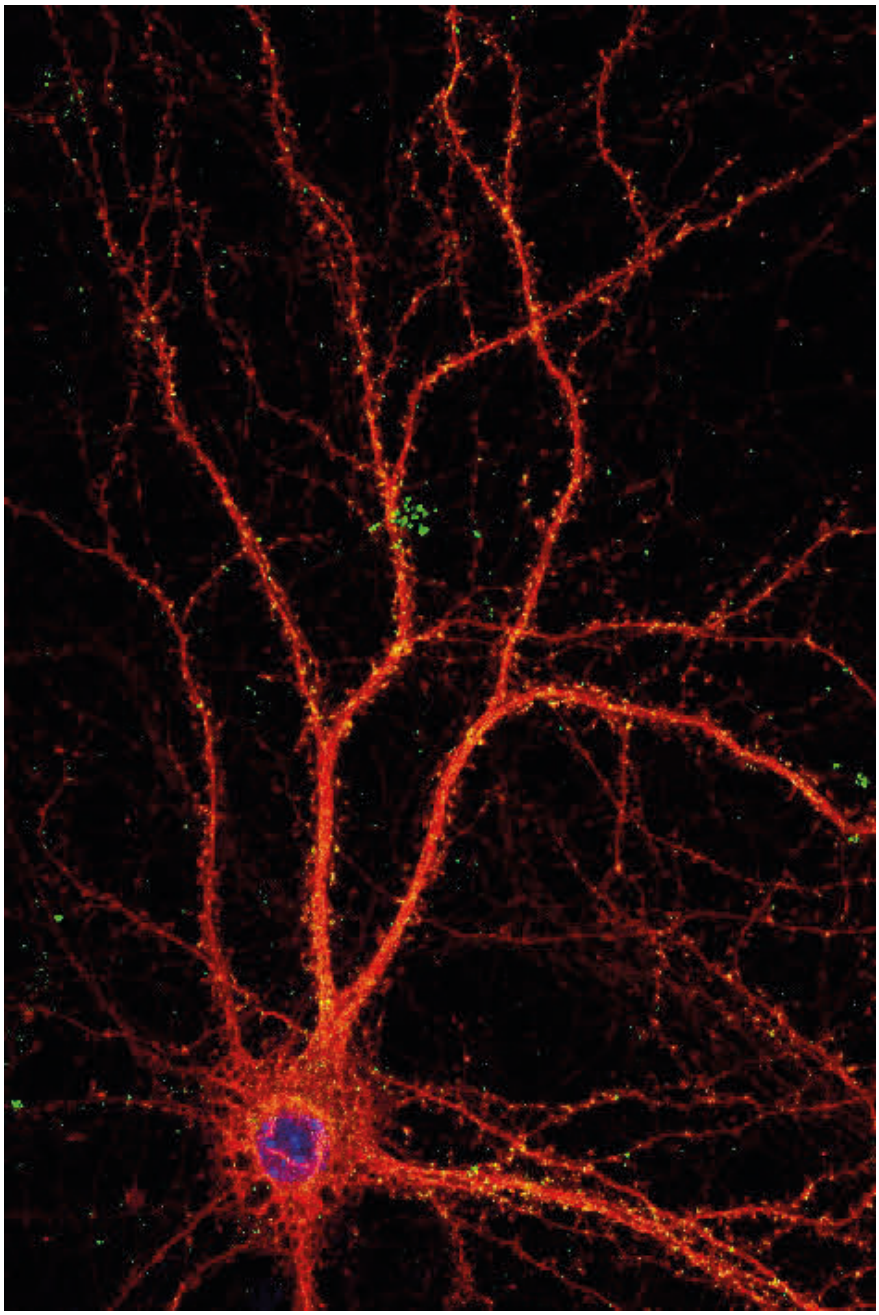
II. Sarayu Ramakrishna, Vishwaja Jhaveri, **Sabine C Konings**, Bharti Nawalpuri, Sumita Chakraborty, Bjørn Holst, Benjamin Schmid, Gunnar K Gouras, Kristine K Freude and Ravi S Muddashetty (2021). APOE4 affects basal and NMDAR mediated protein synthesis in neurons by perturbing calcium homeostasis. Journal of Neuroscience, 41 (42), 8686-8709

III. Isak Martinsson, Luis Quintino, Megg Garcia, **Sabine C Konings**, Alexander Svanbergson, Oliver Stange, Rebecca England, Jia-Yi Li, Cecilia Lundberg and Gunnar K Gouras. Aβ-induced hyperexcitability implicates dysregulation of homeostatic synaptic plasticity in Alzheimer's disease. (Manuscript).

IV. **Sabine C Konings**, Emma Nyberg, Isak Martinsson, Claudia G Almeida, Gunnar K Gouras. Astrocytic-derived ApoE is present in the endosomal-lysosomal system and subcellularly intersects with β-amyloid in neurons. (Manuscript)

Papers outside of this thesis

- Rosalia Fernández-Calle* & **Sabine C Konings*** et al. Unraveling APOE in neuroinflammation: Targeting APOE in neurodegenerative diseases. (Manuscript in preparation). **First co-authorship shared*



ApoE primary neuron treated with ApoE3 astrocyte conditioned media. Neuron was labeld for excitatory marker CAMKIIα (red), major AD risk factor ApoE (green) and nuclear marker DAPI (blue). Yellow signal shows overlap between CAMKIIα and ApoE labeling.

Abstract

Alzheimer's disease (AD) is a progressive, age-related neurodegenerative disorder that is clinically characterized by memory problems. On a neuropathological level, AD is characterized by amyloid plaques consisting of aggregated β-amyloid (Aβ) and neurofibrillary tangles containing Tau. Aging, genes, and lifestyle factors can all influence AD risk. Apolipoprotein E4 (ApoE4) is the major genetic risk factor for AD. The ApoE gene has three major isoforms: ApoE2, ApoE3 and ApoE4, and while ApoE4 increases AD risk, ApoE2, on the other hand, is known to be protective against AD. At early AD stages, prior to clinical onset and the appearance of plaques and tangles, there are already cellular changes, including endosomal and synaptic alterations and intraneuronal Aβ accumulation. ApoE4 has been shown to affect synaptic and endosomal function and intraneuronal Aβ levels. However, the exact link and mechanism(s) involved between ApoE4 and AD remain poorly understood. The overall aim of this thesis was to investigate the role of different ApoE isoforms, in particular the AD-risk variant ApoE4, in early neuronal changes associated with AD.

We found that ApoE is localized at cellular sites that are impacted in early AD, including synaptic terminals and the endosome-lysosome system. ApoE differentially influences neuronal activity depending on the ApoE isoform and on the origin, either astrocytic or neuronal. Additionally, we demonstrated that ApoE4 impairs protein translation, a process needed for synaptic plasticity. Similarly, amyloid precursor protein (APP) and Aβ impact neuronal activity and induce hyperactivity in AD-transgenic neurons overexpressing APP and Aβ in culture, which may be caused by dysregulated homeostatic synaptic plasticity. We also showed that adding astrocyte-derived ApoE3, but not ApoE4, can ameliorate neuronal hyperactivity in AD transgenic neurons. Moreover, we found that astrocyte-derived ApoE predominantly localizes to late endosomes/lysosomes and, interestingly, intracellularly coincides with Aβ.

Altogether, this thesis shows a cellular localization and effect of ApoE in sites altered in early AD stages, including synapses and endosomes. This thesis work reinforces the literature on the involvement of ApoE in cellular changes observed in early AD, including endosomal impairment, synaptic dysfunction and the presence of intracellular Aβ. Our research contributes to a better understanding of the role of ApoE4 in early AD and may help in discovering new therapies for AD.

Popular science summary

Alzheimer's disease (AD) is a progressive, age-related disorder that most prominently causes memory problems. At advanced stages of the disease, AD patients have trouble learning new things and tend to forget many details, including the names and faces of their relatives. At this stage, they are fully dependent on their caregivers. This makes AD not only a devastating disease for the patients themselves, but also for their loved ones. Currently, no effective cure is available to treat, slow down nor prevent AD. Whether or not someone will develop AD during their lifetime depends on many factors, including genetics and lifestyle. Specific gene variants can influence one's risk for developing AD. The ApoE4 gene variant strongly increases the risk to develop AD, and it is relatively common in the general population. In total, there are three ApoE variants: ApoE2, ApoE3 and ApoE4. While ApoE4 increases AD risk, ApoE2 lowers the chance of developing AD. It remains poorly understood how ApoE4 promotes the development of AD.

There is evidence that ApoE4 plays a role in the early stages of AD. Even before the appearance of clinical symptoms, AD already alters the brain by causing lesions and inducing changes on a cellular level. Cellular changes, which are some of the earliest changes seen in AD, include impaired intracellular trafficking, which is the system dealing with the movement of cargo via endosomes within a cell, and dysfunction of synapses, which are needed for neuronal communication and memory formation.

In this thesis, the aim was to study the role of ApoE4 on early cellular changes linked to AD, including endosomal and synaptic changes. We found that ApoE is present at synapses and influences neuronal activity differently depending on whether ApoE3 or ApoE4 is present. We also found that ApoE4 dysregulates the generation of new proteins, a process important for proper synaptic function. In a mouse model of AD, we found that neurons have increased neuronal activity, and this may be caused by the reduced capacity of neurons to adapt to changes in neuronal activity. The presence of ApoE3, but not ApoE4, prevents AD-associated neuronal hyperactivity. Lastly, we found that ApoE is present in endosomes and co-localizes with β-amyloid, a key protein in AD.

Altogether, this thesis work describes the presence and potential role of ApoE at early cellular sites impacted in AD, including endosomes and synapses, and may help to better understand the role of ApoE4 in early AD processes.

Populärvetenskaplig sammanfattning

Alzheimers sjukdom kommer smygande med stigande ålder och orsakar till en början minnesproblem. Med tiden förvärras symptomen och patienterna minns varken namnen eller ansiktena på sina familjemedlemmar och blir helt beroende av vårdgivare i sin omgivning. Detta gör Alzheimers sjukdom till en förödande diagnos både för individen och deras nära och kära. I nuläget saknas effektiva läkemedel som behandlar, saktar ner eller förhindrar sjukdomsförloppet.

Vad som avgör om en person kommer drabbas av Alzheimer sjukdom beror på flera faktorer och inkluderar både genetik och livsstil. Olika genvarianter kan påverka individens risk att utveckla specifika sjukdomar, så som Alzheimers sjukdom. ApoE4 är en sådan genvariant som ökar risken att få Alzheimers sjukdom och samtidigt är ApoE4 relativt vanligt förekommande i samhället. De mest förekommande varianterna av ApoE i befolkningen är ApoE2, ApoE3 och ApoE4. I motsats till ApoE4 som ökar risken för Alzheimer sjukdom, har ApoE2 en skyddande effekt. Varför ApoE4 orsakar en högre risk att insjukna i Alzheimers sjukdom är idag fortfarande oklart.

Tidigare forskning pekar på att ApoE4 verkar tidigt i sjukdomsförloppet. Patologiska förändringar i hjärnan har upptäckts långt innan kliniska symptom uppkommer. Dessa patologiska förändringar inkluderar skador på hjärnans struktur samt mikroförändringar på en cellulär nivå. De cellulära förändringarna är bland de tidigaste som kan upptäckas och är relaterade till den intracellulära transporten av protein, vilket hanteras av vesiklar som kallas endosomer, samt nervcellernas sammankoppling som skapas av synapser. Synapser är väsentliga för nervcellernas förmåga att kommunicera med varandra och för att forma minnen.

Syftet med denna avhandling var att studera ApoEs roll i de tidigt förekommande cellulära förändringarna i Alzheimers sjukdom och inkluderar den intracellulära transporten samt den synaptiska funktionen. Vi upptäckte att ApoE är närvarande vid synapser och att genvarianterna ApoE3 och ApoE4 påverkade nervcellernas elektriska kommunikation på olika sätt. ApoE4 försämrade även genereringen av nya protein, vilket är ett nödvändigt svar på den synaptiska aktiveringen. Med hjälp av musmodeller som efterliknar Alzheimers sjukdom upptäckte vi att deras nervceller har ökad synaptisk aktivitet i jämförelse med nervceller från friska möss. Detta tyder på att Alzheimers sjukdom påverkar nervcellerna kapacitet att anpassa sig till den synaptiska aktiviteten, vilket skapar en patologisk hyperaktivering.

Förekomsten av ApoE3, men inte ApoE4, kunde förhindra en hyperaktivering av nervcellerna. Slutligen fann vi att ApoE är närvarande i intracellulära endosomer tillsammans med β-amyloid, ett av de patologiska kännetecknen för Alzheimers sjukdom.

Sammanfattningsvis beskriver avhandlingen förekomsten av ApoE vid strukturella platser, så som endosomer och synapser, vilka är kopplade till tidiga förändringar vid Alzheimers sjukdom samt beskriver en potentiell roll för ApoE i uppkomsten av dessa cellulära förändringar. Denna avhandling bidrar till en bättre förståelse för betydelsen av ApoE4 i de tidigaste förloppen som påverkar utvecklingen av Alzheimers sjukdom.

Populair-wetenschappelijke samenvatting (NL)

De ziekte van Alzheimer wordt gekenmerkt door geheugenproblemen en deze problemen worden steeds erger naarmate de ziekte vordert. In vergevorderde stadia van de ziekte kan de patiënt zelfs zijn eigen familieleden niet meer herkennen en wordt hij volledig afhankelijk van zijn verpleging. Hierdoor heeft de ziekte van Alzheimer niet alleen een grote impact op de patiënt zelf, maar ook op zijn geliefden. Op dit moment is er geen effectief geneesmiddel om de ziekte te stoppen, te voorkomen of af te remmen.

Verschillende factoren, waaronder veroudering, erfelijkheid en omgevingsinvloeden, kunnen eraan bijdragen of iemand Alzheimer ontwikkelt. Een veelvoorkomend gen dat de kans op Alzheimer drastisch verhoogt, is een gen genaamd apolipoproteïne E4 (ApoE4). Het ApoE gen komt hoofdzakelijk voor in drie varianten: ApoE2, ApoE3 en ApoE4. Dragers van het ApoE4 gen hebben een verhoogde kans op Alzheimer. Daarentegen hebben ApoE2 dragers een verminderde kans om de ziekte te krijgen tijdens hun leven. Het blijft een raadsel hoe ApoE de ontwikkeling van Alzheimer beïnvloedt.

Eerdere onderzoeken wijzen naar een vroege rol van ApoE4 in de ziekte van Alzheimer, zelfs voor de start van klinische symptomen. Voordat de symptomen van Alzheimer zichtbaar zijn in de patiënt, zijn al veel veranderingen aanwezig in de hersenen. De eerste veranderingen in de hersenen zijn te vinden op cellulair niveau, waaronder veranderingen in het intracellulaire transport van eiwitten via endosomen en in neuronale verbindingen, ook wel synapsen genoemd. Synapsen zijn belangrijk in de neuronale communicatie tussen cellen en in het vormen van herinneringen.

Het onderzoeksdoel van deze thesis was om de rol van ApoE4 in vroege Alzheimer-gerelateerde cellulaire veranderingen te bestuderen, met name de veranderingen in endosomen en synapsen. Ons onderzoek laat zien dat ApoE gedetecteerd kan worden in synapsen en endosomen. Daarnaast hebben we aangetoond dat ApoE3 en ApoE4 neuronale activiteit op een verschillende manier beïnvloeden. We hebben ook aangetoond dat ApoE4 zorgt voor een verminderde eiwitproductie in neuronen. De productie van nieuwe eiwitten is belangrijk in de werking van synapsen. In een muismodel voor de ziekte van Alzheimer hebben we aangetoond dat

Alzheimerneuronen een verhoogde neuronale activiteit hebben vergeleken met neuronen van een controlemuis. De abnormale neuronale activiteit in Alzheimerneuronen wordt mogelijk veroorzaakt door een verstoorde adaptatie van neuronen op veranderingen in neuronale activiteit. Het toevoegen van ApoE4 aan de Alzheimerneuronen induceert een abnormale verhoogde neuronale activiteit. Echter, het toevoegen van ApoE3 voorkomt de verhoging in neuronale activiteit in Alzheimerneuronen. Op een sub-cellulair niveau hebben we aangetoond dat de paden van ApoE kruisen met β-amyloïd, een belangrijk eiwit in de ziekte van Alzheimer.

Conclusie: het onderzoek in deze thesis toont aan dat ApoE aanwezig is en mogelijk invloed uitoefent op cellulaire locaties en processen die worden aangetast in vroege stadia van de ziekte van Alzheimer, waaronder endosomen en synapsen. De thesis draagt bij aan een beter begrip van de rol van het genetisch risicogen ApoE4 in de vroege fases van de ziekte van Alzheimer.

List of abbreviations

Aβ	β-amyloid
ABC transporters	ATP-binding cassette transporters
α-CTF	α C-terminal fragment
AD	Alzheimer's disease
AICD	APP intracellular domain
AIS	Axon initial segment
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
Arg	Arginine
BACE1	β-secretase
BBB	Blood brain barrier
BCA	Bicinchoninic acid
BIC	Bicuculine
BSA	Bovine serum albumin
β-CTF	β C-terminal fragments
BSA	Bovine serum albumin
CAMKIIα	$Ca^{2+/}$calmodulin-dependent protein kinase Iiα
CSF	Cerebrospinal fluid
Cys	Cysteine
Dab1	Disabled 1
DAPI	4′,6-diamidino-2-phenylindole
DIV	Days *in vitro*

DMEM	Dulbecco's modified Eagle medium
DMSO	Dimethylsulfoxide
eEF2	Eukaryotic translation elongation factor 2
EOAD	Early onset AD
FBS	Fetal bovine serum
FAs	Fatty acid
FDA	Food and Drug Administration
FUNCAT	Fluorescent non-canonical amino acid tagging
GABA	Gamma-aminobutyric acid
GWAS	Genome wide association studies
HDL	High-density lipoprotein
HSP	Homeostatic synaptic plasticity
IF	Immunofluorescence
ILVs	Intraluminal vesicles
IPSC	Induced pluripotent stem cell
KI	Knock-in
KO	Knock-out
LAMP1	Lysosomal-associated membrane protein 1
LDLR	Low-density lipoprotein receptor
LOAD	Late onset AD
LTD	Long term depression
LTP	Long term potentiation
L-VGCC	L-type voltage-gated Ca^{2+} channels
MAP2	Microtubule associated protein 2
MCI	Mild cognitive impairment
MMSE	Mini Mental State Exam
MRI	Magnetic resonance imaging
MVBs	Multivesicular bodies
N2a or $N2a_{unt}$	Neuro 2a cells that are untransfected

N2a APP_{Swe}	Neuro 2a cells transfected with human APP with the Swedish mutation
NFT	Neurofibrillary tangles
NMDA	N-methyl-D-aspartate
NPC1 and NPC2	Niemann-Pick type C1 and C2
P/S	Penicillin/streptomycin
PBS	Phosphate buffered saline
PBS-T	PBS with 0.1% Tween-20
p-eEF2	Phosphorylated eEF2
PET	Positron emission tomography
PFA	Paraformaldehyde
PHF	Paired helical filaments
PSD-95	Post-synaptic density 95
PSEN1 and PSEN2	Presenilin 1 and Presenilin 2
PTEN	Phosphatase and tensin homolog
PVDF	Polyvinylidine difluoride
RIPA	Radioimmunoprecipitation assay
ROI	Region of interest
sAPPα	Soluble α-cleaved APP
sAPPβ	Soluble β-cleaved APP
SDS PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TBS-T	Tris-buffered saline with 0.1% Tween-20
TGN38	Trans-Golgi network 38
TTX	Tetrodotoxin
Tuj1	β-III-Tubulin
VGAT	Vesicular GABA transporter
vGlut1	Vesicular glutamate transporter 1
WB	Western blot
WT	Wild-type

Introduction

Alzheimer’s disease

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder that is clinically characterized by symptoms such as memory and cognitive decline. AD is the most common cause of dementia and accounts for 60-80% of all dementia cases ("2021 Alzheimer's disease facts and figures,"). According to the World Health Organization, worldwide over 55 million people were suffering from dementia in 2020. As a result of the increasing average life expectancy, this number is expected to almost double every 20 years, reaching 139 million dementia cases in 2050.

AD causes a progressive decline in behavioral, memory and social skills, which eventually results in complete loss of independence ("2021 Alzheimer's disease facts and figures,"). In particular, memory loss is seen as one of the major clinical symptoms of AD. In early stages of the disease, mild memory problems start to appear in the form of e.g. forgetting recent events. As the disease progresses, memory impairment eventually begins to affect daily life (Albert et al., 2011). Clinical AD is preceded by mild cognitive impairment (MCI). MCI patients experience cognitive decline which is higher than observed in normal healthy aging, but their daily life and independence is not yet effected by their memory problems (Petersen, 2001a). MCI can have many different causes and does not always progress into AD or other dementia types, although, MCI patients have an increased risk to develop dementia or AD (Petersen et al., 2001b). Brain positron emission tomography (PET) scan and cerebrospinal fluid (CSF) tests can help predicting whether MCI will eventually develop into AD.

Neuropathological hallmarks of AD

In 1906, clinical psychiatrist and neuroanatomist Alois Alzheimer was the first to describe the disease that is now known as AD (Alzheimer, 1911; Alzheimer et al., 1995). By post-mortem analysis of the brain of Auguste Deter, patient in her fifties that showed symptoms of dementia and confusion, Alois Alzheimer described various neuropathological alterations in the brain, including neuritic plaques, neurofibrillary tangles, gliosis and lipid inclusions (Alzheimer et al., 1995). Even now, amyloid plaques and neurofibrillary tangles are still considered as the two major neuropathological hallmarks of AD.

Amyloid plaques

Amyloid plaques predominantly consist of aggregated β-amyloid (Aβ). Aβ is a cleavage product of amyloid precursor protein (APP), a transmembrane protein. APP can be processed by two main pathways: the amyloidogenic and non-amyloidogenic pathway (**Figure 1**). Aβ is generated by the amyloidogenic processing of APP. In the amyloidogenic pathway, APP is first cleaved by β-secretase (BACE1) into soluble β C-terminal fragments (β-CTFs; also known as C99) and soluble β-cleaved APP (sAPPβ). Subsequently, β-CTF is further cleaved by γ-secretase into Aβ and APP intracellular domain (AICD) (Haass & Selkoe, 1993). In contrast, the non-amyloidogenic pathway starts with APP cleavage by α-secretase generating α-CTFs (also known as C83) and soluble α-cleaved APP (sAPPα). α-CTF is further processed by γ-secretase into p3 peptide and AICD. In the non-amyloidogenic processing of APP, α-secretase cuts inside the Aβ domain, thereby preventing the formation of Aβ (Esch et al., 1990; Sisodia et al., 1990).

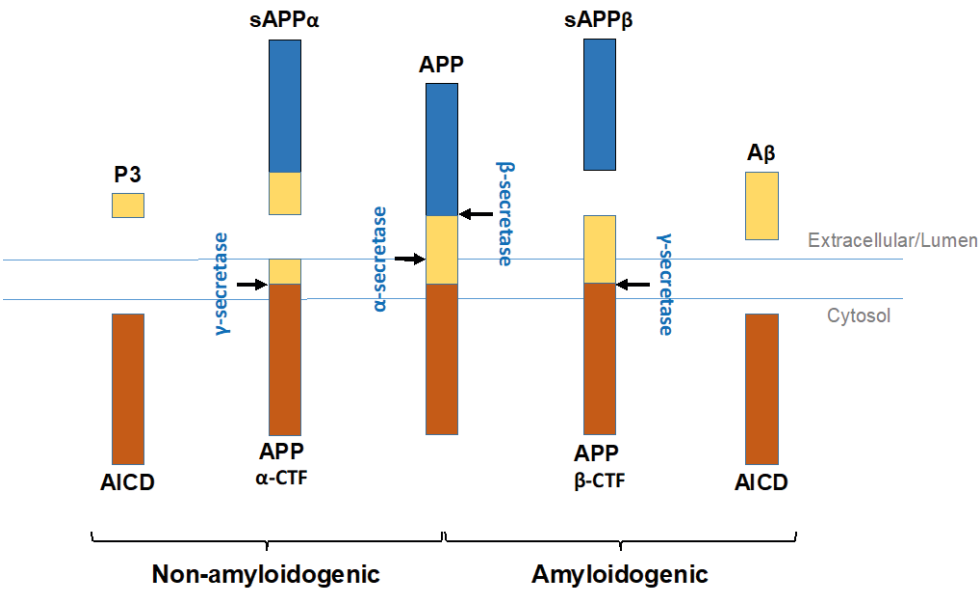


Figure 1: Non-amyloidogenic and amyloidogenic processing of APP. APP can be proteolytically cleaved by two main pathways. In the non-amyloidogenic pathway, APP is initially cleaved by α-secretase, followed by γ-secretase, resulting in the formation of P3 peptide and AICD. In the amyloidogenic pathway, APP is cleaved by β-secretase and subsequently by γ-secretase into AICD and Aβ.

Because γ-secretase cleaves Aβ sequentially, Aβ can exist in various lengths (Takami et al., 2009; Wolfe, 2012). $A\beta_{40}$ is the most abundant form, however longer Aβ peptides such as $A\beta_{42}$ are more associated with AD pathology (Jarrett et al., 1993; McGowan et al., 2005). Longer Aβ species are known to be more hydrophobic

and, as a results, have a stronger tendency to aggregate and form toxic aggregates and fibrils (Iwatsubo et al., 1994; Jarrett et al., 1993; McGowan et al., 2005).

In AD, two forms of plaques are commonly described: dense core plaques and diffuse plaques (DeTure & Dickson, 2019; Dickson & Vickers, 2001; Thal et al., 2006). Dense core plaques, also known as neuritic plaques, have a compact core and stain positive for thioflavin S and Congo red, indicating the presence of fibrillar forms of Aβ inside these plaques (DeTure & Dickson, 2019; Dickson & Vickers, 2001; Thal et al., 2006; Yamaguchi et al., 1988). Dense core plaques are commonly surrounded by dystrophic neurites, i.e. swollen abnormal neurites, and gliosis (DeTure & Dickson, 2019; Serrano-Pozo et al., 2011). Additionally, areas with dense core plaques are associated with synaptic loss. In contrast, diffuse plaques stain negative for thioflavin S and Congo red and differ in morphology from dense core plaques as diffuse plaques show a homogeneous labeling without clear core (DeTure & Dickson, 2019; Dickson & Vickers, 2001).

Neurofibrillary tangles

Intraneuronal neurofibrillary tangles (NFT) are also one of the major hallmarks in AD. NFTs mainly consist of paired helical filaments (PHF) of abnormal hyperphosphorylated Tau, a microtubule-associated protein. The human Tau protein is encoded by the MAPT gene and plays a main role in stabilization of microtubules in neurons (Weingarten et al., 1975). (De)phosphorylation of Tau is known to regulate the normal function of Tau in microtubule assembly. (Lindwall & Cole, 1984). However, the accumulation of abnormal hyperphosphorylated Tau is associated with several diseases, including AD. These diseases are known as tauopathies. In general, three major maturation levels of NFTs are described: pretangles, mature tangles and ghost tangles (Bancher et al., 1989; Moloney et al., 2021). In the pretangle phase, low levels of abnormal Tau aggregates can be detected around the neuronal nucleus while the neuron is still healthy. In mature tangles, inclusions still reside inside neurons, but the neuron is clearly affected as often seen by a dislocated or shrunken nucleus. In the mature phase, the Tau inclusions could fill up the entire cytosol of the neuron. In the final ‘ghost tangle’ phase, observed at late stages of AD, neuronal death occurs and Tau aggregates are found in the extracellular space. The progressive phases and brain distribution of NFT is best described by the Braak stages (Braak & Braak, 1991). In early Braak stages, NFTs are predominantly detected in the entorhinal cortex. At later stages (Braak stage >III), when ghost tangles start to appear, NFTs are known to accumulate in CA1 in the hippocampus and subiculum. In general, the presence of NFTs is a good correlate for neuron loss and AD progression and correlates better with disease severity than amyloid plaques (Arriagada et al., 1992; Bierer et al., 1995).

Diagnosis and treatment

Neuropathological alterations associated with AD play a central role in the development of diagnostic tools and therapies for AD. Traditionally, post-mortem examination of the patient's brain was required to confirm AD. The presence of the neuropathological hallmarks amyloid plaques and neurofibrillary tangles in the patient's brain allowed to diagnose AD with certainty, but exclusively after death. In the clinics, when a patient is expected to be suffering from AD, various cognitive assessments are performed, such as tests for episodic memory, language skills and attention. Cognitive tests commonly used by clinicians are the Mini Mental State Exam (MMSE) and the Montreal Cognitive Assessment (Folstein et al., 1975; Freitas et al., 2013). However, a limitation of the use of neurophysiological tests to diagnosis AD is that they do not allow to make clear distinction between AD and other types of dementia (Mathias & Burke, 2009).

More recently new brain scan methods and CSF biomarker tests were discovered to diagnose AD even before symptom onset. To diagnose future AD, PET and magnetic resonance imaging (MRI) scans detecting abnormal amyloid or Tau, and examination of low $A\beta_{42}$ or high total or phosphor-Tau levels in CSF can be used (Klunk et al., 2004; Mattsson et al., 2017; Motter et al., 1995; Palmqvist et al., 2014). Recently, an accurate blood plasma Aβ test has been developed (Palmqvist et al., 2019), offering a promising, cheaper and less invasive alternative to diagnose AD. Biomarker studies revealed that preclinical AD-related brain changes could start 20 years before clinical onset, thus highlighting the relevance of early (preclinical) diagnosis of AD. An early diagnosis of AD appears to be increasingly important for the development of successful treatments against AD. Early diagnosis, before clinical onset, would allow medical doctors to treat AD at early stages.

Despite the enormous amount of animal studies and clinical trials performed to develop a treatment against AD, a therapy that completely stops or prevents AD does still not exist. For many years, all approved AD treatments on the market only targets clinical AD symptoms, without impacting disease progression. Three approved AD treatments, donepezil, galantamine and rivastigmine, counteract the imbalance in acetylcholine (Yiannopoulou & Papageorgiou, 2020). One approved drug against AD, memantine, is an N-methyl-D-aspartate (NMDA) receptor antagonist. Glutamate is believed to be linked to AD symptoms, and therefore blocking glutamate receptors like NMDA can relieve symptoms related to AD. All four drugs that are approved modestly relieve clinical symptoms in AD patients, without affecting the course of the disease.

Recently, for the first time in over 20 years, a new drug against AD was approved by the Food and Drug Administration (FDA) in the US. The drug Aduhelm (Aducanumab) is a human monoclonal antibody that binds to aggregated but not monomeric Aβ. In human clinical trials, Aducanumab treatment led to reduced number of amyloid plaques in the brain of AD patients (Sevigny et al., 2016).

However, there has been controversy about the ability of Aducanumab to slow down/stop cognitive decline as conflicting data on cognitive decline in AD patients have been published in phase 3 clinical trials of Aducanumab (Knopman et al., 2021; Kuller & Lopez, 2021). A phase 4 clinical trial is currently ongoing to confirm whether Aducanumab truly benefits AD patients while it is already on the market.

Despite the current debate about the efficacy of the FDA approved drug Aducanumab and the high failure of AD treatments in clinical trials caused by lack of beneficial effect, several other drug targets are currently under investigation in human clinical trials. In 2021, 28 therapeutic agents for AD were in phase 3 of clinical trials (Cummings et al., 2021). Most of these drug candidates in phase 3 (82.5%) target underlying biological mechanisms of AD, often related to Aβ or Tau, thereby potentially modifying disease progression. The high number of ongoing phase 3 trials on AD-modifying drugs and the progress in the development of diagnostic tools for AD give hope to future early AD identification and treatments to slow down and/or prevent AD.

Genetics

AD can be sub-divided into two major groups: early onset AD (EOAD) and late onset AD (LOAD). Patients with a clinical AD onset younger than 65 years of age are considered to have EOAD, while an age of onset of 65 years or older is called LOAD. The vast majority of the AD cases (95-99%) are LOAD. Age is considered to be the major risk factor for AD, as the incidence rates for AD and dementia in general increases with age (Corrada et al., 2010; Gao et al., 1998). Besides aging, lifestyle factors and genetics can also influence the risk of developing AD. Although AD is not a complete genetic disorder, twin studies revealed a high heritability link to AD (Gatz et al., 2006), suggesting a strong genetic influence on disease development and onset.

EOAD is strongly associated with rare dominant mutations that directly cause AD. The genetic mutations causing AD are mostly related to APP processing into Aβ. Three causative genes that are commonly linked to EOAD are APP, presenilin 1 (PSEN1) and presenilin 2 (PSEN2) (Goate et al., 1991; Rogaev et al., 1995; Sherrington et al., 1995) (**Figure 2, top**). PSEN1 and PSEN2 are critical proteins in the γ-secretase complex. To date, over 200 genetic variants predominantly linked to APP and PSEN1/2 are identified that could directly cause EOAD, also known as familial AD (Giau et al., 2019) (https://www.alzforum.org/mutations). In general, the causative AD mutations in APP, PSEN1 or PSEN2 are promoting either the relative levels of longer Aβ species, such as $A\beta_{42}$, or promote Aβ aggregation (Scheuner et al., 1996; Selkoe & Hardy, 2016). A commonly studied mutation in APP, often used in generating AD mouse models, is the APP Swedish mutation (KM670/671NL). The Swedish mutation has been shown to increase total Aβ levels (Mullan et al., 1992).

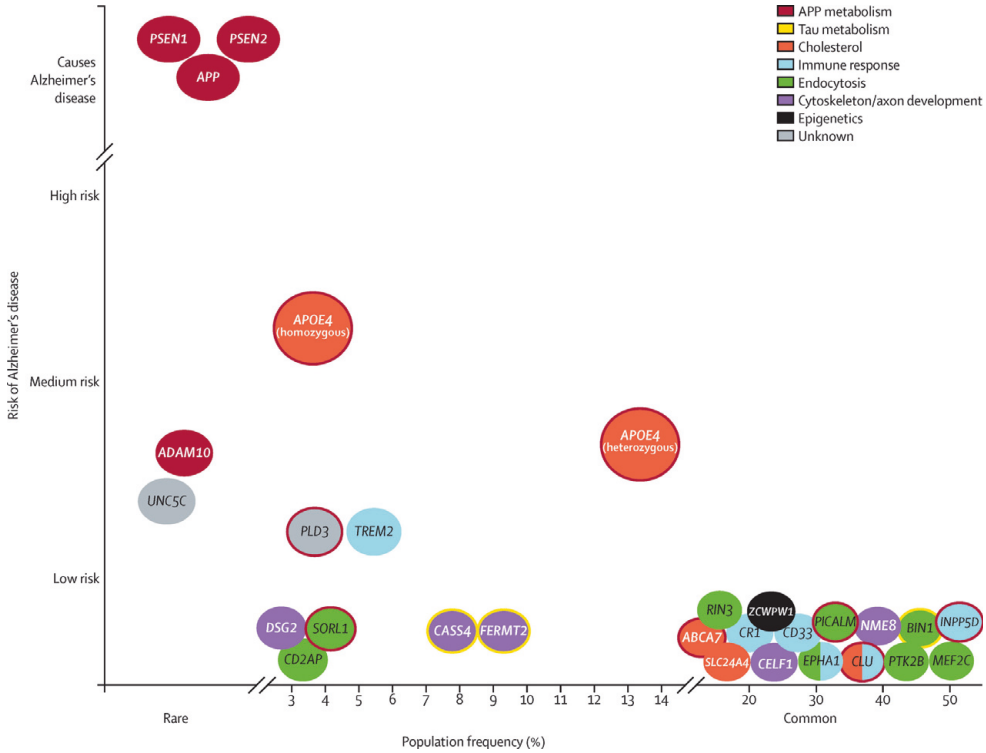


Figure 2: Overview of genes related to Alzheimer's disease. Genes known to directly cause AD disease in humans are shown at the top of the graph. Genes associated with increased risk for AD are distributed over the graph based on their population frequency (%) (rare to common) on the x-axis and risk level (low to high risk) on the y-axis. The colors indicate the pathways where the gene is involved in. The figure was adapted from Scheltens et al. (2016), with permission from Elsevier (license number: 5195891182177).

The most common form of AD, LOAD, is not associated with dominant genetic mutations causing AD, but rather with genetic risk factors increasing the risk of developing AD. Over the years, genome-wide association studies (GWAS) have identified numerous risk genes and variants related to AD (Lambert et al., 2013; Schwartzentruber et al., 2021) (**Figure 2**). Although most risk genes are very rare, it could give clues to identify the most crucial cellular mechanisms underlying AD. Most genes linked to AD risk are involved in endosomal trafficking (e.g. BIN1, PICALM and SORL1), lipid metabolism (e.g. ApoE, CLU and ABCA7) and/or inflammation (e.g. TREM2 and CLU) (Giri et al., 2016; Jones et al., 2010; Van Acker et al., 2019). The strongest and most common genetic risk factor for LOAD is apolipoprotein E4 (ApoE4). The presence of one or two ApoE4 allele(s) increases the risk for AD by around 4 and 14 fold, respectively, compared to ApoE3/ApoE3 carriers (Raber et al., 2004).

Apolipoprotein E

ApoE is a 299 amino acids long lipoprotein and has a molecular weight of 34-36 kDa. In humans, the ApoE gene is located on chromosome 19. The ApoE protein can be sub-divided into two main regions: The N-terminal domain (residues 1-167) and the C-terminal domain (residues 206-299) that are connected via a hinge region (**Figure 3**) (Chen et al, 2011; Weisgraber, 1994). The receptor binding region of ApoE is located in the N-terminal domain (residues 136-150), and the lipid binding region in the C-terminal domain (residues 244-272).

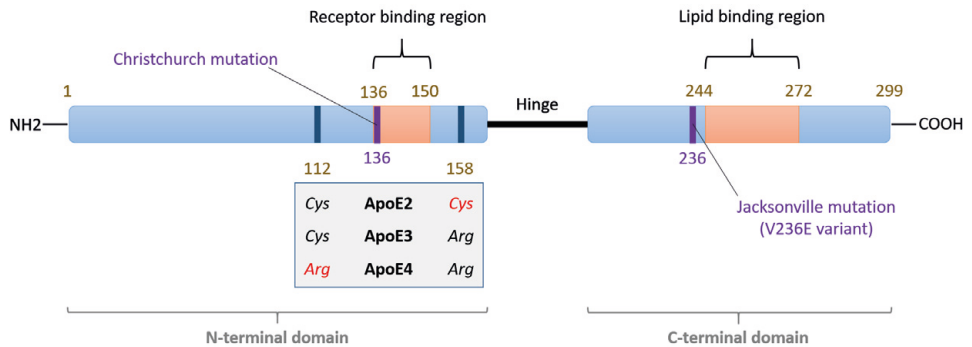


Figure 3: Schematic representation of the structure of human ApoE. The human ApoE protein consists of a N-terminal and C-terminal domain that are connected by a hinge region. The receptor binding region is located at the N-terminal domain (136-150). The lipid-binding domain is located at the C-terminal domain (244-272). The different human ApoE isoforms, ApoE2, ApoE3 and ApoE4, differ in structure at location 112 and 158. Two recent mutations in ApoE were associated with reduced risk for AD. These include the ApoE Christchurch mutation at position 136 and Jacksonville mutation (V236E variant) at position 236. Cys = Cysteine, Arg = Arginine.

In humans, ApoE consists in three major isoforms: ApoE2, ApoE3 and ApoE4. ApoE3 is the most common form with a prevalence of 77.9% in Caucasians. ApoE2 and ApoE4 have a prevalence of 8.4% and 13.7%, respectively (Farrer et al., 1997; Liu et al., 2013). The E3/E3 genotype is by far the most common with a frequency of 60.9% in Caucasians, followed by the E3/E4 genotype (21.3%). The E2/E4, E4/E4 and E2/E2 genotypes are relatively rare with a genotype frequency of 2.6%, 1.8%, and 0.8%, respectively (Farrer et al., 1997; McKay et al., 2011). An ApoE4 genotype is strongly associated with an increased risk for AD (Corder et al., 1993). On the other hand, an ApoE2 genotype seems to be protective against AD (Corder et al., 1994). Remarkably, the ApoE allele frequency and the impact of ApoE on AD risk differs between different ethnicities, with East-Asians being most vulnerable to the effects of ApoE4 (Belloy et al., 2019; Farrer et al., 1997). Besides AD, ApoE is also highly associated with aging and longevity, with ApoE2 positively and ApoE4 negatively impacting longevity in humans (McKay et al., 2011; Shinohara et al., 2020).

Structurally, the human ApoE isoforms differ at just two amino acid positions: 112 and 158. The ApoE3 protein has a cysteine (Cys) at position 112 and an arginine (Arg) at position 158 (**Figure 3**). The Cys on position 112 is replaced by an Arg in the ApoE4 protein, while in the ApoE2 protein the Arg on position 158 is substituted by Cys (Rall et al., 1982; Weisgraber et al., 1981). The amino acid differences between the different ApoE isoforms seem to impact the receptor binding and lipidation of ApoE. The exact native structure of ApoE and how it effects its functioning remains poorly understood. In contrast to ApoE2 and ApoE3, ApoE4 can form a salt-bridge between Arg61 and Glu255, resulting in an interaction between the N- and C-terminal domain of ApoE4, causing a closed structure (Dong & Weisgraber, 1996; Yu et al., 2014). This interaction seems to affect the lipid-binding properties of ApoE (Dong & Weisgraber, 1996). In addition to differences in ApoE isoforms, the lipidation status itself also can affect the structure of ApoE. Although the exact native lipidated structure of ApoE needs to be better defined, ApoE lipidation appears to cause a higher structural separation between the two domains, thus altering the receptor binding capacity (Yamazaki et al., 2019).

Normal function of ApoE

ApoE was first described as a plasma lipoprotein produced by liver cells that transports cholesterol and lipids (Shore & Shore, 1973; Utermann, 1975). It was later discovered that ApoE is also highly expressed in the brain, where it is the most abundant lipoprotein. The main function of ApoE in the brain is to transport cholesterol and lipids from astrocytes to neurons. The blood brain barrier (BBB) prevents the exchange of both ApoE and cholesterol between the periphery and brain (Bjorkhem & Meaney, 2004; M. Liu et al., 2012). Therefore, the brain is largely dependent on local production of ApoE and cholesterol (Bjorkhem & Meaney, 2004). The major source of ApoE in the brain is from astrocytes (Pitas et al., 1987), although microglia, neurons, smooth muscle cells and choroid plexus cells can also produce ApoE to a lesser extent (Xu et al., 2006). Neurons only express ApoE under stress conditions, such during as excitotoxic stress (Boschert et al., 1999; Xu et al., 2006).

Cholesterol metabolism is essential for healthy neuronal function and synapse formation (Goritz et al., 2005; Mauch et al., 2001). Neurons cannot produce endogenous cholesterol efficiently and are therefore highly dependent on external sources, like cholesterol produced by astrocytes (Feringa & van der Kant, 2021; Nieweg et al., 2009). ApoE is a central player in cholesterol transfer from astrocytes to neurons. ApoE is produced by astrocytes and thereafter becomes lipidated by ATP-binding cassette (ABC) transporters, such as ABCA1 and ABCG1, whereby it forms a high-density lipoprotein (HDL)-like lipid particle consisting of cholesterol and other lipids. In turn, neurons can take up the HDL-like ApoE lipid particles via endocytosis. The internalization of the ApoE-lipid complex is initiated by ApoE

binding to receptors from the low-density lipoprotein receptor (LDLR) family. In the endosome-lysosome system free cholesterol is released from the ApoE lipid particle and subsequently escapes the endosomal system to traffic to other cellular compartments (Ikonen, 2008). In general, ApoE4 is considered to be poorly lipidated, suggesting that ApoE3 is more efficient in transporting and providing cholesterol to neurons (Gong et al., 2002).

In addition to the commonly described ApoE-related astrocyte to neuron lipid transport, recently ApoE was also found to play a role in a lipid shuttle transferring fatty acids (FA) from neurons to astrocytes (Ioannou et al., 2019; Liu L. et al., 2017; Qi et al., 2021). Excessive cytosolic FAs are toxic to neurons. This in turn causes mitochondrial impairment and eventually even neurodegeneration (Schonfeld & Reiser, 2017; Sultana et al., 2013). Catabolizing FAs or storing FA in lipid droplets could prevent lipid toxicity, however, neurons have a limited capacity to store FAs and form lipid droplets; and are inefficient in oxidizing lipids (Schonfeld & Reiser, 2013). Astrocytes are very efficient in neutralizing the toxic effects of free FAs and ApoE plays a crucial role in transporting toxic free FAs from neurons to astrocytes for neutralization (Ioannou et al., 2019). ApoE4 transfers and neutralizes FAs less efficiently from neurons to astrocytes than ApoE3 (Qi et al., 2021), again suggesting a more efficient lipid trafficking by ApoE3.

ApoE and AD

Already in the early 1990s, ApoE was described to localize inside amyloid plaques (Namba et al., 1991). In 1993, ApoE4 was discovered to be the major genetic risk factor for AD (Corder et al., 1993; Strittmatter et al., 1993). The presence of one or two ApoE4 alleles do not only strongly increase the risk to develop AD, an ApoE4 genotype is also associated with an earlier age of onset in an allele dose dependent manner (Corder et al., 1993). While in the general population the prevalence of an ApoE4 allele is around 14%, in AD patients this is increased to almost 40% (Farrer et al., 1997), highlighting the impact of ApoE4 in AD pathogenesis.

Recently, rare mutations in the ApoE gene were associated with reduced AD risk. Two copies of the ApoE3 Christchurch mutation (Arg to Serine substitution at amino acid position 136) (**Figure 3**) protected an individual carrying an autosomal dominant AD PSEN1 gene variant from developing major clinical cognitive deficits and Tau pathology but not amyloid pathology before her 70s (Arboleda-Velasquez et al., 2019). Other individuals carrying the same PSEN1 AD mutation, but without being homozygous for the ApoE3 Christchurch mutation, develop AD before their 50s (Acosta-Baena et al., 2011). In addition, another rare ApoE mutation, the ApoE3 Jacksonville mutation (Valine to Glutamic acid at amino acid position 236) (**Figure 3**), also dramatically reduces AD risk, potentially caused by reduced ApoE aggregation and increased lipid-binding (Liu et al., 2021; Medway et al., 2014).

ApoE4 and amyloid plaques

Since the discovery of ApoE4 as a major risk gene for AD, the number of studies on ApoE4 and amyloid pathology has increased tremendously. ApoE4, both in mouse models as well as in human ApoE4 carriers, is strongly associated with increased plaque load and density compared to non-ApoE4 mice and human carriers (Fagan et al., 2002; Holtzman et al., 2000; Rebeck et al., 1993; Schmechel et al., 1993; Tiraboschi et al., 2004) (**Figure 4, amyloid pathology**). Human ApoE impacts amyloid deposition in an isoform-dependent manner, with ApoE2 showing the least and ApoE4 showing the highest Aβ plaque deposition (Fagan et al., 2002). In the complete absence of ApoE, amyloid plaques, in particular neuritic/dense core thioflavin S-positive plaques, were dramatically reduced (Bales et al., 1997; Holtzman et al., 2000), highlighting the crucial role of ApoE on amyloid pathology.

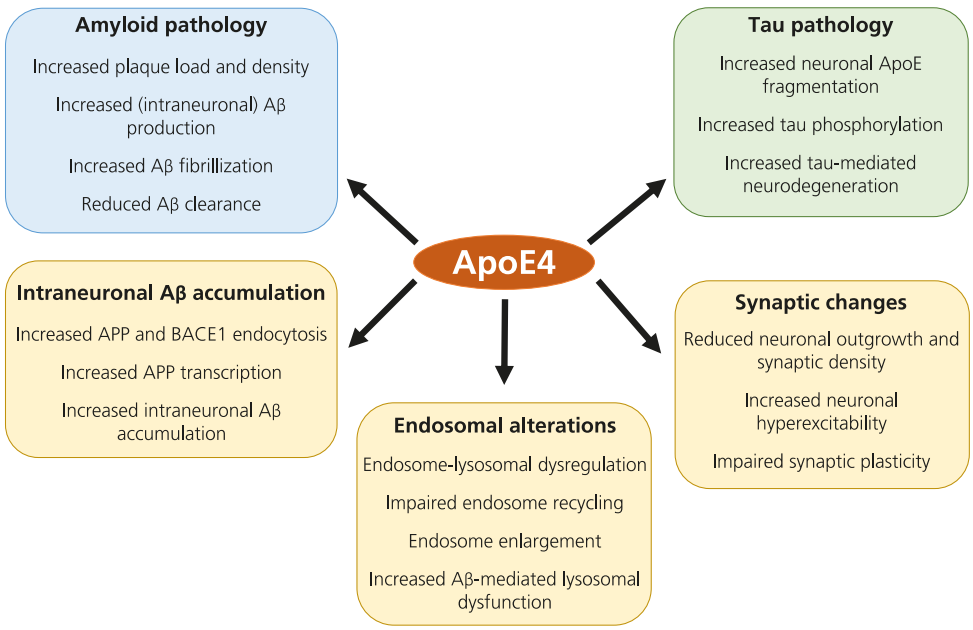


Figure 4: ApoE4-related effects on the major AD hallmarks and early cellular changes in AD. Literature suggests a role of ApoE in multiple processes related to AD pathology. This schematic illustration shows the ApoE4-induced alterations in processes related to AD pathology that are relevant for the scope of this thesis.

The precise mechanism between ApoE4 and plaque development remains poorly understood. Studies transiently suppressing or overexpressing ApoE4 showed a critical effect of ApoE on plaque seeding but not plaque growth, as ApoE4 suppression or overexpression only affected plaque load prior to plaque onset (Huynh et al., 2017; Liu C. et al., 2017). No clear effect of ApoE3 on plaque formation was found in these studies, suggesting an ApoE4-specific effect on early amyloid pathology prior to plaque seeding (Huynh et al., 2017; Liu C. et al., 2017).

Amyloid plaque formation is commonly hypothesized to be caused by an imbalance in Aβ production and/or aggregation on one hand, and Aβ clearance on the other hand. In general, ApoE4 increases intraneuronal Aβ levels and enhances Aβ fibril formation (Castano et al., 1995; He et al., 2007; Huang et al., 2017; Ma et al., 1994). Intraneuronal Aβ production and the effect of ApoE4 on this will be further discussed in the chapters 'Intraneuronal Aβ accumulation' and 'ApoE4 and intraneuronal Aβ accumulation'. Besides ApoE genotype, Aβ fibrillization is also influenced by the lipidation status of ApoE. Poorly lipidated ApoE, caused by depletion of the ABCA1 transporter, caused increased Aβ deposition in AD transgenic mouse models (Koldamova et al., 2005; Wahrle et al., 2005). As previously discussed, ApoE4 is generally considered to be poorly lipidated compared to the ApoE2 and ApoE3 isoforms (Gong et al., 2002), suggesting the ApoE4-induced effects on plaque pathology might be linked to its poor lipidation state.

Besides altered Aβ production and aggregation, amyloid pathology is also influenced by Aβ clearance. In the brains, there are different pathways to remove Aβ from the brain, including enzymatic degradation, removal of Aβ via the BBB and cellular lysosomal degradation (Yamazaki et al., 2019). Overall, Aβ clearance is decreased in the presence of ApoE4 (Kanekiyo et al., 2014; Yamazaki et al., 2019). However, the exact mechanism of ApoE4 influencing Aβ clearance in the brain is complex. Proteolytic degradation is suggested to be decreased in ApoE4 carriers as proteases associated with Aβ degradation, insulin-degrading enzyme and neprilysin, were shown to be decreased in post-mortem AD brains from ApoE4 carriers compared to non-ApoE4 carriers (Cook et al., 2003; Miners et al., 2006). ApoE4 is also associated with reduced Aβ clearance by the BBB (Deane et al., 2008; Q. Ma et al., 2018; Robert et al., 2017). Although the mechanism(s) of ApoE4 on BBB clearance of Aβ remain(s) poorly understood, ApoE4 was shown to clear Aβ at a slower rate via LDL receptors at the BBB than ApoE2 and ApoE3 (Deane et al., 2008). Additionally, the cellular clearance of Aβ is also influenced by ApoE4. In astrocytes and microglia Aβ internalization and subsequent degradation was reduced in the presence of ApoE4 (Lin et al., 2018). Consistent with that, neuronal Aβ degradation was also lower in ApoE4 as ApoE4 causes less efficient Aβ trafficking and degradation by lysosomes compared to the ApoE3 isoform (Li et al., 2012).

Altogether, although the precise mechanisms of the effect of ApoE4 on enhanced amyloid pathology remain poorly understood, it is clear that ApoE4 negatively influences both Aβ production and aggregation on the one hand and the clearance of Aβ on the other hand. This could cause an imbalance in Aβ homeostasis that could eventually lead to amyloid plaques formation.

ApoE4 and Tau neurofibrillarly tangles

Although the link between ApoE4 and Aβ has been intensively studied, the connection between ApoE4 and Tau is less clear. The current understanding on how ApoE isoforms affect Tau-related pathology is contradictory in the field. Some studies support a detrimental effect of ApoE4 on Tau pathology (**Figure 4, Tau pathology**). In a Tau mouse model (carrying the Tau P301S mutation) without amyloid pathology, ApoE induced Tau-mediated neurodegeneration in an ApoE isoform dependent way, with ApoE4/P301S mice showing the strongest neurodegeneration and ApoE2/P301S the weakest (Shi et al., 2017). In the absence of ApoE, Tau-mediated neurodegeneration was almost prevented, suggesting a critical role of ApoE in Tau-associated neurodegeneration (Shi et al., 2017). This Tau-mediated neurodegeneration seems to be dependent on ApoE produced by astrocytes as selectively removing astrocytic ApoE4 was shown to reduced tau pathology and neurodegeneration in these mice (Wang C. et al., 2021).

In contrast, other studies support a detrimental effect of ApoE2, rather than ApoE4, on Tau pathology. ApoE2 but not ApoE4 is associated with a higher incidence of primary tauopathies, disorders in which Tau plays a major role (Ikeda et al., 1997; Robinson et al., 2020; Zhao et al., 2018). The high incidence of ApoE2 in primary tauopathies is reflected by the presence of stronger tau pathology in an ApoE2 target replacement mouse models carrying the Tau P301L mutation and in progressive supranuclear palsy (PSP) patients carrying ApoE2 (Zhao et al., 2018). AD is considered to be a secondary tauopathy. In the presence of amyloid pathology, an ApoE4 genotype was strongly associated with higher and ApoE2 with lower levels of NFTs, while ApoE isoforms do not affect NFT density in the absence of amyloid pathology (Farfel et al., 2016). This indicates distinct effects of ApoE isoforms on primary tauopathies and AD.

In contrast to the finding of Wang C. and colleagues (2021) that Tau-induced pathology is dependent on astrocytic ApoE, the expression of ApoE4 in neurons also led to high Tau phosphorylation in both mouse and induced pluripotent stem cell (IPSC)-derived neuronal models (Tesseur et al., 2000; Wang et al., 2018). Although neurons are not the major source of ApoE in the brain, neurons can produce ApoE under certain circumstances such as stress (Boschert et al., 1999). Neuronal ApoE, but not astrocytic ApoE, can be cleaved into C-terminal truncated ApoE fragments, fragments that have been associated with increased Tau phosphorylation (Brecht et al., 2004; Ljungberg et al., 2002). ApoE4 is more prone to cleave into C-terminal truncated fragments than ApoE3 (Harris et al., 2003; Y. Huang et al., 2001; Wang et al., 2018), potentially explaining the higher levels of phosphorylated Tau in neurons in the presence of ApoE4.

Early cellular changes in AD

In the pre-clinical phase of AD, prior to the formation of amyloid plaques and NFTs, early alterations are already observed at a cellular level. Among the earliest changes in AD are intraneuronal accumulation of Aβ, endosome enlargement and synaptic alterations. The mechanism(s) how these early cellular changes affect AD pathology, and which cellular process happens first in AD remain poorly understood.

Intraneuronal Aβ accumulation

Over 20 years ago, intraneuronal accumulation of Aβ, in particularly $A\beta_{42}$, was first described in the post-mortem human brains (Gouras et al., 2000; Mochizuki et al., 2000) and has since then been confirmed in human AD brains (Cataldo et al., 2004; D'Andrea et al., 2001; Tabira et al., 2002) and AD transgenic mouse models (Knobloch et al., 2007; Oakley et al., 2006; Shie et al., 2003; Takahashi et al., 2002) by many other research labs. Aβ-positive neuronal cell bodies were mainly observed in brain regions known to be vulnerable for AD, such as hippocampus and entorhinal cortex (Gouras et al., 2000). Interestingly, the most prominent Aβ labeling was described in MCI patients with early amyloid and Tau pathology, while the extent of intraneuronal Aβ aggregates decreases with severity of AD and amyloid plaques. Thus, this supports that intraneuronal Aβ is an early event in AD and occurs before the onset of amyloid plaques.

Both Aβ production and aggregation is known to occur intracellularly. BACE1, the initial proteolytic enzyme involved in the amyloidogenic APP cleavage, has an optimal activity at a low acidic pH as present in endosomes (Vassar et al., 1999) and was shown to cleave APP in endosomal compartments (Rajendran et al., 2006; Sannerud et al., 2011). Although the exact subcellular site of Aβ formation by γ-secretase remained unclear (van der Kant & Goldstein, 2015), recently, PSEN2, a subunit of γ-secretase, was shown to specifically localize to late endosomes/lysosomes (Sannerud et al., 2016). Intriguingly, while PSEN1 is normally localized throughout the entire cell, AD mutations in the PSEN1 gene results in PSEN1 re-localization to endosomes/lysosomes. AD PSEN1 mutations are known to increase the intracellular $A\beta_{42}/A\beta_{40}$ ratio, highlighting endosomes/lysosomes as a potential intracellular site for $A\beta_{42}$ generation. In addition to Aβ production in endosomes, $A\beta_{40}$ and $A\beta_{42}$ were shown to internalize and aggregate in acidic compartments of the endosomal pathway (Esbjorner et al., 2014). Aβ was shown to predominantly accumulate in late endosomal multivesicular bodies (MVBs) in neurites and synaptic terminals (Langui et al., 2004; Takahashi et al., 2002).

Endosomal impairment

Abnormal endosomes in neurons are among the earliest pathological changes linked to AD. In healthy conditions, cargo is internalized and initially transported to early endosomes (Hu et al., 2015; Neefjes & van der Kant, 2014) (**Figure 5**). In early endosomes, the internalized cargo are sorted and either (1) transported via late endosomes to lysosomes for degradation, (2) recycled back to the plasma membrane via recycling endosomes, or (3) transported to the trans Golgi network via the retromer pathway (Hu et al., 2015; Jovic et al., 2010; Neefjes & van der Kant, 2014). During the endosomal maturation from early into late endosomes, late endosomal MVBs containing intraluminal vesicles (ILVs) are formed. ILVs can be secreted as then exosomes into the extracellular space via exocytosis or are degraded when MVBs fuse with lysosomes (Eitan et al., 2016). Compared to the cytosol (pH 7), the endosomal system has an acidic environment which progressively drops during endosomal maturation towards lysosomes (early endosomes pH 6.5; lysosome pH 4.5) (Hu et al., 2015). The endosome-lysosome system in neurons is less well characterized. Neurons are highly polarized cells with long extended processes. Endocytosis predominantly takes place at synaptic and growth sites, and endosomal vesicles mature towards late endosomes and lysosomes while transported retrogradely towards the cell soma (Overly & Hollenbeck, 1996). Endosomal recycling occurs locally in neurites, without the involvement of the cell soma.

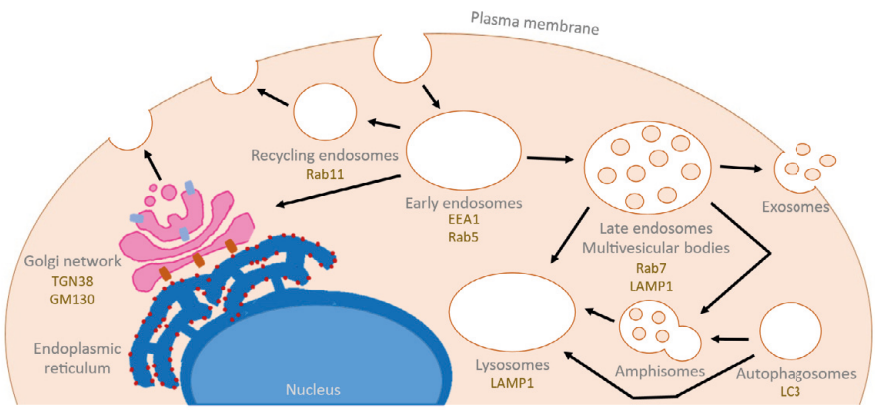


Figure 5: The endosomal-lysosomal system. Cargo are internalized into cells from the outside and trafficked towards early endosomes. In early endosomes the cargo are sorted and either recycled back towards the cell membrane via recycling endosomes, retrogradely transported towards the trans Golgi network, or transported via late endosomes towards lysosomes for degradation. In late endosomes, intraluminal vesicles are formed thereby creating multivesicular bodies. The intraluminal vesicles can be either seceted into the extracellular space by exocytosis or degraded by lysosomes. Intracellular proteins can be taken up by autophagy into autophagosomes and fused with late endosomes into amphisomes or directly with lysosomes for degradation. In brown, some subcellular markers to detect certain intracellular cell organelles are shown.

Accumulating evidence suggests that endosomal dysfunction plays a crucial role in early AD. Several papers reported alterations in the endosome-lysosome system related to AD prior to amyloid plaque onset. This observation was also investigated in Down syndrome, where AD-like dementia occurs invariably with age. These observed alterations include, but are not limited to, enlargement of endosomes (Cataldo et al., 2000), increased levels of endosomal genes such as Rab5 and Rab7 (Ginsberg et al., 2010), and lysosomal impairment (Jiang et al., 2019). Additionally, as discussed in the previous section, the endosomal system appears to be a major site in the generation of Aβ in neurons. Interestingly, many genes associated with increased risk for LOAD are related to endosomal trafficking, for instance BIN1, PICALM, SORL1 and ApoE (Van Acker et al., 2019). This further highlights the potential relevance of the endosome-lysosome system in early stages of AD.

Enlarged endosomes are observed in very early stages of AD in human post-mortem Down syndrome and AD brains (Cataldo et al., 2000). Interestingly, Aβ immunolabeling was detected inside the enlarged Rab5-positive endosomes in neurons and the intraneuronal Aβ was the strongest prior to the appearance of extracellular Aβ deposits (Cataldo et al., 2004), indicating enlarged endosomes might cause Aβ accumulation. Intriguingly, overactivation of Rab5 does not only impaired early endosomes, but also further intracellular trafficking at late endosomes and lysosomes (Cataldo et al., 2008).

Synaptic dysfunction

In the brain, neurons can communicate with each other via synapses. Synaptic plasticity is a way to strengthen or weaken synaptic terminals in response to increased or decreased neuronal activity. Synaptic plasticity is highly linked to learning and memory and consists of two main processes: long-term potentiation (LTP) and long-term depression (LTD). LTP induces synaptic strengthening by increasing the number of glutamatergic α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors on the cell membrane, activating NMDA receptors and increasing Ca^{2+} influx. On the other hand, LTD internalizes AMPA receptors, thereby reducing the number of AMPA receptors on the membrane. This, in turn, induces reduced NMDA receptor activation and Ca^{2+} influx, leading to weakening of the synapse. In relation to synaptic plasticity, neuronal activity is also shown to influence local protein synthesis by impacting protein translation (Costa-Mattioli et al., 2009; Sutton & Schuman, 2006; Sutton et al., 2004). Well-regulated synaptic plasticity is crucial for learning and memory in a healthy brain.

Synaptic dysfunction is an early pathological event in AD and during disease progression, synapse loss correlates well with cognitive impairment in AD (DeKosky & Scheff, 1990; Terry et al., 1991). In line with increased synapse loss, pre- and post-synaptic proteins levels were shown to be reduced in post-mortem

brains of AD patients compared to age-matched control brains (Davidsson & Blennow, 1998; Reddy et al., 2005). Prior to plaques being formed, synaptic terminals are among the neuronal sites were Aβ accumulates (Koffie et al., 2009; Takahashi et al., 2002). Oligomeric Aβ species, not Aβ associated with amyloid plaques, are associated with synaptic toxicity, causing synaptic dysfunction, synapse loss and impaired synaptic plasticity. Impairments in synaptic plasticity include alterations in LTP and LTD, and dysregulated local protein synthesis (Koffie et al., 2009; Li S. et al., 2009; Ma et al., 2013; Walsh et al., 2002). In particular oligomeric Aβ located in synaptic terminals near plaques is associated with reduced synaptic density and has been shown to disrupt synapses (Koffie et al., 2009; Takahashi et al., 2004).

AD also features aberrant network activity. Already before plaque onset, brain regions vulnerable to AD were shown to be hyperactive in AD mouse models (Busche et al., 2012; Palop et al., 2007). In humans, hyperactivity in the hippocampus appears to be a good predictor of cognitive deficits in MCI patients (Miller et al., 2008; Quiroz et al., 2010). AD-linked proteins Aβ and Tau can both alter neuronal network function. Aβ has been mainly associated with hyperexcitability, and Tau has been seen to induce either hypo- or hyper-excitability in different studies (Angulo et al., 2017; Bright et al., 2015; Busche et al., 2019).

In sum, synaptic and neuronal network dysfunction are among the earliest alterations seen in AD and correlate well with AD-related cognitive decline.

The role of ApoE4 in early cellular changes in AD

The major AD genetic risk factor ApoE4 has been shown to affect many processes linked to AD, however, the most critical mechanism(s) and cell types involved remain(s) to be determined. ApoE4 primarily affects amyloid plaques prior to seeding, not after plaque onset (Huynh et al., 2017; Liu C. et al., 2017), thus suggesting an early effect of ApoE4 on AD pathogenesis. In this chapter, the effect of ApoE4 on early cellular events in AD, including intraneuronal Aβ, endosome alterations and synaptic dysfunction, is discussed.

ApoE4 and intraneuronal Aβ accumulation

Preceding amyloid plaques, Aβ is shown to be produced and could accumulate inside neurons. ApoE4 was previously seen to significantly enhance the accumulation of intraneuronal Aβ compared to ApoE2 and ApoE3 (Zhao et al., 2014) (**Figure 4, intraneuronal Aβ accumulation**). In melanocytes, ApoE was shown to regulate fibril formation of the amyloid protein premelanosomal protein in late endosomes (van Niel et al., 2015), however it needs to be determined whether

ApoE has a similar regulatory effect on Aβ in neurons. In the context of Aβ production, ApoE was shown to influence intraneuronal Aβ production by stimulating APP and BACE1 endocytosis (He et al., 2007). The effects on intraneuronal Aβ production is suggested to be dependent on ApoE isoforms: ApoE4 induces the highest APP and BACE1 internalization and consequently leads to higher intracellular Aβ levels than the ApoE2 and ApoE3 isoforms. ApoE also seems to impact APP transcription and does that also in an ApoE isoform-dependent manner (Huang et al., 2017). ApoE4 induced the strongest increase in APP transcription, followed by ApoE3. The weakest increase in APP transcription was seen in the presence of ApoE2. All in all, ApoE, in particular ApoE4, could increase intraneuronal Aβ levels.

ApoE is the main cholesterol carrier in the brain. Astrocyte-derived cholesterol, transferred to neurons via ApoE, was shown to regulate the production of Aβ in neurons (Wang H. et al., 2021). Increased cholesterol levels in neurons were shown to affect Aβ production by increasing the interaction between APP and APP processing enzymes BACE1 and γ-secretase. In contrast, when neuronal cholesterol levels were low, a preferentially interaction of APP with α-secretases was observed. Neuronal cholesterol also seems to play a role in intracellular Aβ accumulation. Niemann-Pick disease, a neurodegenerative disorder characterized by cholesterol accumulation in neuronal endosomes, shows striking similarities with AD. Like in AD, Niemann-Pick disease was shown to also cause Aβ accumulation in endosomes, endosomal trafficking impairment and neurofibrillary tangle formation (Jin et al., 2004; Nixon, 2004). Niemann-Pick disease is caused by mutations in Niemann-Pick type C1 (NPC1) and C2 (NPC2), which normally are involved in the trafficking of cholesterol from endosomal/lysosomal vesicles to other cellular compartments (Nixon, 2004). In Niemann-Pick disease, cholesterol fails to efficiently exit the endosome-lysosome system, causing cholesterol accumulation. Although the exact mechanisms by which cholesterol and ApoE alter intraneuronal Aβ need to be better defined, both ApoE and cholesterol seem to play an important role in intraneuronal Aβ formation and aggregation.

ApoE4 and endosomal impairment

ApoE delivers lipids to neurons by binding to ApoE receptors. ApoE bound to receptors is then internalized and forms an endosome. Although the exact endosomal trafficking of ApoE remains poorly understood, ApoE was shown to be recycled after internalization (Heeren et al., 1999; Rensen et al., 2000). In addition, internalized recombinant human ApoE was reported to be present in early and late endosomes and lysosomes (DeKroon & Armati, 2001; Li et al., 2012; Ljungberg et al., 2003), suggesting that ApoE is either recycled or degraded after internalization. Using electron microscopy, Van Niel et al. (2015) showed that ApoE was localized to ILVs in late endosomes of melanocytes. It has to be noted that most studies on

ApoE trafficking have been done using recombinant, non-lipidated, ApoE or in non-neuronal cell types and it remains to be determined whether astrocyte-derived lipidated ApoE is present in the same endosomal compartments in neurons.

The ApoE4 isoform is associated with endosomal dysfunction (**Figure 4, endosomal alterations**). Genes linked to the endosome-lysosome system are upregulated in humanized ApoE4 compared to ApoE3 mice, suggesting a dysregulated endosome system in the presence of ApoE4 (Nuriel et al., 2017b). ApoE4 was also shown to impair endosome recycling, and as a result, traps various receptors including ApoE, glutamatergic and insulin receptors inside vesicles (Chen et al., 2010; Heeren et al., 2004; Zhao et al., 2017) (**Figure 6**). The ApoE4-induced impairment of endosome recycling is potentially caused by the molten globule state of ApoE4, but not other ApoE isoforms. In the acidic environment found in early endosomes, ApoE4 forms a molten globule state, which dysregulates dissociation of ApoE from its receptor (Pohlkamp et al., 2021; Xian et al., 2018). Reducing the pH in early endosomes was shown to prevent ApoE4-induced impairment of endosome recycling (Xian et al., 2018).

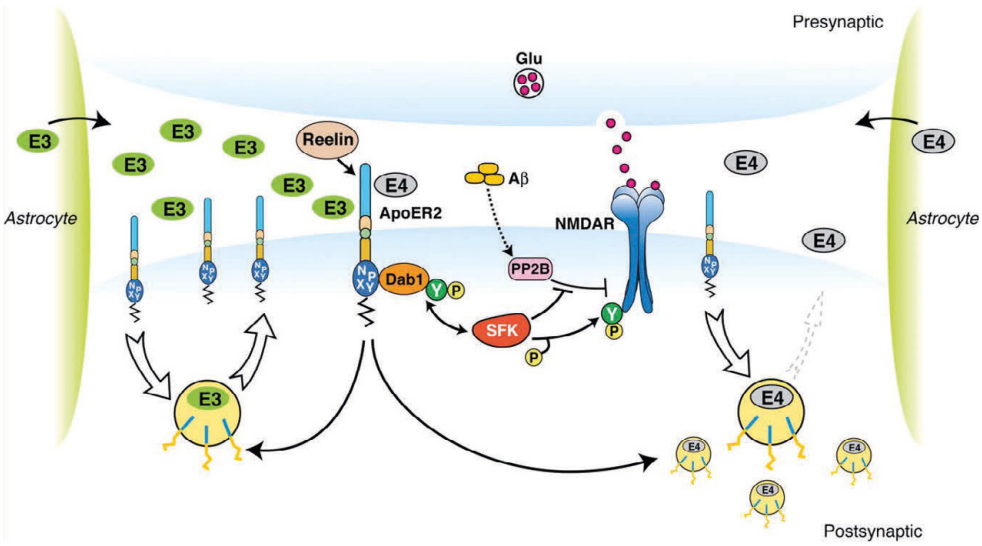


Figure 6: ApoE4 impairs endosome recycling, traps receptors and impairs Reelin's effects on synaptic plasticity. ApoE3 and ApoE4 both bind to ApoER2 receptors and internalize together with the receptors. After internalization, ApoE3 and receptors are efficiently recycled back to the plasma membrane. However, ApoE4 impairs endosome recycling, trapping ApoER2 receptors inside vesicles. Reelin, a protein involved in synaptic plasticity and learning and memory, also binds to ApoER2 receptors and the reduced presence of ApoER2 receptors at the plasma membrane caused by ApoE4-induced endosome recycling impairment consequently reduces the downstream Reelin signalling. Impaired endosome recycling induced by ApoE4 prevents Reelin to bind to ApoER2 receptors, eventually causing synaptic dysfuction. This figure was adapted from Chen et al. (2010).

In AD, an ApoE4 genotype is highly associated with enlargement of endosomes. While endosome enlargement is an early event in AD in general, endosomes are enlarged to an even greater extent in brains of ApoE4 carriers (Cataldo et al., 2000). Larger size and increased number of early endosomal vesicles was also observed in humanized ApoE4 mice (Nuriel et al., 2017b) and an increase in number of early endosomes was also seen in human IPSC-derived neurons (Lin et al., 2018), further supporting the involvement of ApoE4 in relation to endosomal abnormalities. *In vitro* studies also described a role of ApoE4 in Aβ-related lysosomal leakage (Ji et al., 2002; Ji et al., 2006). In addition, ApoE4 was shown to be less efficient in endosomal trafficking of Aβ to lysosomes for degradation than ApoE3 (Li et al., 2012). Altogether, ApoE4 associates with a dysfunctional endosome-lysosome system in both the absence and presence of AD pathology.

ApoE4 and synaptic changes

Many risk genes for LOAD relate to impaired synaptic function in AD, including ApoE4 (Perdigao et al., 2020). Various altered synaptic phenotypes are seen in the presence of the ApoE4 isoform (**Figure 4, synaptic changes**). Neuronal outgrowth and synaptic density are reduced by ApoE4 compared to ApoE3 expression (Dumanis et al., 2009; Nathan et al., 1994). In addition, ApoE4 is associated with neuronal network dysfunction. ApoE4 knock-in (KI) mice were shown to have increased neuronal hyperexcitability in the entorhinal cortex (Nuriel et al., 2017a). The loss of gamma-aminobutyric acid (GABA) inhibitory neurons was shown in aged ApoE4 mice (Knoferle et al., 2014), which potentially explains the hyperexcitability as a result of reduced inhibitory tone in ApoE4 mice. Furthermore, ApoE4 can impair synaptic plasticity. The effect of ApoE isoforms on LTP is most extensively studied, and the majority of studies support that ApoE4 is associated with reduced LTP (Y. Chen et al., 2010; Trommer et al., 2004). Along with impaired synaptic plasticity, ApoE4 mice perform worse in memory-related behavior tests (Andrews-Zwilling et al., 2010; Bour et al., 2008; Rodriguez et al., 2013), indicating that ApoE4 negatively impacts memory, most likely by impaired synaptic functioning.

The exact mechanisms underlying ApoE4-mediated synaptic dysfunction are poorly understood. Impaired endosome recycling of synaptic receptors, previously described in section ‘ApoE4 and endosomal impairment’, has been proposed as a potential mechanism for synaptic dysregulation by ApoE4. By trapping ApoE receptor ApoER2 and glutamate receptors AMPA and NMDA, the regulation of synaptic plasticity is impaired (Chen et al., 2010; Pohlkamp et al., 2021; Xian et al., 2018) (**Figure 6**). On top of that, the binding of Reelin, a protein involved in learning and memory (Weeber et al., 2002), to ApoER2 receptors is reduced, consequently resulting in dysfunctional synaptic regulation.

Whether the effects of ApoE on synaptic function is direct or indirect remains to be determined. Human ApoE was shown to co-localize with synaptic proteins in the brain, suggesting a synaptic localization (Koffie et al., 2012). In AD, ApoE localizes preferably to synapses also containing accumulated Aβ oligomers. A synaptic presence of ApoE was further supported by the presence of ApoE/Aβ complexes in synaptosomes of human brains (Bilousova et al., 2019). The co-localization of ApoE with synaptic markers or in synaptosomes was also seen in control brains (Bilousova et al., 2019; Koffie et al., 2012), suggesting ApoE is present at synapses in both healthy and pathological conditions.

Rationale of the study

At the start of this study, ApoE4 was known as the major risk factor for AD for many years, however the most critical mechanism(s) and cell types involved in the pathogenesis of AD remained unclear. Endosomal alterations, synaptic dysfunction and intraneuronal Aβ accumulation are among the earliest cellular changes observed in AD and ApoE4, the major genetic risk gene for AD, was shown to play a role in these early AD events. However, whether ApoE is present at these cellular sites impacted at early AD stages and how ApoE influences endosomes, synapses and intraneuronal Aβ remained poorly understood.

The major role of ApoE in the brain is to transport lipids, primarily cholesterol, to neurons. Lipids are delivered to neurons via ApoE internalization, suggesting an intraneuronal presence of ApoE. Because cholesterol plays a crucial role in synapse formation (Goritz et al., 2005), as cholesterol carrier, ApoE is expected to play a role in synaptic function. Synapses are sites affected early in AD, however, at the start of this PhD project, only one study described the potential localization of ApoE at synaptic terminals (Koffie et al., 2012). Also, the presence of ApoE at the endosome-lysosome system, another site affected early in AD, was poorly studied. Multiple studies described the presence of internalized ApoE at endosomes and/or lysosomes (DeKroon & Armati, 2001; Li et al., 2012; Ljungberg et al., 2003), however, most studies used recombinant human ApoE produced by E coli. It remained unknown whether more physiological astrocyte-secreted human ApoE also localizes to endosomes/lysosomes.

Neuronal network dysfunction has been repeatedly associated with AD. AD transgenic mouse models overexpressing APP, and thereby Aβ, were shown to induce neuronal hyperactivity (Angulo et al., 2017; Busche et al., 2012). However, it remained unknown whether APP, Aβ, or both were inducing the observed abnormal neuronal activity. In addition, the underlying mechanisms causing the hyperactivity of neurons in AD mouse models remained poorly studied.

Although neuronal network hyperactivity was described for mice overexpressing APP, the link between network dysfunction and sporadic AD was less clear. In 2017, at the very start of this PhD project, ApoE4 was shown to also induce neuronal hyperexcitability *in vivo* (Nuriel et al., 2017a). This study on ApoE4 and abnormal neuronal network dysfunction opened up many new research questions; e.g. whether the abnormal neuronal network dysfunction can also be studied in culture and if the

ApoE4-induced effects on neuronal activity are dependent on the cellular source of ApoE. Astrocytes are the main producer of ApoE in the brain, however, neuronal ApoE was previously shown to cause GABAergic interneuron loss (Knoferle et al., 2014), potentially causing impaired inhibitory network control. Moreover, the effect of different ApoE isoforms on neuronal network activity in AD mouse models was poorly studied.

In addition to neuronal network abnormalities, ApoE4 was previously described to impair synaptic plasticity. Although several mechanisms for ApoE4-induced synaptic plasticity have been proposed, the most crucial one remains unknown. The control of mRNA translation into new proteins plays a crucial role in synaptic plasticity (Costa-Mattioli et al., 2009) and dysfunctional ribosomes are observed in early AD (Ding et al., 2005). Global protein translation related to neuronal activity has never been studied in the context of ApoE4. It is therefore of high relevance to determine how ApoE4 impacts activity dependent protein synthesis as this might potentially explain the altered synaptic function caused by ApoE4.

The subcellular localization of ApoE, in particular astrocyte-derived ApoE, remained poorly understood in neurons, but ApoE presumably localized at endosomes and/or lysosomes. Interestingly, Aβ is extensively described to also be present at endosomal compartments (Rajendran et al., 2006; Takahashi et al., 2002). ApoE is known to co-deposit with Aβ in amyloid plaques (Namba et al., 1991). Additionally, intraneuronal Aβ and ApoE have been previously detected within the same neurons and synapses (Gouras et al., 2000; Koffie et al., 2012), but it remained unknown whether ApoE and Aβ intersect at a subcellular level. The potential subcellular intersection of ApoE and Aβ could be of high relevance in AD research as this might be important in early AD pathology.

Over the last decades, AD research has progressed tremendously and has led to many new insights and discoveries about the disease, which will be important for future therapeutics. With the research done in this thesis, we hope to fill some of the knowledge gaps in the current AD literature and contribute to advancing the understanding of AD.

Aims of the thesis

The overall research aim of this thesis was to study early cellular changes related to Alzheimer's disease and more specifically to determine the role of different ApoE isoforms, in particularly the major AD genetic risk factor ApoE4, on early AD changes including synaptic and endosomal changes, and intracellular Aβ accumulation. The specific key research objectives addressed in this thesis were as follows:

1. To examine the synaptic presence of human ApoE3 and ApoE4 isoforms and their cell source specific effects on neuronal activity in normal and AD conditions (**Paper I**).
2. To investigate the underlying mechanism(s) of ApoE- and AD transgenic APP/Aβ-related neuronal and synaptic (dys)function (**Paper II and III**).
    - To study the effect of different ApoE isoforms on global translation response in neurons (**Paper II**).
    - To examine the role of dysregulated homeostatic synaptic plasticity in Aβ-induced hyperexcitability (**Paper III**).
3. To study the subcellular trafficking of human ApoE3 and ApoE4, in particularly at endosomes/lysosomes, and whether ApoE and APP/Aβ intersect at intracellular sites (**Paper IV**).

Methodological considerations

In this section, a brief description is given of the key methods performed in the papers included in this thesis. The focus of the methods described here is on the procedures I performed myself. Detailed descriptions of methods can be found at the methods sections of the papers included in this thesis (see appendices).

Animals

Animals, mostly mice, were used for part of the research performed in this thesis. All experiments performed on animals were approved by the ethical committee for animal research of Lund University (ethical permit number: M5983-19) and the Institutional Animal Ethics Committee and Institutional Biosafety Committee of InStem, India. At Lund University, all mice were kept under a 12h/12h light/dark cycle and had ad libitum access to food and water. Several mouse models have been used for this thesis (**Table 1**). ApoE knock-out (KO), humanized ApoE3 knock-in (KI), and humanized ApoE4-KI mice were used to study different (human) ApoE conditions. To study AD conditions, AD transgenic APP/PS1 mice were used. APP/PS1 mice contain transgenic human APP carrying the Swedish mutation and PSEN1 containing an EOAD-associated mutation (L166P), causing increased human $A\beta_{42}$ over $A\beta_{40}$ levels and AD-like Aβ deposition in these mice. Humanized ApoE-KI and APP/PS1 mouse model allowed us to study neuronal activity and endosomal trafficking of different ApoE isoforms and in an AD-like context. Because of the heterozygous breeding of ApoE3 KI and APP/PS1 mice, wild-type littermates of these mouse models were used as wild-type controls.

Table 1: List of mouse models used in this thesis

Animal model	Full name	Company
ApoE KO	B6.129P2-Apoe<tm1Unc>/J	Jackson Laboratories
ApoE3-KI	B6.Cg-Apoeem2(APOE*)Adiuj/J	Jackson Laboratories
ApoE4-KI	B6(SJL)-Apoetm1.1(APOE*4)Adiuj/J	Jackson Laboratories
APP/PS1	B6.Cg-Tg (APPswe, PSEN1dE9)85Dbo/Mmjax	Jackson Laboratories

Primary cell cultures

Primary neuron cultures

To study neuronal cells in culture, primary neurons were derived from cortical and hippocampal tissue from embryonic mouse brains at embryonic day E15-E17. Cortices and hippocampi were dissected, incubated in 0.25% trypsin (Thermo Fisher Scientific, 15090046), and manually dissociated using glass pipets in fetal bovine serum (FBS) medium (**Table 2**). Neurons were seeded on poly-D-lysine coated (Sigma-Aldrich, P7405) coverslips, 8-well plates, or 6-well plates for immunofluorescence, live cell imaging, and Western blot, respectively. 3-5 hours after cell seeding, the FBS media was replaced by complete neurobasal medium (**Table 3**). Primary neurons were kept at 37°C with 5% CO_2 for 15 days *in vitro* (DIV) (**Paper II**) or 18-21 DIV (**Paper I, III and IV**) before collection or fixation. The generated neuron cultures were not 100% pure neuron cultures and contain approximately 5-10% of astrocytes. The primary neuron cultures were obtained from embryonic tissue and grown in neuron-specific neurobasal media to favor the growth of neurons inside the cultures. The presence of astrocytes in neuron cultures promotes healthy neuronal and synaptic function (Pfrieger & Barres, 1997). However, we need to keep in mind that astrocytes, although in small amounts, might still produce and secrete ApoE in our cultures. Therefore, for most of our studies on the effects of ApoE isoforms, ApoE KO neuron cultures were used to exclude the effects induced by endogenously produced ApoE.

Table 2: FBS media

	Concentration	Company	Catalogue number
Dulbecco's modified Eagle medium (DMEM)	89%	Nordic Biolabs	SH30243
Fetal bovine serum (FBS)	10%	Gibco	10082147
Penicillin/streptomycin (P/S)	1%	Thermo Fisher Scientific	SV30010

Table 3: Complete neurobasal media

	Concentration	Company	Catalogue number
Neurobasal medium	96.3%	Gibco	21103049
B27 supplement	1x (from 50x)	Gibco	17504044
L-Glutamine	1.4 mM	Gibco	25030081
P/S	1%	Thermo Fisher Scientific	SV30010

Primary astrocyte cultures

Astrocytes are the main cellular source of ApoE in the brain. Therefore, to create a physiologically relevant source of ApoE, human ApoE was obtained from humanized ApoE-KI astrocytes. Primary astrocyte cultures were derived from ApoE KO, ApoE3 KI and ApoE4 KI post-natal mouse brains at post-natal day P1-P3. Cortical and hippocampal tissue was dissected from post-natal brains and incubated in 0.25% trypsin for 15 minutes. Subsequently, the brains were washed in 0.45% glucose-Hanks buffer solution and dissociated into single cells in FBS medium (**Table 2**) by manual dissociation using plastic Pasteur pipets. The dissociated cells suspension was run through a 70 µm cell strainer (Falcon™, 352350) prior to seeding to get rid of bigger cell clumps. FBS medium was replaced by AstroMACS medium (**Table 4**) 3-5 hours after seeding. The media of primary astrocytes were replaced every 2-3 days by fresh AstroMACS medium to maintain a healthy astrocyte culture. Primary astrocytes were cultured at 37°C and 5% CO_2 until at least 80% confluence was reached before further use.

Table 4: AstroMACS

	Concentration	Company	Catalogue number
MACS® Neuro medium	97.55%	Miltenyi Biotec	130-117-031
MACS® NeuroBrew MACS®-21	2%		
AstroMACS supplement	0.2%		
L-Glutamine	(0.25%) 0.5 mM	Gibco	25030081

Collection of astrocyte conditioned media

To obtain a physiological source of human ApoE, astrocyte conditioned media of ApoE KO, ApoE3 KI and ApoE4 KI primary astrocyte were collected. Mature astrocyte cultures were shortly washed in phosphate buffered saline (PBS) and cultured in complete neurobasal medium (**Table 3**) for 48 hours. Conditioned astrocyte media were collected, centrifuged and stored for long-term at -80°C. Astrocyte conditioned media were divided into small aliquots to avoid freeze-thaw cycles. A Western Blot analysis was performed after each medium collection to confirm the presence of human ApoE in the ApoE3 KI and ApoE4 KI conditions.

Cell lines

N2a cells

Mouse neuro-2a (N2a) neuroblastoma cells (ATCC® CCL-131™) were used for several experiments in **paper IV**. N2a cells are neuron-like cells, are easy to work

with, and have the advantage of being immortal, divide and grow fast compared to primary neuron cultures. Although N2a cells are a less advanced model compared to neuron cultures, studying ApoE in N2a cells could give us clues to better understand the cell biology of the intracellular localization of ApoE. N2a cells used in this thesis were either not transfected ($N2a_{unt}$) or were stably transfected with human APP containing the Swedish mutation (N2a APP_{Swe}) (Thinakaran et al., 1996) to study the endosomal trafficking of ApoE in both normal and AD-like conditions, respectively. Both N2a cells lines were cultured in N2a media (**Table 5**) and kept at 37°C and 5% CO_2. N2a media of N2a APP_{Swe} cells also contained Geneticin (Gibco, 10131027) to positively select stably transfected cells. $N2a_{unt}$ and N2a APP_{Swe} cells were seeded on glass coverslips coated with poly-D-lysine one day prior to ApoE treatments.

Table 5: N2a media

	Concentration	Company	Catalogue number	Remarks
DMEM, high glucose	44.5%	Nordic Biolabs	SH30243	
Opti-MEM	44.5%	Gibco	31985062	
FBS	10%	Gibco	10500064	
P/S	1%	Thermo Fisher Scientific	SV30010	
Geneticin	50 mg/ml	Gibco	10131027	*Only used for N2a APP_{Swe} cells

Astrocyte conditioned media treatment

Astrocyte conditioned media were added to primary neuron cultures and N2a cells to provide primary neurons and neuron-like cultures with astrocyte-derived ApoE. Complete media change in primary neurons could stress neurons in culture, therefore only half of the complete neurobasal media was removed from the primary neuron cultures and the same volume of astrocyte conditioned media was added to the remaining complete neurobasal media, resulting in 50% neuron-conditioned and 50% astrocyte-conditioned media. Neurons were cultured in astrocyte-conditioned media for various time points before further use. N2a cells were treated with astrocyte conditioned media in a similar way as primary neurons. Astrocyte conditioned media was added in a 1:1 ratio to the N2a media to get 50% N2a-conditioned and 50% astrocyte-conditioned media.

Recombinant and synthetic proteins

Besides astrocyte-derived ApoE, recombinant human ApoE3 (Sigma-Aldrich, SRP4696) and ApoE4 (Sigma-Aldrich, A3234) have been used as a source for human ApoE. Lyophilized ApoE proteins were reconstituted in 0.1% bovine serum

albumin (BSA) in milli-Q water. A final concentration of 2.5 µg/ml recombinant ApoE was used for treating primary neurons or N2a cells (**Paper IV**). An advantage of recombinant compared to astrocyte-produced ApoE is that recombinant ApoE is cheap and highly accessible. Importantly, recombinant ApoE is produced in E. coli bacteria and therefore unlipidated when added to cells. It is known from previous literature that FBS in culture media, like in our N2a media (**Table 5**), can provide ApoE with cholesterol (Wang H. et al., 2021). In this thesis, recombinant ApoE is mainly used in exploratory studies, whereby most findings in recombinant ApoE-treated cells were repeated with astrocyte-derived ApoE.

Synthetic human $A\beta_{42}$ (Tocris, 1428) was reconstituted in dimethylsulfoxide (DMSO) to a concentration of 250 µM. Prior to use, $A\beta_{42}$ proteins were sonicated for 10 min and subsequently centrifuged for 15 minutes at 12,000 rpm, to prevent Aβ from aggregation. Synthetic $A\beta_{42}$ was used to study the effect of human $A\beta_{42}$ on neurons. For immunofluorescence experiments, neurons were treated with 0.5 µM synthetic $A\beta_{42}$ (**Paper III and IV**); for live-cell microscopy, 200 pM or 0.5 µM was added to neurons (**Paper III**).

Biochemical analyses

SDS PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) was used to detect the presence of, or to determine the relative protein levels of our proteins of interesting in cell lysate and media. Cells were lysed in NP40 buffer (20 mM Tris, 150mM KCl, 5 mM $MgCl_2$ and 1% NP40) (**Paper I and II**) or modified radioimmunoprecipitation assay buffer (RIPA) buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM EGTA, 1% Nonidet P-40, 0.25% sodium deoxycholate) (**Paper III**), depending on the protein of interest. Both lysis buffers also contained protease ((Thermo Fisher Scientific, 78430) and phosphatase inhibitors ((Thermo Fisher Scientific, 78428). The total protein concentration in lysate was determined by bicinchoninic acid (BCA) assay. Equal concentrations of neuron lysate were added to the gel. Media samples were directly mixed with sample buffer and added to the gel.

Blue native-PAGE

The native structure of ApoE complexes and physiological lipidation state of ApoE in different types of media were studied using non-denaturing blue native-PAGE. Astrocyte media, neuron media and recombinant ApoE samples were prepared in NativePAGE™ sample buffer (Invitrogen, BN2003) and subsequently run on a

NativePAGE™ 4-16% Bis-Tris gel (Invitrogen, BN1002BOX) at 150 V. A protocol previously described by (Heinsinger et al., 2016), including membrane boiling in PBS and 10% formic acid incubation, was followed to detect ApoE under native conditions.

Western blot

The protein samples were separated by electrophoresis based on their molecular weight and afterwards transferred to polyvinylidine difluoride (PVDF) membranes. Unspecific signal on the membrane was blocked by incubating the membrane in 5% BSA in tris-buffered saline containing 0.1% Tween 20 (TBS-T) (**Paper II**), or in 5% (for SDS PAGE) or 10% (Native PAGE) skim milk powder (Millipore, 70166) in PBS containing Tween 20 (PBS-T) (**Paper I, III and IV**). The membranes were incubated in primary antibodies overnight at 4°C and subsequently in secondary antibodies for 1 hour at room temperature (**Table 6 and 7**). All washes were performed in either TBS-T (**Paper II**) or PBS-T (**Paper I, III and IV**). To visualize the signal on the membranes, the membranes were developed in ECL Western Blotting Substrate (Thermo Fisher Scientific; 32106) and imaged using the Sapphire Biomolecular imager (Azure Biosystems). To detect ApoE, recombinant human ApoE3 was added to the gel as a positive control. For relative protein quantification, the proteins were normalized to loading control β-actin (**Paper I and III**) or Tuj1 (**Paper II**). Quantifications were performed using ImageJ 1.53c software.

Immunofluorescence

Immunofluorescence labeling and subsequent fluorescence microscopy were performed to identify certain proteins or neuron types in culture and to study the synaptic and subcellular localization of ApoE and Aβ. Primary mouse neurons (19 DIV) (**Paper I, III and IV**) and N2a cells (**Paper IV**) were grown on glass coverslips and fixed in 4% paraformaldehyde (PFA) and 4% sucrose in PBS at room temperature for 15 min prior to immunofluorescence labelling. The cells were permeabilized and blocked for 1 hour in blocking solution containing 0.1% saponin (Sigma-Aldrich, 84510), 1% BSA, 2% normal goat serum (Jackson ImmunoResearch, 005-000-121) in PBS. Subsequently, the cells on coverslips were incubated in primary antibodies (**Table 6**), diluted in PBS containing 2% normal goat serum, overnight at 4°C. The second day, the cells on coverslips were incubated with secondary antibodies and nuclear DAPI and/or F-actin phalloidin staining (**Table 7**). All washes during the immunofluorescence procedure were done in PBS. The coverslips were mounted using ProLongTM Diamond Antifade Mountant (Invitrogen, P36961).

Epifluorescence microscopy

Different types of microscopes have been used for taking the fluorescence images in this thesis. An inverted Olympus IX70 epifluorescence microscope, equiped with 405, 488, 568 and 647 nm filters was used to capture images where there was no need to control the depth/z-axis. The microscope was equipped with an X-Cite® 120Q excitation light source (Excelitas Technologies), a C11440 ORCA-Flash4-oIT digital camera, and 40x NA 1.3 and 60x NA 1.4 oil immersion objectives.

Since our samples consisted of only one cell layer, there was in most samples no need to control the z-axis. A disadvantage of epifluorescence microscopy is that all signal is acquired in once, without being able to control the depth. As a result, small intracellularly detected puncta could appear to be co-localized, while in reality the puncta are just on top of each other at a different z-level. As in some studies we were specifically interested in determining the subcellular localization of our protein of interest, a more enhanced microscope technique was used.

Confocal microscopy

Higher resolution confocal microscopy was performed to better distinguish between two antibodies in an x, y and z-plane. Two confocal microscopes were used during the thesis work. In **Paper I and III**, a Leica TCS SP8 laser scanning confocal microscope was used equipped with a Diode 405/504 nm and 405, 488, 514, 552 and 638 nm Argon lasers. In **Paper IV**, a newer version of a Leica TCS SP8 laser scanning confocal microscope was used, which was equipped with 405, 488, 552 and 638 nm lasers. During image acquisition of stacks, a Z-step size of 0.5 µm was used.

Image analyses

Images obtained by epifluorescence or confocal microscopy were further processed or analyzed using ImageJ 1.53c, ICY or Imaris (version 9.3).

3D reconstruction of neurons and synaptic terminals (Paper I)

To assess in 3D whether ApoE is present at vGlut1-positive synaptic terminals, confocal images of primary neurons labeled for CAMKIIα, ApoE and vGlut1 were 3D reconstructed using Imaris. In the Imaris software, a surface of each individual channel was created. The settings used to create a surface for human ApoE were corrected based on ApoE KO controls.

Orthogonal images of ApoE with a subcellular/Aß markers (Paper IV)

To be able to look at our images of N2a cells from different angles, orthogonal images were obtained to provide an image in the x, y and z direction. Fluorescent images of $N2a_{unt}$ and N2a APP_{Swe} cells treated with ApoE3 and ApoE4 astrocyte conditioned media were captured using confocal microscopy. Orthogonal images were obtained using Imaris software.

Quantification of co-localization of ApoE with subcellular markers (Paper IV)

To define the subcellular localization of recombinant ApoE, the co-localization of ApoE with late endosomal marker Rab7, late endosomal/lysosomal marker LAMP1, cis-Golgi marker GM130 and trans-Golgi marker TGN38 was studied in N2a cells treated with recombinant ApoE3 or ApoE4 for 4 hours. The co-localization of ApoE with the subcellular markers was measured based on the percentage of ApoE pixels overlapping with the subcellular marker. To exclude extreme values, only N2a cells containing 5-20 ApoE puncta inside the cell were included in the co-localization analyses.

Quantification of ApoE at synaptic terminals (Paper I)

The percentage of ApoE present at neurites and vGlut1-positive synaptic terminals were quantified using ICY software. For each imaged neuron, 7 neurites were selected as region of interest (ROI). The ROI of neurites was set a little wider than the CAMKII-positive neurite to include all synaptic terminals. The number of puncta positive for human ApoE at/close to neurites was quantified using the spot detector in the ICY software. The settings used for the spot detector to detect ApoE puncta were adjusted based on unspecific puncta detected in ApoE KO neurons or ApoE KO astrocyte media-treated neurons. The percentage of ApoE puncta at/close to neurites co-localizing with vGlut1-positive synaptic terminals was quantified by using the ICY colocalizer protocol.

Quantification of dendritic branching (Paper I)

The dendritic branching and neuronal outgrowth of different ApoE neurons were studied using Sholl analysis in ImageJ (Neuroanatomy plugin) (Ferreira et al., 2014). Sholl analysis automatically detect intersections, reflecting dendrites, at each radius step of 30 µm in binary images of MAP2-labeled neurons. 5 neurons from each embryo were used for analysis, with a total of 5 embryos per conditions.

Quantification of excitatory and inhibitory synaptic density (Paper I)

The excitatory and inhibitory synaptic density in primary neurons labeled for excitatory synaptic marker vGlut1, inhibitory synaptic marker VGAT and phalloidin were analyzed in ImageJ. First, neuronal processes were selected based on phalloidin staining by the use of a plugin called NeuroJ (Meijering et al., 2004). Subsequently, the excitatory and inhibitory synaptic density per 100 µm was

determined by vGlut1-phalloidin and VGAT-phalloidin co-localization analyses, respectively, by the SynapCount v2 plugin (Mata et al., 2017). For each embryo, 5 neurons were analyzed.

Quantification of percentage of excitatory neurons in a culture (Paper III)

To quantify the percentage of excitatory neurons in wild-type and APP/PS1 cultures, primary neurons were labeled for dendritic marker MAP2, excitatory neuronal marker CAMKIIα and DAPI. By using the cell counter plugin in ImageJ, the number of CAMKII- and MAP2-positive neurons were determined. The percentage of excitatory neurons in a culture was calculated by the total number of CAMKII-positive neurons / the total number of MAP2-positive neurons.

Lists of antibodies

Table 6: List of primary antibodies used in this thesis

Antibody	Host	Dilution WB	Dilution IF	Company	Catalogue number
APP/Aβ (6E10)	Mouse	-	1:500	Biolegend	803003 (previously SIG-39320)
Aβ N-terminal (82e1)	Mouse	-	1:100	Immuno-Biological Laboratories	10323
ApoE	Rabbit	1:250	1:500	Invitrogen	16H22L18
ApoE (WUE-4)	Mouse	1:500	-	Novus Biologicals	NB110-60531
β-actin	Mouse	1:2000	-	Sigma-Aldrich	A5316
CAMKIIα	Mouse	-	1:500	Sigma-Aldrich	05-532
eEF2	Rabbit	1:1000	-	Cell Signaling Technology	2332
GM130	Mouse	-	1:500	BD Bioscience	610822
LAMP1	Rabbit	-	1:1000	Abcam	ab24170
LAMP1	Rat	-	1:1500	Abcam	ab25245
MAP2	Chicken	-	1:1000	Abcam	ab92434
Phospho-eEF2 (Thr56)	Rabbit	1:1000	-	Cell Signaling Technology	2331
Rab7	Mouse	-	1:500	Abcam	ab50533
TGN38	Mouse	-	1:200	Santa Cruz	sc-166594
Tuj1	Mouse	1:1000	-	Sigma-Aldrich	T8578
VGAT	Rabbit	-	1:500	Synaptic Systems	131013
vGlut1	Guinea pig	-	1:500	Sigma-Aldrich	Ab5905

Table 7: List of secondary antibodies and fluorescent dyes used in this thesis

Antibody	Host	Dilution WB	Dilution IF	Company	Catalogue number
Alexa Fluor 488 AffiniPure anti-Rabbit IgG	Goat	-	1:500	Jackson ImmunoResearch	111-545-144
Alexa Fluor 568 anti-Mouse IgG	Goat	-	1:500	Invitrogen	A-11004
Alexa Fluor 568 anti-Rat IgG	Goat	-	1:500	Invitrogen	A11077
Alexa Fluor 633 anti-Guinea pig IgG	Goat	-	1:500	Invitrogen	A-21105
Alexa Fluor 647 AffiniPure anti-Chicken IgY (IgG)	Donkey	-	1:500	Jackson ImmunoResearch	703-605-155
Alexa Fluor 647 AffiniPure anti-Mouse IgG	Goat	-	1:500	Jackson ImmunoResearch	115-605-003
Alexa Fluor 647 AffiniPure Anti-Rat IgG	Goat	-	1:500	Jackson ImmunoResearch	112-605-003
DAPI	-	-	1:2000	Sigma-Aldrich	D9542
DyLight™ 405 AffiniPure Goat Anti-Chicken IgY (IgG)	Goat	-	1:500	Jackson ImmunoResearch	103-475-155
Filipin	-	-	1:1000	Sigma-Aldrich	SAE0088
HRP-conjugated Rabbit IgG	Goat	1:2000	-	R&D systems	HAF008
HRP-conjugated Mouse IgG	Goat	1:2000	-	R&D systems	HAF007
Rhodamine phalloidin	-	-	1:2000	Invitrogen	R415

Live-cell calcium imaging

In vitro live-cell Ca^{2+} imaging can be used as a measure to study neuronal firing in culture. To study Ca^{2+} fluctuations, a Ca^{2+} dye called Fluo-4 AM (Thermo Fisher Scientific, F14201) was used to trace intracellular Ca^{2+} levels over time using live-cell microscopy. Prior to live-cell imaging, primary neurons (18-21 DIV) were incubated with 3 µM Fluo-4 AM in DMSO for 30 min at 37°C. For a total duration of 2 minutes, the neurons were imaged every 100 ms for each selected region using a Nikon Eclipse Ti microscope. During the live-cell imaging, the neurons were kept at a constant temperature of 37°C and at 5% CO_2.

Calcium imaging analysis

Different analysis programs for Ca^{2+} imaging were used in different papers. In **Paper I**, live cell Ca^{2+} data were analyzed using NETCAL software (Orlandi et al., 2017). In **Paper III**, the Ca^{2+} data were analyzed using ImageJ and PeakCaller (Artimovich et al., 2017). While the PeakCaller program provides a quicker

approach to obtain data on spike frequency and amplitude, the NETCAL program allows to specifically analyze Ca^{2+} intensity changes in a neuron sub-group.

In short, for the analysis in **Paper I** the obtained Ca^{2+} images files were first pre-processed and, when required, corrected for drifting of the imaging platform in the NETCAL program. Subsequently, each neuronal cell body was selected as ROI to enable the analysis of individual cells. Ca^{2+} traces were generated based on fluctuations in Ca^{2+} fluorescent intensity over time. Each ROI was semi-automatically classified into either the neuron, glia or silent group using a pre-defined algorithm. In short, ROIs showing characteristic neuron, astrocyte or silent Ca^{2+} patterns were selected as representative traces for each respective sub-group to learn the program to recognize neuronal, astrocytic and silent traces. After the classification by the algorithm, the traces in each sub-group were always manually checked to avoid false classification. Since we were interested in neuronal activity, the cells classified in the neuron sub-group were used for further analysis. A spike interference method was used to determine spike features of each ROI in the neuron sub-group based on the Ca^{2+} traces. In the spike analysis, a burst was defined as a group of connected spikes within an interval < 1 second. A neuron was considered to be active when > 2 spikes per 2 minutes were detected. The NETCAL software provides several spike outputs including the firing rate of neurons (spike frequency), bursting rate, amplitude of the spikes, proportion of neurons that are active in the cultures and proportion of spikes that are not associated to a burst activity (independent spikes).

In **Paper III**, the Ca^{2+} live-cell imaging files were initially opened in ImageJ to manually select ROIs. Each neuronal cell body was selected as a ROI. Further analysis was performed in the MATLAB PeakCaller script. The Ca^{2+} image files were pre-processed and normalized to extract the Ca^{2+} fluorescent intensities over time. To detect spikes, the threshold was set to 10% above baseline. Using the PeakCaller script, spike frequency, amplitudes of the spikes and inter-spike intervals were obtained. In the analysis of amplitudes and inter-spike intervals, cells without spikes were excluded from the analysis.

Statistical analyses

All statistical analyses were performed using Graphpad Prism 8.4.1 software. Prior to statistical testing, the normal distribution of the data was assessed by using histograms, QQ-plots and Shapiro-Wilk normality tests. Normal distributed data was statistically analyzed using student t-tests for 2 independent groups, or one-way ANOVA for >2 independent groups. In case of significant differences detected by one-way ANOVA, multiple comparison analyses were performed to determine which specific groups showed significant differences. Non-normalized data were

statistically analyzed using non-parametric Mann-Whitney tests for 2 groups and Kruskal-Wallis tests for >2 groups. When the Kruskal-Wallis test showed significance, the significant differences were further analyzed using Dunn's tests. In our studies, α-level of ≤ 0.05 was considered significant. The significant differences between different groups were shown as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

Results

In this section, the main results from the four papers included in this thesis are summarized. Detailed descriptions of the results can be found in the full papers in the appendices.

Astrocyte conditioned media provides a physiologically source of human ApoE (Paper I, II, IV)

In the brain, ApoE is primarily produced by astrocytes (Boyles et al., 1985), and the function and receptor binding affinity of ApoE can change depending on whether ApoE is lipidated. Therefore, consideration of the physiological source of ApoE is important. To obtain human ApoE in a physiological way *in vitro*, astrocyte conditioned medium was obtained from humanized ApoE3-KI and ApoE4-KI primary mouse astrocytes (**Figure 7A**).

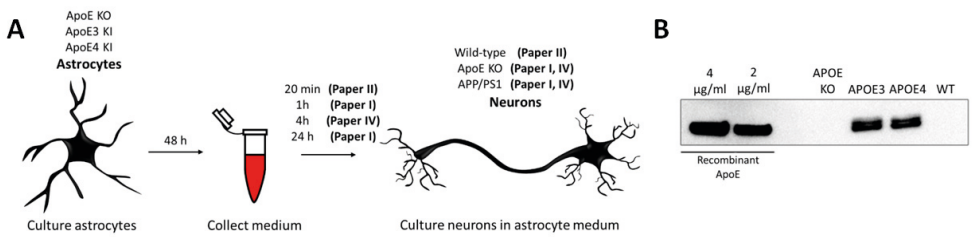


Figure 7: Astrocyte conditioned media from human ApoE3- and ApoE4-KI mice were used as a source of human ApoE in experiments on N2a cells and primary neurons. A. Schematic overview of astrocyte media collection and treatment. Astrocyte conditioned media was collected from ApoE KO, ApoE3-KI and ApoE4-KI primary astrocyte cultures 48 h after media change. Subsequently, the collected astrocyte conditioned media was used to treat primary neurons at different time points depending on the research question. **B.** The presence of human ApoE in the collected media from ApoE3-KI and ApoE4-KI primary astrocyte cultures was confirmed by western blot before media use in experiments. Recombinant human ApoE3 was used as a postive control. Figure is adapted from paper I.

Both human ApoE3 and ApoE4 were secreted and detected in astrocyte conditioned media collected 48 hours after change to fresh neurobasal media (**Figure 7B**). As controls, no human ApoE was detected in conditioned media from ApoE KO and wild-type primary astrocytes (**Figure 7B**). To detect human ApoE, an antibody

generated against human ApoE was used that does not detect mouse ApoE in western blots. ApoE3-KI and ApoE4-KI astrocyte conditioned media were used as a source of human ApoE in experiments on N2a cells and primary neurons in **Papers I, II and IV**.

Human ApoE localizes to synaptic terminals and affects neuronal activity in an ApoE isoform- and cell source-dependent manner (Paper I)

As discussed in the introduction of this thesis, ApoE has been shown to be involved in many processes related to synaptic activity and plasticity. However, it was unclear whether ApoE is present at synaptic terminals. Here, we observed that exogenously added astrocyte-derived ApoE targets synaptic terminals positive for the pre-synaptic marker vGlut1 (**Figure 8A**). Both astrocytic ApoE3 and ApoE4 were detected at or near synaptic terminals, suggesting that the synaptic localization of ApoE is not ApoE isoform-specific (**Figure 8B**).

Although the majority of the ApoE in the brain is produced by astrocytes, neurons can also generate ApoE though mainly when stressed (Boschert et al., 1999). Still, certain neuronal and synaptic alterations have been specifically associated with neuron-derived ApoE (Knoferle et al., 2014; Lin et al., 2018). Therefore, we also studied whether ApoE endogenously expressed by humanized ApoE3- and ApoE4-KI neurons is present at synaptic terminals. Like astrocyte-derived ApoE, endogenous neuronal human ApoE co-localized at or near vGlut1-positive pre-synaptic terminals in both ApoE3 and ApoE4 neurons (**Figure 8C-D**), suggesting a synaptic localization of neuronal ApoE. Of all the ApoE detected in neurites, about 40% localized in close proximity to synapses independent of cell origin and ApoE isoform (**Figure 8B and D**).

Since ApoE appeared to localize at synapses, we next examined whether ApoE could influence neuronal activity. Changes in neuronal activity were studied *in vitro* using live-cell Ca^{2+} imaging with the Ca^{2+} dye Fluo-4 AM. Depolarization of neurons due to action potentials is linked to a fast increase in intracellular Ca^{2+} levels, making live-cell Ca^{2+} imaging a useful tool to study neuronal activity.

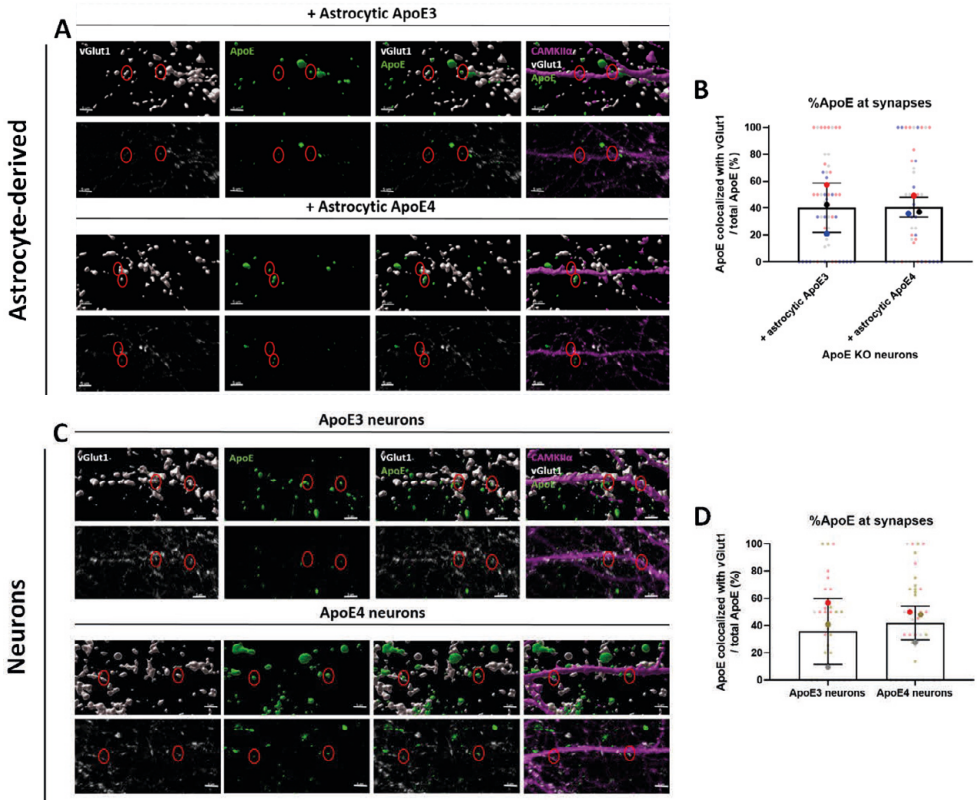


Figure 8: Astrocytic and neuronal ApoE localizes at or close to synaptic terminals. A. Confocal microscope images showing astrocyte-derived ApoE at or near vGlut1-positive synaptic terminals from CAMKIIα-positive excitatory neurons. Instances of ApoE co-localizing with vGlut1 are circled in red. **B.** Quantification of the percentage of astrocytic ApoE localized at vGlut1-positive synapses. **C.** Neuronal ApoE localizes to vGlut1-positive pre-synapses as observed with confocal microscopy. Red circles highlight co-localization between ApoE and pre-synaptic marker vGlut1. **D.** Quantification of the percentage of neuronal ApoE co-localized with pre-synaptic marker vGlut1. Data in the graphs are shown as mean ± SD in superplots. Scale bars in images represent 5 µm. Figure is adapted from paper I.

Exposing of ApoE KO neurons to ApoE4 astrocyte conditioned media for 1 hour resulted in increased spike frequency and a reduced proportion of independent spikes compared to ApoE KO and ApoE3 astrocyte conditioned media (**Figure 9A-B**). Independent spikes are spikes that are not associated with burst activity, and the proportion of independent spikes is used as a measure of the efficacy of the spikes and negatively correlates with coordinated activity (Fernandez-Garcia et al., 2020). This suggests that the 1 hour treatment with astrocyte-derived ApoE4 significantly increased coordinated activity compared to treatment with ApoE KO or ApoE3 astrocyte conditioned media. In contrast, no differences in spike frequency were detected in neurons cultured in the different astrocyte conditioned media for 24 hours (**Figure 9C**). Interestingly, the proportion of independent spikes in neurons

significantly increased after long-term ApoE4 exposure compared to ApoE KO control (**Figure 9D**). Opposite to what was observed after 1 hour of treatment, this result suggests that astrocyte-derived ApoE4 addition results in less coordinated neuronal activity with longer exposure.

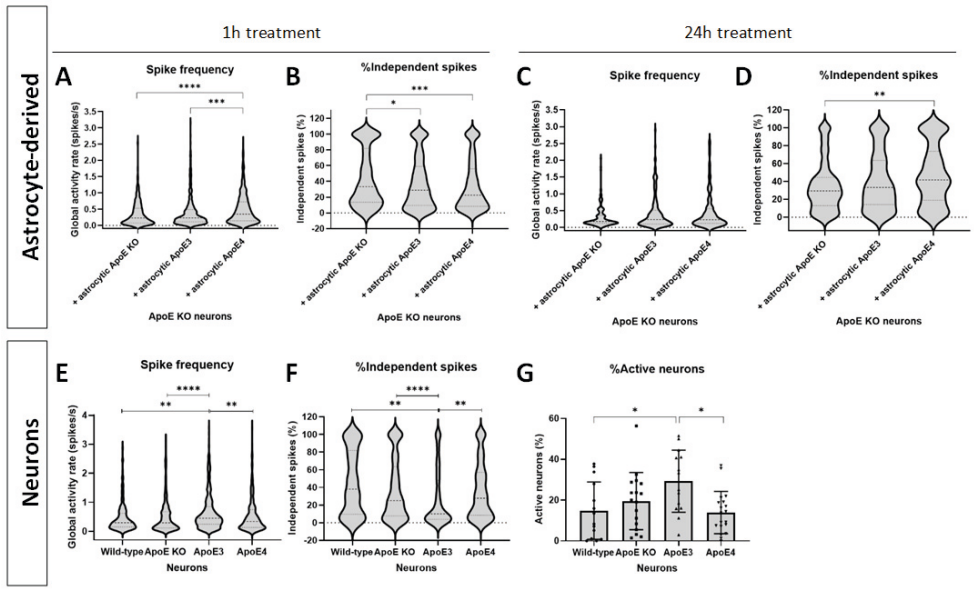


Figure 9 : Neuronal activity is differentially influenced depending on ApoE isoform and cellular origin. A-B. Quantification of the spike frequency (spikes/s) (**A**) and percentage of independent spikes (%) (**B**) in ApoE KO neuronal cultures treated with ApoE KO, ApoE3 or ApoE4 astrocyte conditioned media for 1 hour. **C-D.** Quantification of spike frequency (**C**) and independent spikes (%) (**D**) in ApoE KO neuronal cultures treated with ApoE KO, ApoE3 and ApoE4 astrocyte conditioned media for 24 hours. **E-G.** Quantification of spike frequency (**E**), proportion of independent spikes (%) (**F**) and active neurons (%) (**G**) in neuronal cultures from wild-type (WT), ApoE KO, ApoE3 and ApoE4 mice. Data is shown as violin plots (**A-F**) or mean ± SD (**G**). Statistical differences are shown as * p value < 0.05, ** p value < 0.01, *** p value < 0.001 and **** p value < 0.0001. Figure is adapted from paper I.

As neuronal ApoE was also detected at synaptic terminals, we therefore studied whether neuronal ApoE could affect activity similarly to astrocyte-derived ApoE. Neuronal ApoE4, but not astrocytic ApoE4, has been shown to induce GABAergic cell loss with aging (Knoferle et al., 2014), which might indicate that neuronal ApoE can influence neuronal activity. Interestingly, we observed that neurons expressing ApoE3, but not ApoE4, had significantly increased spike frequencies (**Figure 9E**) and network coordination, which was evidenced by a reduced proportion of independent spikes in ApoE3 cultures (**Figure 9F**). Remarkably, while astrocyte-derived ApoE did not alter the proportion of neurons that were active in culture, ApoE3-expressing neuronal cultures had a significantly higher percentage of active neurons compared to wild-type and ApoE4 neuronal cultures (**Figure 9G**). Together, these data suggest that ApoE can influence neuronal activity *in vitro* depending on ApoE isoform and cellular origin.

ApoE4 alters the NMDAR-mediated translation response via distinct Ca^{2+} influx (Paper II)

The synthesis of new proteins plays a critical role in activity-related synaptic plasticity and memory formation (Sutton & Schuman, 2006; Sutton et al., 2004). In **Paper II**, we asked whether ApoE isoforms differentially affect protein synthesis. Global protein translation was studied by measuring the phosphorylation status of eukaryotic elongation factor 2 (eEF2). Increased phosphorylation of eEF2 correlates with reduced mRNA to protein translation (Heise et al., 2014). Here, ApoE4-treated primary neurons and synaptoneurosomes showed significantly higher levels of eEF2 phosphorylation, indicating that ApoE4 inhibits global protein translation both in neurons and at synapses (**Figure 10A-B**). The ability of ApoE4 to inhibit protein synthesis was corroborated via the use of a technique called fluorescent non-canonical amino acid tagging (FUNCAT). FUNCAT can directly detect the synthesis of new proteins by fluorescently labeling newly translated proteins. With this method, we observed that the levels of newly synthesized proteins were reduced in ApoE4-treated neurons (**Figure 10C-D**), which is in line with reduced protein translation. The effect of ApoE4 on protein translation appears to require ApoE receptors as ApoE receptor antagonist RAP prevented the ApoE4-induced increase in eEF2 phosphorylation in ApoE4-treated neurons (**Figure 10E**).

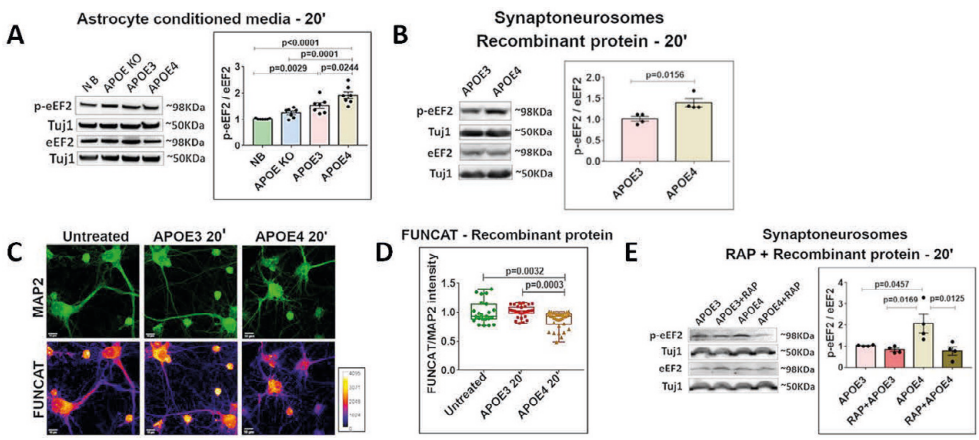


Figure 10: ApoE4 decreases global protein translation in neurons and synaptoneurosomes after 20 min treatment. A. Representative western blot and quantification of phosphorylated eEF2 to total eEF2 levels in primary mouse neurons (15 DIV) cultured in neurobasal (NB), ApoE KO, ApoE3 or ApoE4 astrocyte conditioned media for 20 min. **B.** Represenative western blot membrane and quantification of phosphorylated eEF2 to total eEF2 levels in synaptoneurosomes derived from rat brain (P30) treated with recombinant ApoE3 or ApoE4 for 20 min. **C.** Representative images of primary rat neurons (15 DIV) treated with recombinant ApoE3, ApoE4 or no treatment for 20 min showing MAP2-labeling and FUNCAT fluorescent signal. **D.** Quantification of FUNCAT fluorescent signal normalized to MAP2 fluorescent signal in primary rat neurons (15 DIV) treated with recombinant ApoE3, ApoE4 or no treatment for 20 min. **E.** Representative western blot bands and quantification of phosphorylated eEF2 to total eEF2 from rat brain-derived synaptoneurosomes treated with recombinant ApoE3 or ApoE4 in the absence or presence of ApoE receptor antagonist RAP. Data is expressed as mean ± SEM. Figure is adapted from paper II.

Alterations in protein translation related to neuronal activity are typically mediated by stimulation of NMDA receptors. The translational response mediated by NMDA receptor activation induces an overall inhibition of protein synthesis though the translation of specific proteins, such as post-synaptic density 95 (PSD95) and phosphatase and tensin homolog (PTEN), is upregulated (Kute et al., 2019) (**Figure 11A-C**). PTEN is a protein known to inhibit the Akt/protein kinase B pathway, a pathway involved in neuronal outgrowth and synaptogenesis (Cuesto et al., 2011; Markus et al., 2002).

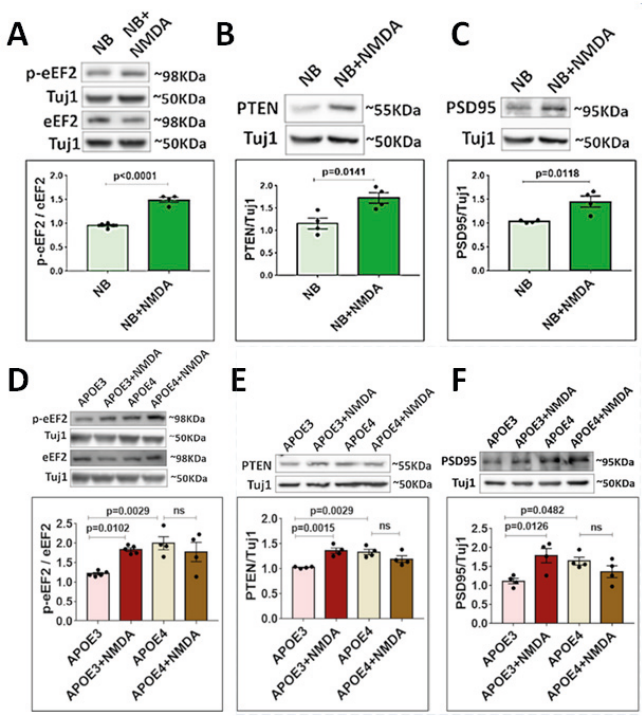


Figure 11: Translational response induced by NMDAR activation is lost in ApoE4-treated neurons. A-C. Representative western blots and quantification of eEF2 phosphorylation (**A**), PTEN (**B**) and PSD95 (**C**) levels in wild-type primary neurons treated with 20 μM NMDA for 5 min. **D-F.** Representative western blot bands and quantification of wild-type primary rat neurons treated with ApoE3 or ApoE4 for 20 min in the presence or absence of 20 μM NMDA for 5 min. Data are shown as mean ± SEM. Figure is adapted from paper II.

In ApoE3-treated primary rat neurons, adding NMDA activated a similar translational response similar to adding NMDA alone (**Figure 11D-F**). Interestingly, in primary neurons treated with ApoE4, the global inhibition of protein translation, as indicated by eEF2 phosphorylation and increase in PTEN and PSD95 levels was not detected after NMDA addition. This suggest that the NMDA-induced translational response is impaired in the presence of ApoE4 but not ApoE3.

NDMA receptor activation induces a strong Ca^{2+} influx in neurons. Likewise, Ca^{2+} influx seems to be critical for the effect of ApoE4 on eEF2 phosphorylation and protein translation as the ApoE4-induced increase in eEF2 phosphorylation was abolished in the absence of Ca^{2+} (**Figure 12A**). In ApoE3-treated primary rat neurons, global protein translation was not altered in the absence of extracellular Ca^{2+}.

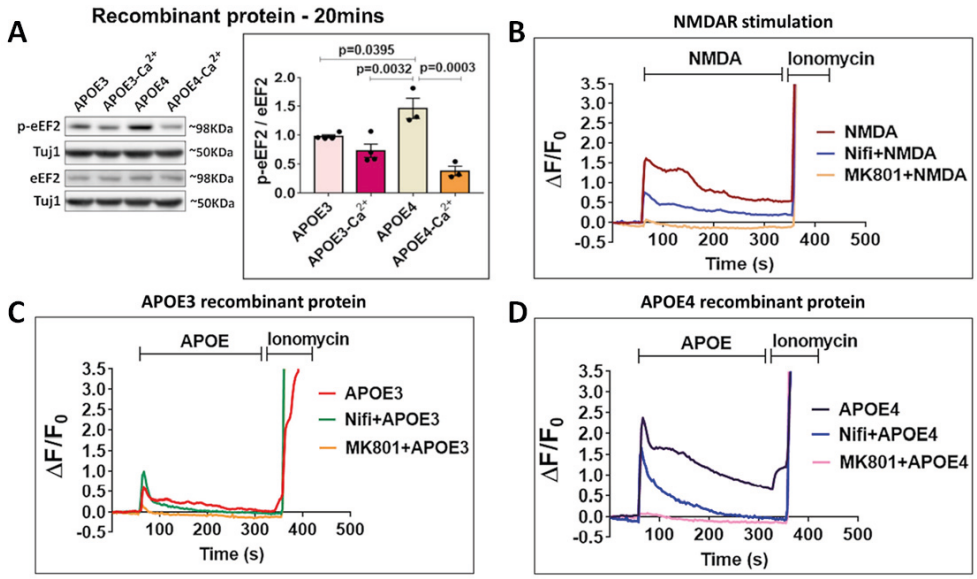


Figure 12: ApoE3 and ApoE4 induce distinct intracellular Ca^{2+} patterns in rat primary neurons. A. Representative western blot and quantification of rat primary neurons treated with ApoE3 or ApoE4 for 20 min in the absence or presence of extracellular Ca^{2+}. Data are indicated as mean ± SEM. **B-D.** Representative graphs showing the change in fluorescent intensity of Ca^{2+} indicator Fluo-4 AM in neurons treated with NMDA (**B**), ApoE3 (**C**) or ApoE4 (**D**) over a total duration of 5 min (360 s). Nifedipine (Nifi) and MK801 were added to study the involvement of L-VGCC and NMDA receptors, respectively. Figure is adapted from paper II.

To further study how ApoE3 and ApoE4 affect Ca^{2+} influx and how this relates to the NMDA-induced Ca^{2+} influx, intraneuronal Ca^{2+} fluctuations were traced acutely over time after NMDA, ApoE3 or ApoE4 treatment. After NMDA addition, intracellular Ca^{2+} levels strongly increased within the first few seconds, and the higher Ca^{2+} level was sustained for over 5 minutes (**Figure 12B**). Adding NMDA to primary neurons in combination with NMDA receptor antagonist MK801 led to no change in Ca^{2+} levels, supporting the specificity of NMDA for this response in neurons. In comparison, ApoE3 treatment of neurons led to a small increase in intracellular Ca^{2+} levels but this increase was not sustained (**Figure 12C**). Interestingly, ApoE4 treatment strongly increased Ca^{2+} levels similarly to NMDA treatment and led to sustained levels of increased Ca^{2+} (**Figure 12D**). With ApoE3 and ApoE4 treatment, the Ca^{2+} increase was completely blocked after adding

MK801, suggesting that the change in intracellular Ca^{2+} levels with ApoE3 and ApoE4 is mostly dependent on NMDA receptor activation. Inhibition of L-type voltage-gated Ca^{2+} channels (L-VGCC) receptors via nifidipine (Nifi) partially abolished the Ca^{2+} response after ApoE4 treatment, indicating that L-VGCC receptors also partially contribute to the changes in Ca^{2+} levels induced by ApoE4.

Altogether, we found that ApoE4 inhibits global protein translation and strongly induces Ca^{2+} influx predominantly via NMDA receptors, while ApoE3 only induces a short burst in NMDA receptor-related Ca^{2+} influx. Therefore, ApoE4 may promote synaptic dysfunction via dysregulation of the NMDA receptor-mediated protein translation response and altered Ca^{2+} homeostasis.

APP/PS1 neurons are hyperactive *in vitro* potentially due to dysregulated homeostatic synaptic plasticity (Paper III)

Aberrant neuronal activity is a feature observed in early AD, and increased neuronal hyperactivity has been observed in mouse models overexpressing human APP and Aβ (Angulo et al., 2017; Busche et al., 2012; Busche et al., 2019). In line with this, we observed that AD transgenic APP/PS1 neurons had, overall, increased spontaneous neuronal activity as indicated by significantly increased spike frequency and amplitude compared to wild-type neurons (**Figure 13A-B**).

To study whether the increased neuronal activity in our APP/PS1 neuronal cultures was from excitatory or inhibitory neurons, we sub-divided the neurons in our cultures into either CAMKII-positive, which were considered excitatory neurons, or CAMKII-negative neurons, which were most likely reflecting inhibitory neurons. We first observed no significant difference in the proportion of CAMKII-positive excitatory neurons in wild-type and APP/PS1 neuron cultures (**Figure 13C**), suggesting that APP/PS1 neuronal cultures does not alter the proportion of excitatory neurons compared to control. From there, live neurons were transduced with an AAV viral vector for Td-Tomato under a CAMKII promoter, allowing us to selectively identify CAMKII-positive excitatory neurons in live cultures (**Figure 13D).** Interestingly, in APP/PS1 neuronal cultures, only CAMKII-positive neurons showed significantly increased spontaneous neuronal spike frequency and amplitude, and CAMKII-negative neurons, which were likely GABAergic inhibitory interneurons, did not show any significant difference in neuronal activity compared to wild-type controls (**Figure 13D-E**). This suggests that excitatory neurons specifically drive the observed hyperactivity in APP/PS1 neurons.

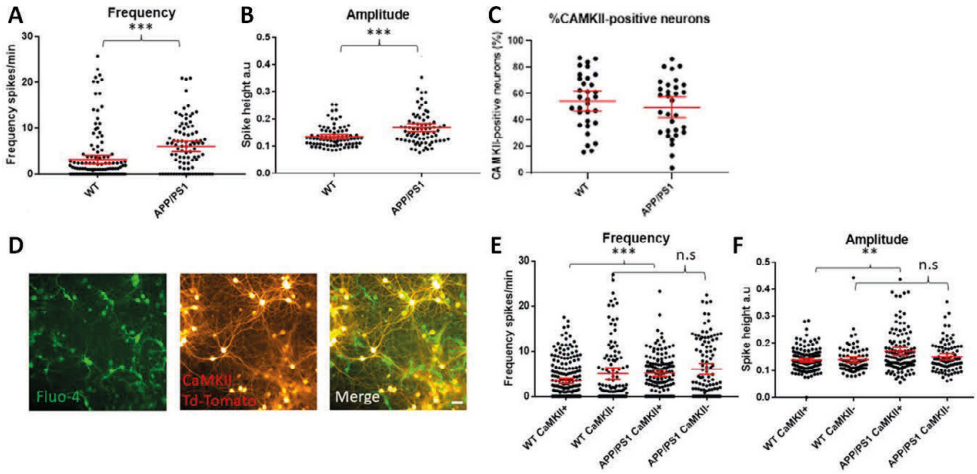


Figure 13: AD transgenic APP/PS1 neurons, particularly CAMKIIα-positive excitatory neurons, show increased spontaneous neuronal activity. A-B. Quantification of spike frequency (**A**) and amplitude (**B**) in wild-type (WT) and APP/PS1 primary neuron cultures measured by live-cell Ca^{2+} imaging. **C.** Quantification of the proportion of CAMKIIα-positive excitatory neurons (%) of all MAP2-positive neurons in wild-type and APP/PS1 cultures. **D.** Representative fluorescent image of neurons labeled with Ca^{2+} dye Fluo4 (green) and expressing CAMKII-Td-Tomato (red). Scale bar represents 50 µm. **E-F.** Quantification of spike frequency (**E**) and spike amplitude (**F**) in wild-type and APP/PS1 neuronal cultures sub-divided into CAMKII-positve and CAMKII-negative neurons based on the expression of CAMKII-Td-Tomato. Data are expressed as mean ± 95% confidence interval. ** p value < 0.01, *** p value < 0.001, and ns is not significant. Figure is adapted from paper III.

Homeostatic synaptic plasticity (HSP) is a vital mechanism maintaining the average neuronal firing rate around a homeostatic set point to ensure proper neuronal function (Turrigiano, 2012; Turrigiano, 2008). We hypothesized that the altered neuronal activity in APP/PS1 neurons could be due to dysregulated HSP in these neurons. To test this hypothesis, wild-type and APP/PS1 neurons were treated with compounds known to alter neuronal activity: tetrodotoxin (TTX) and bicuculine. As expected, acute treatment with TTX and bicuculine induced decreased and increased neuronal activity, respectively (**Figure 14A-B**), and these effects were seen in both wild-type and APP/PS1 neurons. Chronic treatment with TTX or bicuculine is a well-established method to study HSP (Turrigiano et al., 1998). When treating neurons chronically with TTX or bicuculine, neuronal activity is expected to adapt to the 'new' situation via HSP mechanisms and, eventually, normalize to the baseline level of activity. Supporting this, we chronically treated wild-type neurons with TTX or bicuculine and, upon removal, we observed no difference in activity in TTX-treated neurons and a decrease in activity in bicuculine-treated neurons (**Figure 14C**). Interestingly, APP/PS1 neurons were unable to adapt to the chronic administration of TTX or bicuculine and showed decreased and increased neuronal activity, respectively, when TTX or bicuculine was removed after 48 hours (**Figure 14D**). This suggests that APP/PS1 neurons have a reduced ability to adapt to changes in neuronal activity.

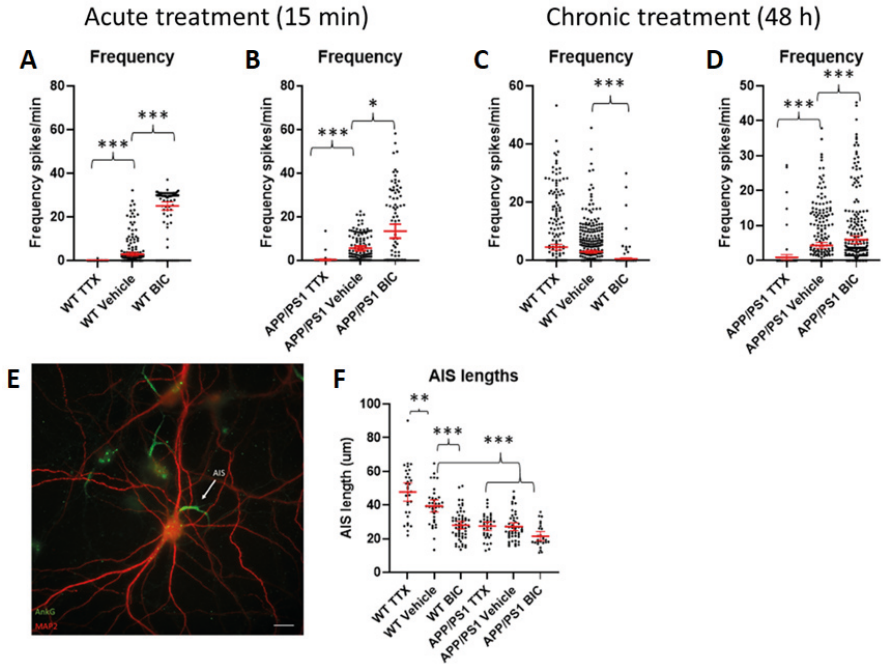


Figure 14: APP/PS1 neurons are less able to adapt to changes in neuronal activity. A-B. Quantification of spike frequency via Ca^{2+} imaging in wild-type (**A**) and APP/PS1 (**B**) primary neuronal cultures acutely treated with TTX, bicuculine (BIC) or vehicle control for 15 min. **C-D.** Quantification of spike frequency in wild-type (**C**) and APP/PS1 (**D**) primary neuronal cultures chronically treated with TTX, BIC or vehicle control for 48 h. Scale bar represents 20 µm. **E.** Representative fluorescence image of a wild-type neuron labeled for ankyrin-G (green) and MAP2 (red). The white arrow indicates the axon initial segment (AIS). **F.** Quantification of AIS length (in µm), detected by ankyrin-G labeling, in wild-type and APP/PS1 neuronal cultures treated with TTX, BIC or vehicle control for 48 h. Data are shown as mean ± 95% confidence interval. . * p value < 0.05, ** p value < 0.01, *** p value < 0.001. Figure is adapted from paper III.

To further test whether the inability of APP/PS1 neurons to adapt to changes in neuronal activity was caused by impaired HSP mechanisms, we measured the length of the axon initial segment (AIS). The AIS is known to be involved in the regulation of HSP by modulating the intrinsic neuronal output (Grubb & Burrone, 2010). Changes in the AIS reflects the ability of neurons to adapt to changes in activity by modifying its intrinsic excitability. To visualize the AIS, we labeled for ankyrin-G. As expected, in wild-type neuronal cultures, long-term treatment with TTX or bicuculine resulted in significant changes in AIS length (**Figure 14E-F**). However, in APP/PS1 neuronal cultures, neither TTX nor bicuculine treatment induced modifications in AIS length, further supporting the inability of APP/PS1 neurons to adapt to changes in neuronal activity.

ApoE3, but not ApoE4, rescues APP/PS1-induced hyperexcitability in neurons (Paper I)

As shown in **Paper III**, AD transgenic APP/PS1 neurons were hyperactive in culture compared to wild-type neurons (**Figure 13**). Since ApoE could affect neuronal activity by itself (**Figure 9**), we wanted to investigate whether different ApoE isoforms influence neuronal activity differently in AD transgenic neurons. We found that APP/PS1 neurons, compared to wild-type neurons, remain hyperactive when cultured in ApoE KO and ApoE4 astrocyte conditioned media as we observed increased spike frequency, a reduced proportion of independent spikes and increased amplitude of spikes (**Figure 15A-C**). Interestingly, culturing APP/PS1 neurons in ApoE3 astrocyte conditioned media did not significantly alter neuronal spike frequency, number of independent spikes nor spike amplitude compared to wild-type neurons (**Figure 15A-C**), suggesting that ApoE3 ameliorates the hyperactivity in APP/PS1 neurons.

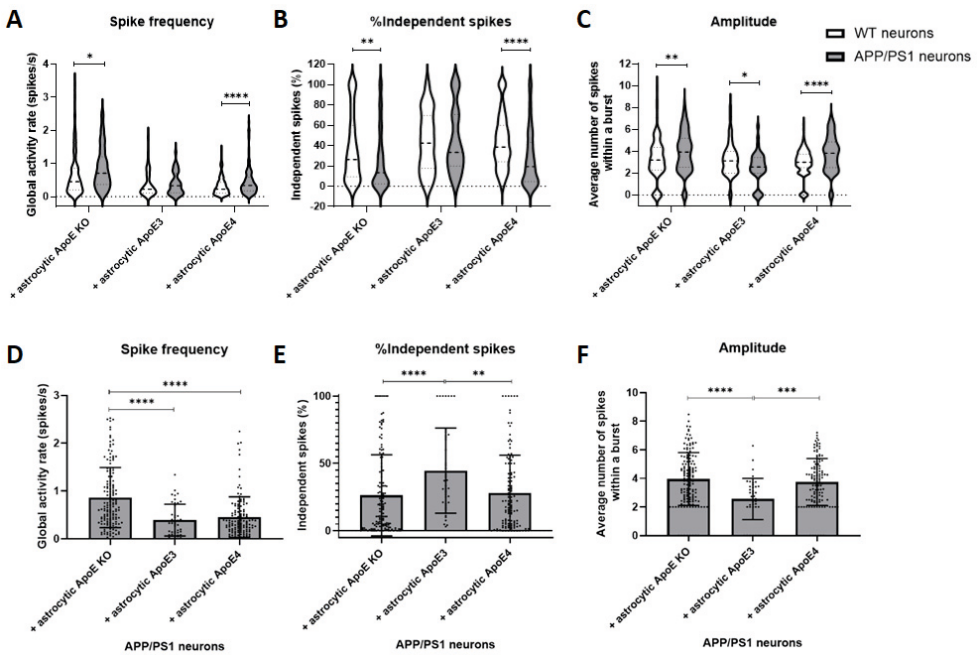


Figure 15: Astrocyte-secreted ApoE3 ameliorates neuronal hyperexcitability in AD transgenic APP/PS1 neurons. A-C. Quantification of spike frequency (**A**), percentage of independent spikes (**B**) and amplitude (**C**) in wild-type (white) and APP/PS1 (grey) neuronal cultures treated with ApoE KO, ApoE3 or ApoE4 astrocyte conditioned media for 24 hours. Data was obtained by live-cell Ca^{2+} imaging. Data are expressed as violin plots. **D-F.** Quantification of spike frequency (**D**), percentage of independent spikes (**E**), and amplitude (**F**) in APP/PS1 primary neuronal cultures treated for 24 hours with conditioned media from ApoE KO, ApoE3 or ApoE4 primary astrocyte cultures. Data are expressed as mean ± SD. * p value < 0.05; ** p value < 0.01; *** p value < 0.001; **** p value < 0.0001. Figure is adapted from paper I.

Further investigation into the effects of different ApoE isoforms on neuronal activity in an AD context, showed that, in APP/PS1 neuronal cultures, the absence of ApoE and the presence of ApoE4 affect neuronal activity in a similar way (**Figure 15D-F**). Interestingly, ApoE3 influences neuronal activity in APP/PS1 neurons differently compared to the other conditions, leading to a reduction in spike frequency, amplitude and coordinated activity in the form of increased independent spikes (**Figure 15D-F**). These findings indicate that ApoE3 differentially affects neuronal activity in APP/PS1 neurons compared to ApoE4 and no ApoE conditions and that ApoE4 affects neuronal activity in APP/PS1 neurons in a similar manner to the complete absence of ApoE.

ApoE subcellularly intersects with Aβ/β-CTFs in the endosome-lysosome system (Paper IV)

Endosomal alterations are among the earliest changes seen in AD, implicating endosomes as important cellular sites in early AD pathology. ApoE has been noted as being in the endosome-lysosome system (DeKroon & Armati, 2001; Li et al., 2012; Ljungberg et al., 2003). However, these studies were performed using recombinant human ApoE produced by E. coli, and recombinant ApoE is generally considered to be unlipidated (Monteilhet et al., 1993). In the brain, ApoE is predominantly produced by astrocytes, and therefore in **Paper IV**, we studied the subcellular localization of ApoE3 and ApoE4 produced by astrocytes in neurons and neuron-like N2a cells. We found that internalized astrocyte-derived ApoE co-localizes with LAMP1-positive late endosomes/lysosomes in N2a cells 4 hours after treatment (**Figure 16A**). We further studied the endosomal localization of ApoE in primary neuron cultures and observed that a small subset of ApoE puncta also co-localized with LAMP1-positive vesicles in neurites (**Figure 16B**). However, it is important to note that the majority of the ApoE detected in neurites was negative for late endosome/lysosome markers, suggesting that further research on the subcellular localization of ApoE in neurons is required.

ApoE has been shown to, at least partially, localize within the endosome-lysosome system. Likewise, a defined endosomal localization has been described for Aβ in neurons (Rajendran et al., 2006; Takahashi et al., 2002). While it has been known that neurons burdened with high levels of intraneuronal Aβ also label positive for ApoE (Gouras et al., 2000), it remains unclear whether ApoE and Aβ intersect at a subcellular level. To study this, N2a cells expressing human APP with the Swedish mutation (N2a APP_{Swe}) were treated with astrocytic ApoE3 or ApoE4 for 4 hours. There, we found that ApoE co-localized with 82e1, an antibody specific for the N-terminus of APP-CTF and Aβ (**Figure 17A**).

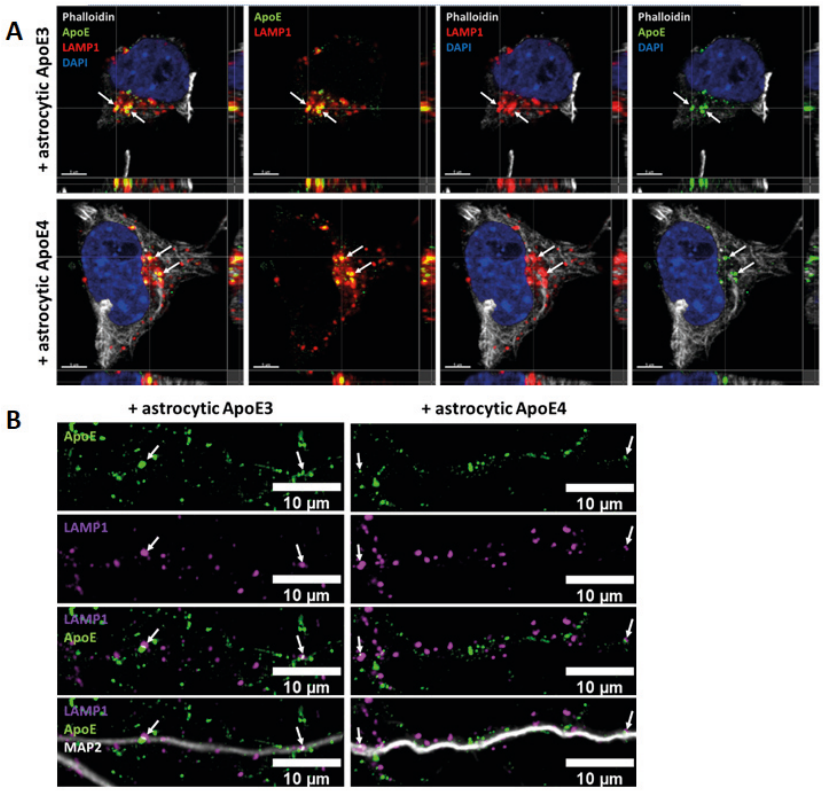


Figure 16: Astrocyte-derived ApoE3 and ApoE4 localize to late endosomes/lysosomes in N2a cells and to a lesser extent in neurons. A. Representative orthogonal confocal images of N2a cells treated with ApoE3 or ApoE4 astrocyte conditioned media for 4 hours. White arrows indicate co-localization between ApoE (green) and late endosomal/lysosomal marker LAMP1 (red). Scale bar represents 5 µm. **B.** Representative confocal images of APP/PS1 neurites after treatment with ApoE3 or ApoE4 astrocyte conditioned media for 4 hours. Primary neurons were labeled for MAP2 (grey), ApoE (green) and LAMP1 (magenta). Arrows indicate co-localization between ApoE and LAMP1 puncta. Scale bar represents 10 µm. Figure is adapted from paper IV.

We showed that internalized ApoE predominantly localizes to LAMP1-positive late endosomes/lysosomes (**Figure 16**). We, therefore, hypothesized that the potential intracellular site where ApoE and Aβ/APP-CTF intersect is late endosomes/lysosomes. In line with this hypothesis and the previous literature (Willen et al., 2017), we observed that 82e1-positive APP metabolites APP-CTF and Aβ also co-localized with LAMP1-positive vesicles in N2a APP_{Swe} cells (**Figure 17B**).

We then tested whether ApoE and Aβ intracellularly intersect in APP/PS1 primary neurons and treated them with conditioned media from ApoE3 and ApoE4 primary astrocytes for 4 hours. Like in N2a APP_{Swe} cells, co-localization between ApoE and antibody 82e1-positive APP-CTF/Aβ was observed in our APP/PS1 primary neuron cultures (**Figure 17C**). Together, these findings help to uncover a potential neuronal

intersection between ApoE and Aβ and its subcellular localization, which may help explain the role of ApoE in AD.

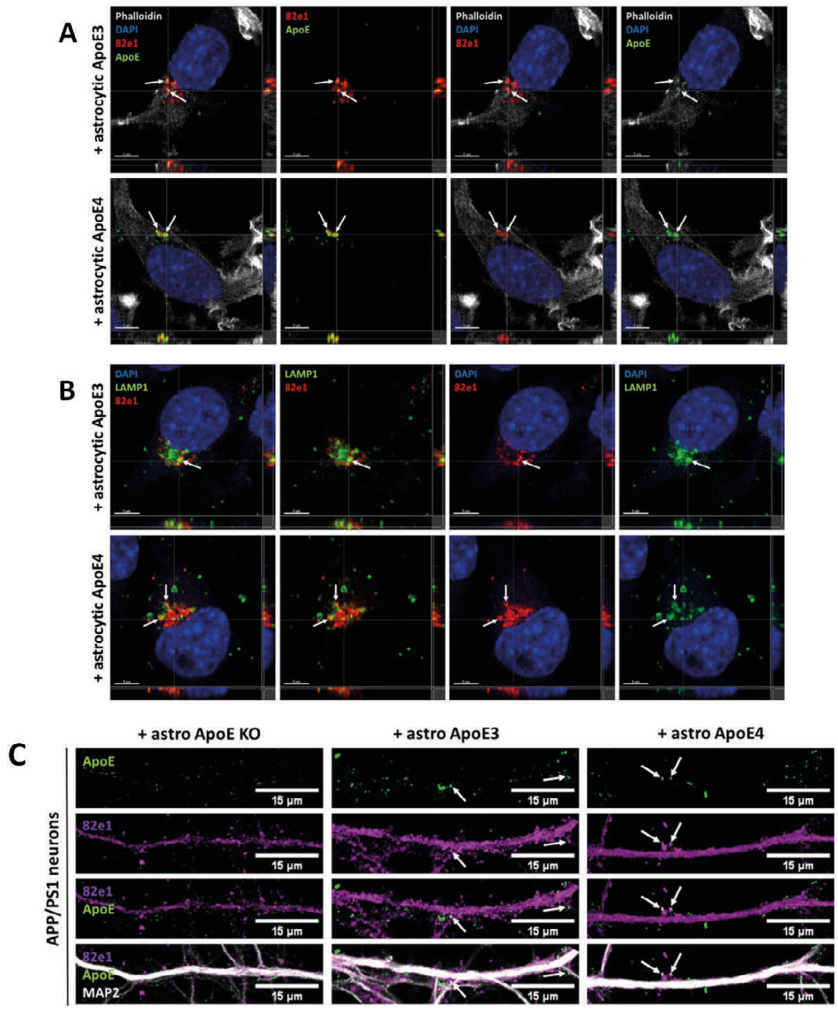


Figure 17: Astrocyte-derived ApoE co-localizes with APP metabolites APP CTF and/or Aβ in N2a cells and neurons. A. Representative confocal orthogonal images of N2a APP_{Swe} cells treated with astrocyte-derived ApoE3 or ApoE4 for 4 hours. Overlap between ApoE (green) and 82e1-positive puncta is pointed out by white arrows. Scale bar represents 5 µm. **B.** Representative orthogonal confocal images of N2a APP_{Swe} cells cultured in ApoE3 or ApoE4 astrocyte conditioned media for 4 hours. White arrows indicate co-localization between late endosomal/lysosomal marker LAMP1 (green) and 82e1-positive APP metabolites (red). Scale bar represents 5 µm. **C**. Representative confocal images of APP/PS1 neurites after treating with ApoE KO, ApoE3 or ApoE4 astrocyte conditioned media for 4 hours. Neurons were labeled for ApoE (green), 82e1 (magenta) and MAP2 (grey). White arrows highlight the co-localization of ApoE with 82e1. Scale bar represents 15 µm. Figure is adapted from paper IV.

Discussion and concluding remarks

Already in 1993, ApoE4 was identified as the major genetic risk factor for AD (Corder et al., 1993). However, to date, the underlying mechanisms causing the increased risk on AD pathology by ApoE4 are still poorly understood. The focus of this thesis was to investigate the involvement of ApoE4 in early cellular alterations linked to AD, particularly synaptic dysfunction, endosomal alterations and intraneuronal Aβ accumulation.

Synaptic terminals are considered important sites in early AD. Aβ was shown to accumulate intracellularly at synaptic terminals (Koffie et al., 2009; Takahashi et al., 2002) and loss of synaptic terminals correlates best with cognitive decline in AD (DeKosky & Scheff, 1990; Terry et al., 1991). In **Paper I**, we demonstrated that ApoE targets synaptic terminals in culture, both after addition of astrocyte-derived ApoE and when neurons exogenously express ApoE. The synaptic appearance of ApoE is supported by previous research showing ApoE and pre-synaptic marker synapsin-1 co-localization by array tomography (Koffie et al., 2012) and by the detection of ApoE in synaptosomes from human brains (Bilousova et al., 2019).

In **Paper I**, we also demonstrated that ApoE isoforms differentially affect neuronal activity in culture. Previous literature has shown that ApoE4 induced abnormal neuronal activity, mainly in the form of neuronal hyperactivity (Filippini et al., 2009; Klein et al., 2014; Nuriel et al., 2017a). We found that the effects of ApoE on spontaneous neuronal activity is influenced by the cellular source of ApoE. The majority of ApoE in the brain is produced by astrocytes. In accordance with previous findings in brain studies (Nuriel et al., 2017a), we demonstrated that astrocyte-derived ApoE4 induces increased spontaneous neuronal activity in culture compared to ApoE3 or the absence of ApoE. Remarkably, the increased neuronal activity induced by astrocyte-derived ApoE4 appears to be only transient, as it was only observed after 1 hour, but not 24 hours treatment. ApoE was still detectable in neuronal media after 24 hours, suggesting that the loss of ApoE4-induced effects on neuronal activity after 24 hours was not caused by lack of ApoE4 in the media. A possible explanation for the acute, but not chronic effects of astrocytic ApoE4 on increasing neuronal activity might be the ability of neurons to adapt to changes in neuronal activity and their ability to maintain relatively constant activity levels in the long-term via HSP. The ApoE4-induced increased neuronal activity might thereby be set back to baseline after 24 hours, preventing chronic abnormal neuronal activity after ApoE4 treatment.

Acute treatment with astrocytic ApoE4 caused increased neuronal activity in culture. According to Nuriel at al. (2017a), a loss of inhibitory tone could be the reason of ApoE4-induced neuronal hyperexcitability seen *in vivo* in ApoE4 mice. Even earlier, ApoE4 mice were shown to have inhibitory GABAergic interneuron loss and memory deficits with age (Andrews-Zwilling et al., 2010; Li G. et al., 2009). Interestingly, a specific deletion of ApoE in neurons was able to rescue the ApoE4-induced GABAergic cell loss (Knoferle et al., 2014). In contrast, deletion of ApoE in astrocytes had no effect on GABAergic neuron loss, suggesting that a loss in inhibitory tone in the form of losing GABAerigic inhibitory neurons might be specifically caused by neuron-derived ApoE. Surprisingly, we found that in the presence of neuron-derived ApoE, ApoE3, but not ApoE4, is associated with increased spontaneous neuronal activity in culture. This suggests that neuronal ApoE4 does not induce hyperactivity like astrocytic ApoE4 but instead decreases spontaneous neuronal activity compared to neuronal ApoE3. The ApoE4 genotype has been associated with reduced neuronal outgrowth and synaptic density (Dumanis et al., 2009; Nathan et al., 1994). In our experiments, ApoE3 and ApoE4 neurons did not differ in neuronal outgrowth, however, the synaptic density, as measured by excitatory vGlut1- and inhibitory VGAT-synaptic puncta, was significantly reduced in ApoE4 neurons. A loss in synaptic terminals could potentially explain the reduced neuronal activity observed in ApoE4 compared to ApoE3 neurons. It remains unknown whether this reduced synaptic density with ApoE4 is caused by lower synapse formation during development or increased synapse loss during aging. In summary, astrocytic and neuronal ApoE can both influence neuronal activity in culture, but affect neuronal activity differently depending on the cellular origin.

ApoE4 was shown to impair synaptic plasticity, but the underlying mechanism of ApoE4-induced synaptic impairment remains largely unknown. The synthesis of new proteins in response to synaptic activation plays an important role in synaptic plasticity (Sutton & Schuman, 2006; Sutton et al., 2004). In **Paper II**, we demonstrated that ApoE4 impairs protein translation in neurons. ApoE4 is shown to mimic the early phase of NMDA-induced translational response, however, differently from NMDA induction, the intracellular Ca^{2+} response induced by ApoE4 has a slower recovery to baseline intracellular Ca^{2+} levels. During the recovery, primary neurons treated with ApoE4 are non-responsive to NMDAR stimulation. The translational response induced by ApoE3 recovers quicker, and as a result, ApoE3 treated cells are able to induce translational inhibition upon NMDA stimulation. The precise mechanism of ApoE4-induced translational inhibition remains to be determined. ApoE signaling was previously shown to interact with NMDA receptors, for example in relation to the phosphorylation of disabled 1 (Dab1) (Hoe et al., 2005). Reelin, a protein involved in learning and memory via binding to ApoE receptors, can also modulate NMDA receptor activation and Ca^{2+} influx through Dab1 dependent mechanisms (Chen et al., 2005). ApoE4 impaired the receptor recycling of both Reelin/ApoE receptor ApoER2 and NMDA receptors,

causing a reduced expression of ApoER2 and NMDA receptors on the cell surface (Chen et al., 2010). In addition, because of ApoE4's molten-globule state in the acidic environment of trapped endosomes, ApoER2 receptors can cluster and constantly phosphorylate Dab1, rather than dissociate from receptors for recycling to the cell surface (Xian et al., 2018). The constant activation of Dab1 in combination with reduced expression of ApoER2 and NMDA receptors on the cell membrane induced by ApoE4 could potentially explain the reduced responsiveness to NMDA activation and increased Ca^{2+} influx in ApoE4 treated neurons.

AD is associated with aberrant neuronal network dysfunction. MCI and AD patients who had epileptiform activity present with stronger cognitive decline than patients with no epilepsy (Vossel et al., 2013). Additionally, many AD transgenic mouse models show epileptic seizures and hyperactivity (Minkeviciene et al., 2009; Palop et al., 2007). In **Paper III**, we showed that APP/PS1 primary neurons are hyperactive in culture compared to wild-type neurons. Overexpression of APP and Aβ, independently from each other, increase spontaneous neuronal firing in our cultures, indicating that both APP and Aβ can separately induce neuronal hyperactivity. Interestingly, increased spontaneous neuronal activity in APP/PS1 is specifically seen in CAMKII-positive excitatory neurons, and not in inhibitory neurons. The proportion of excitatory neurons and synapses in our cultures did not significantly differ between wild-type and APP/PS1 cultures, suggesting that the ratio excitatory-to-inhibitory neurons is not impaired in APP/PS1 neurons. Interestingly, we demonstrated in **Paper I** that addition of astrocyte-derived ApoE3, but not ApoE4, can rescue increased neuronal activity in APP/PS1 neurons, suggesting that ApoE3 potentially can ameliorate aberrant neuronal activity in AD conditions.

The precise mechanism of APP/PS1-induced neuronal hyperactivity has been poorly understood for many years. We discovered a dysfunction in HSP in APP/PS1 neurons as a possible mechanism causing abnormal neuronal activity in AD-like conditions. HSP is an important mechanism to respond to changes in neuronal activity and to restore baseline activity (Turrigiano, 2012; Turrigiano, 2008). In wild-type conditions, we demonstrated that, as expected, acute TTX and bicuculine treatment induces decreased and increased activity, respectively, in neurons. Then in conditions employed to induce HSP, wild-type neurons adapt to the 48 hours of TTX- and bicuculine treatment and set their activity level back to a baseline level like in non-treated controls. Remarkably however, we see that APP/PS1 neurons are unable to adapt to a chronically TTX-induced decrease and bicuculine-induced increase in activity. This indicates an impaired HSP adaptation of APP/PS1 neurons to changes in neuronal activity. The AIS is a site involved in initiation action potentials and its length and position can influence HSP by fine tuning intrinsic neuronal excitability (Grubb & Burrone, 2010; Wefelmeyer et al., 2016). Our work shows that while wild-type neurons adjust their AIS after altered neuronal activity induced by TTX and bicucline, APP/PS1 neurons were unable to adjust the AIS

length after 48 hours of TTX or bicuculine treatment, suggesting impaired homeostatic intrinsic plasticity in APP/PS1 neurons. Consistent with our findings, Aβ was shown to be involved in HSP and can lead to an overcompensation of homeostatic scaling (Galanis et al., 2021; Gilbert et al., 2016), supporting our finding of an aberrant HSP response in the presence of Aβ.

Like synaptic terminals, the endosome-lysosome system is a site affected early in AD. Internalized recombinant ApoE was previously shown to be present within the endosomal system in early endosomes, late endosomes and lysosomes (DeKroon & Armati, 2001; Li et al., 2012; Ljungberg et al., 2003). In **Paper IV**, we demonstrated that astrocyte-derived ApoE also localizes to late endosomes and/or lysosomes in neuron-like N2a cells and to a lesser extent in primary neurons. In this thesis, we found no difference in the endosomal localization of ApoE3 and ApoE4. Previous studies on the endosomal trafficking of ApoE were also unable to define a consistent difference in intracellular trafficking of different ApoE isoforms, as previous studies showed conflicting findings by indicating either increased (DeKroon & Armati, 2001), decreased (Li et al., 2012) or no difference (Ljungberg et al., 2003) in the late endosomal/lysosomal presence of ApoE4 compared to ApoE3. The conflicting data on ApoE isoforms might be caused by the fact that ApoE traffics differently in different cell types like we see in N2a cells and neurons.

The endosomal system is highly associated with early AD as it is a site involved in Aβ production and intraneuronal accumulation. We demonstrated that added astrocyte-derived ApoE intersects subcellularly with endogenous APP cleavage products APP-CTF and/or Aβ in N2a cells and primary neurons. The intersection of ApoE and APP-CTF/Aβ in N2a cells most likely occurs in late endosomes/lysosomes. Additional research is necessary to further understand the physiological and potential pathological role of the subcellular intersection of ApoE and APP-CTF/Aβ. He et al. (2007) supports that ApoE plays a role in APP and BACE1 internalization and subsequent intraneuronal Aβ production, which would suggest ApoE and Aβ might intersect during Aβ generation. Furthermore, in post-mortem studies, neurons that contained prominent intraneuronal Aβ accumulation also showed evident ApoE labeling (Gouras et al., 2000). Accumulated Aβ detected with APP/Aβ antibody 4G8 was even detected in the same intracellular granules as ApoE (LaFerla et al., 1997), suggesting ApoE might intersect with accumulated Aβ. Our work and previous data support a neuronal intersection of ApoE and Aβ, possibly during Aβ production and/or accumulation.

In summary, in the studies that are part of this thesis, we have investigated early processes linked to AD, like endosomal alterations, synaptic changes and intraneuronal Aβ accumulation, with a focus on the major AD risk factor ApoE4. We demonstrated that ApoE localizes in cellular sites that are affected early in AD, including synapses and endosomes. Additionally, we showed that ApoE can alter neuronal activity and global protein translation, depending on the ApoE isoform. We also showed that AD transgenic APP/PS1 neurons are hyperactive in culture,

potentially caused by impaired HSP, which can be prevented by adding astrocytic ApoE3. Lastly, we demonstrated that ApoE intersects with APP-CTF and/or Aβ, presumably in the endosomal system. We hope that our work contributes to the better understanding of AD, particularly on how ApoE4 impacts AD processes. Future studies on early AD biological mechanisms and how ApoE4 impacts these will be important to increase our knowledge about AD pathogenesis and could hopefully open up ways to treat or prevent AD in the future.

Future perspectives

As part of this thesis, we tried to elucidate the role of ApoE, in particular ApoE4, in early stages of AD, with the focus on cellular alterations related to synapses, endosomes and intraneuronal Aβ. Although our research contributes to the better understanding of ApoE in normal and AD biology, many questions remain unanswered. In this section, potential future research directions are discussed based on the research performed in this thesis.

ApoE has been shown to be expressed by many cell types in the brain. At this moment it remains unknown which cellular source of ApoE is the most critical for AD. In this thesis, we studied the effect of astrocytic and neuronal ApoE on neuronal activity. Recently, microglial ApoE has become more emphasized in AD (Keren-Shaul et al., 2017; Krasemann et al., 2017). Therefore, it will be important to also study the effects of microglial ApoE on early cellular changes in AD, including neuronal activity. A recent paper showed microglial ApoE significantly increases plaque volume, but not number, suggesting microglial ApoE is only involved at later disease stages in plaque homeostasis and not formation (Henningfield et al., 2021). It remains to be determined whether microglial ApoE affect processes linked to early AD. Additional studies could help us to better understand the role of different cellular sources of ApoE on early alterations in AD, such as synaptic activity.

In humans, ApoE occurs as three major isoforms. In this thesis, only the effects of ApoE3 and ApoE4 on early AD changes were studied. ApoE2 has been shown to be protective against AD, and therefore it would be of high interest to also study ApoE2 in the context of early neuronal changes linked to AD. Additionally, recently a mutation in ApoE3, called the ApoE3 Christchurch mutation, was shown to protect against AD (Arboleda-Velasquez et al., 2019). Studying ApoE2 and ApoE3-Christchurch could potentially help to identifying future therapeutic targets against AD.

The main function of ApoE is transporting lipids and therefore lipids may play a crucial role in the normal and AD-related function of ApoE. In this thesis, astrocyte-conditioned media from different ApoE isoform conditions were used as a way to study differences in ApoE isoforms. Since we did not characterize the lipid and protein content of ApoE KO, ApoE3 and ApoE4 astrocyte conditioned media, it remains to be determined whether the observed differences in ApoE3 and ApoE4

were directly caused by ApoE isoform differences or indirectly via ApoE-related lipid changes. ApoE4 was shown to reduce cholesterol efflux in primary astrocytes, resulting in less cholesterol in astrocytic media (Rawat et al., 2019), suggesting that the observed changes in neurons induced by different ApoE isoforms might be caused by an altered lipid composition related to ApoE4. Studying the lipid and protein content in ApoE KO, ApoE3 and ApoE4 astrocyte media could provide insights in the differences we observe between ApoE3 and ApoE4. In addition, by using astrocyte conditioned media, only the indirect interaction between astrocytes and neurons is studied. Future experiments using a neuron-astrocyte co-culture system could help us to better understand the role of astrocytic ApoE on neurons in a more direct approach.

The further investigation of the role of ApoE at cellular sites affected in early AD, including synaptic terminals and endosomes, are of high interest to better understand early changes in AD in relation to its major genetic risk factor. Previous studies reported a synaptic localization of ApoE (Bilousova et al., 2019; Koffie et al., 2012), and we now showed that added astrocyte-derived ApoE targets synaptic terminals in culture. However, the precise pre- and/or post-synaptic localization of ApoE remains unknown. The use of more advanced imaging techniques, such as electron microscopy and super-resolution microscopy will be required to better define the exact synaptic location of ApoE.

In N2a cells, ApoE was shown to clearly co-localize with late endosomal and lysosomal marker LAMP1, suggesting an endosomal localization of ApoE. Remarkably, in primary neurons only a small subset of ApoE co-localized with LAMP1-positive puncta, remaining an unknown subcellular pool of ApoE in neurons. Additional studies will be required to better define the neuronal localization of ApoE. ApoE showed a vesicle-like pattern in neurons, and therefore additional experiments studying the cellular localization of ApoE in early endosomes, recycling endosomes and autophagosomes would be relevant to better understand the neuronal subcellular trafficking of ApoE. In addition, live-cell microscopy using fluorescently-tagged ApoE could provide additional insights into the cellular trafficking of ApoE at different time points after ApoE addition.

Interestingly, in this thesis we demonstrated that ApoE and APP metabolites APP-CTF/Aβ co-localize at a subcellular level in N2a cells and primary neurons. In addition, we showed that added ApoE and Aβ both target synaptic terminals. Previous studies have described ApoE and intraneuronal Aβ at the same neurons and synapses (Bilousova et al., 2019; Gouras et al., 2000; Koffie et al., 2012), however the subcellular intersection between ApoE and Aβ remains poorly defined. It also remains unclear whether the neuronal intersection of ApoE and Aβ will affect AD pathogenesis. Additional studies on the cellular localization and interplay between ApoE and Aβ, in particularly in AD models, could potentially help us to better understand the early link between ApoE and Aβ pathology in AD.

In **Paper II**, we demonstrated that ApoE4 impairs global protein synthesis. Additional studies on how ApoE4-induced protein translation inhibition influences synaptic activity and behavior in mice would be of high importance to better understand the relevance of the effects of ApoE and protein translation in relation to synaptic and memory deficits. Additionally, studying the effect of ApoE4 on global translation in an AD model could help us better understand the underlying mechanisms of ApoE4, synaptic regulation and Aβ in an AD context. Interestingly, although FAD was previously linked to an altered protein translation response (Cefaliello et al., 2020; Elder et al., 2021), it remains unclear how APP and/or Aβ influence global protein translation. Therefore, studying the effect of APP or Aβ on protein translation related to synaptic activity could potentially help to better understand the role of altered protein translation in early AD.

We showed a potential role of aberrant HSP regulation in neuronal hyperactivity observed in APP/PS1 neurons. Interestingly, in **Paper I** we also observed an ApoE-isoform dependent effect on neuronal activity in APP/PS1 neuron cultures. Therefore, additional studies on how ApoE isoforms affect HSP could be of high relevance in the better understanding of ApoE in AD. Future experiments on the role of ApoE3 and ApoE4 in HSP will potentially provide new insights in how ApoE3 reduces neuronal hyperactivity in AD.

On a therapeutic level, the most recent FDA-approved AD drug Aducanumab is shown to have specific severe side-effects called amyloid-related imaging abnormality (ARIA)-edema, which is predominantly observed in ApoE4 carriers (Sevigny et al., 2016). It remains unknown why the severe ARIA side-effects particularly manifest in ApoE4 carriers after treatment. Therefore, future studies on the exact mechanism of ApoE4-related brain inflammation and swelling in response to Aβ antibody treatment, like Aducanumab, are required to provide better and optimize future therapies for ApoE4 carriers.

One of the biggest questions that remains is how early cellular alterations in AD are linked and in which sequence they occur. The link between synaptic impairment, neuronal hyperexcitability, endosomal dysfunction and intraneuronal Aβ production and accumulation remains poorly defined. Studying and carefully assessing the cellular changes in AD mouse models and MCI and Down syndrome human post-mortem brains could potentially contribute to the better understanding of all cellular changes occurring in early AD changes, define which cellular pathological event happens first in AD and help to therapeutically intervene at early stages of AD before clinical symptoms appear.

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References

2021 Alzheimer's disease facts and figures. (2021). *Alzheimers Dement, 17*(3), 327-406. doi:10.1002/alz.12328

Acosta-Baena, N., Sepulveda-Falla, D., Lopera-Gomez, C. M., Jaramillo-Elorza, M. C., Moreno, S., Aguirre-Acevedo, D. C., . . . Lopera, F. (2011). Pre-dementia clinical stages in presenilin 1 E280A familial early-onset Alzheimer's disease: a retrospective cohort study. *Lancet Neurol, 10*(3), 213-220. doi:10.1016/S1474-4422(10)70323-9

Albert, M. S., DeKosky, S. T., Dickson, D., Dubois, B., Feldman, H. H., Fox, N. C., . . . Phelps, C. H. (2011). The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement, 7*(3), 270-279. doi:10.1016/j.jalz.2011.03.008

Alzheimer, A. (1911). über eigenartige Krankheitsfälle des späteren Alters. *Zeitschrift für die gesamte Neurologie und Psychiatrie, 4*(1), 356. doi:10.1007/BF02866241

Alzheimer, A., Stelzmann, R. A., Schnitzlein, H. N., & 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

Andrews-Zwilling, Y., Bien-Ly, N., Xu, Q., Li, G., Bernardo, A., Yoon, S. Y., . . . Huang, Y. (2010). Apolipoprotein E4 causes age- and Tau-dependent impairment of GABAergic interneurons, leading to learning and memory deficits in mice. *J Neurosci, 30*(41), 13707-13717. doi:10.1523/JNEUROSCI.4040-10.2010

Angulo, S. L., Orman, R., Neymotin, S. A., Liu, L., Buitrago, L., Cepeda-Prado, E., . . . Moreno, H. (2017). Tau and amyloid-related pathologies in the entorhinal cortex have divergent effects in the hippocampal circuit. *Neurobiol Dis, 108*, 261-276. doi:10.1016/j.nbd.2017.08.015

Arboleda-Velasquez, J. F., Lopera, F., O'Hare, M., Delgado-Tirado, S., Marino, C., Chmielewska, N., . . . Quiroz, Y. T. (2019). Resistance to autosomal dominant Alzheimer's disease in an APOE3 Christchurch homozygote: a case report. *Nat Med, 25*(11), 1680-1683. doi:10.1038/s41591-019-0611-3

Arriagada, P. V., Growdon, J. H., Hedley-Whyte, E. T., & Hyman, B. T. (1992). Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology, 42*(3 Pt 1), 631-639. doi:10.1212/wnl.42.3.631

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

Bales, K. R., Verina, T., Dodel, R. C., Du, Y., Altstiel, L., Bender, M., . . . Paul, S. M. (1997). Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat Genet, 17*(3), 263-264. doi:10.1038/ng1197-263

Bancher, C., Brunner, C., Lassmann, H., Budka, H., Jellinger, K., Wiche, G., . . . Wisniewski, H. M. (1989). Accumulation of abnormally phosphorylated tau precedes the formation of neurofibrillary tangles in Alzheimer's disease. *Brain Res, 477*(1-2), 90-99. doi:10.1016/0006-8993(89)91396-6

Belloy, M. E., Napolioni, V., & Greicius, M. D. (2019). A Quarter Century of APOE and Alzheimer's Disease: Progress to Date and the Path Forward. *Neuron, 101*(5), 820-838. doi:10.1016/j.neuron.2019.01.056

Bierer, L. M., Hof, P. R., Purohit, D. P., Carlin, L., Schmeidler, J., Davis, K. L., & Perl, D. P. (1995). Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. *Arch Neurol, 52*(1), 81-88. doi:10.1001/archneur.1995.00540250089017

Bilousova, T., Melnik, M., Miyoshi, E., Gonzalez, B. L., Poon, W. W., Vinters, H. V., . . . Gylys, K. H. (2019). Apolipoprotein E/Amyloid-beta Complex Accumulates in Alzheimer Disease Cortical Synapses via Apolipoprotein E Receptors and Is Enhanced by APOE4. *Am J Pathol, 189*(8), 1621-1636. doi:10.1016/j.ajpath.2019.04.010

Bjorkhem, I., & Meaney, S. (2004). Brain cholesterol: long secret life behind a barrier. *Arterioscler Thromb Vasc Biol, 24*(5), 806-815. doi:10.1161/01.ATV.0000120374.59826.1b

Boschert, U., Merlo-Pich, E., Higgins, G., Roses, A. D., & Catsicas, S. (1999). Apolipoprotein E expression by neurons surviving excitotoxic stress. *Neurobiol Dis, 6*(6), 508-514. doi:10.1006/nbdi.1999.0251

Bour, A., Grootendorst, J., Vogel, E., Kelche, C., Dodart, J. C., Bales, K., . . . Mathis, C. (2008). Middle-aged human apoE4 targeted-replacement mice show retention deficits on a wide range of spatial memory tasks. *Behav Brain Res, 193*(2), 174-182. doi:10.1016/j.bbr.2008.05.008

Boyles, J. K., Pitas, R. E., Wilson, E., Mahley, R. W., & Taylor, J. M. (1985). Apolipoprotein E associated with astrocytic glia of the central nervous system and with nonmyelinating glia of the peripheral nervous system. *J Clin Invest, 76*(4), 1501-1513. doi:10.1172/JCI112130

Braak, H., & Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol, 82*(4), 239-259. doi:10.1007/BF00308809

Brecht, W. J., Harris, F. M., Chang, S., Tesseur, I., Yu, G. Q., Xu, Q., . . . Huang, Y. (2004). Neuron-specific apolipoprotein e4 proteolysis is associated with increased tau phosphorylation in brains of transgenic mice. *J Neurosci, 24*(10), 2527-2534. doi:10.1523/JNEUROSCI.4315-03.2004

Bright, J., Hussain, S., Dang, V., Wright, S., Cooper, B., Byun, T., . . . Griswold-Prenner, I. (2015). Human secreted tau increases amyloid-beta production. *Neurobiol Aging, 36*(2), 693-709. doi:10.1016/j.neurobiolaging.2014.09.007

Busche, M. A., Chen, X., Henning, H. A., Reichwald, J., Staufenbiel, M., Sakmann, B., & Konnerth, A. (2012). Critical role of soluble amyloid-beta for early hippocampal

hyperactivity in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A, 109*(22), 8740-8745. doi:10.1073/pnas.1206171109

Busche, M. A., Wegmann, S., Dujardin, S., Commins, C., Schiantarelli, J., Klickstein, N., . . . Hyman, B. T. (2019). Tau impairs neural circuits, dominating amyloid-beta effects, in Alzheimer models in vivo. *Nat Neurosci, 22*(1), 57-64. doi:10.1038/s41593-018-0289-8

Castano, E. M., Prelli, F., Wisniewski, T., Golabek, A., Kumar, R. A., Soto, C., & Frangione, B. (1995). Fibrillogenesis in Alzheimer's disease of amyloid beta peptides and apolipoprotein E. *Biochem J, 306 (Pt 2)*, 599-604. doi:10.1042/bj3060599

Cataldo, A. M., Mathews, P. M., Boiteau, A. B., Hassinger, L. C., Peterhoff, C. M., Jiang, Y., . . . Nixon, R. A. (2008). Down syndrome fibroblast model of Alzheimer-related endosome pathology: accelerated endocytosis promotes late endocytic defects. *Am J Pathol, 173*(2), 370-384. doi:10.2353/ajpath.2008.071053

Cataldo, A. M., Petanceska, S., Terio, N. B., Peterhoff, C. M., Durham, R., Mercken, M., . . . Nixon, R. A. (2004). Abeta localization in abnormal endosomes: association with earliest Abeta elevations in AD and Down syndrome. *Neurobiol Aging, 25*(10), 1263-1272. doi:10.1016/j.neurobiolaging.2004.02.027

Cataldo, A. M., Peterhoff, C. M., Troncoso, J. C., Gomez-Isla, T., Hyman, B. T., & Nixon, R. A. (2000). Endocytic pathway abnormalities precede amyloid beta deposition in sporadic Alzheimer's disease and Down syndrome: differential effects of APOE genotype and presenilin mutations. *Am J Pathol, 157*(1), 277-286.

Cefaliello, C., Penna, E., Barbato, C., Di Ruberto, G., Mollica, M. P., Trinchese, G., . . . Crispino, M. (2020). Deregulated Local Protein Synthesis in the Brain Synaptosomes of a Mouse Model for Alzheimer's Disease. *Mol Neurobiol, 57*(3), 1529-1541. doi:10.1007/s12035-019-01835-y

Chen, J., Li, Q., & Wang, J. (2011). Topology of human apolipoprotein E3 uniquely regulates its diverse biological functions. *Proc Natl Acad Sci U S A, 108*(36), 14813-14818. doi:10.1073/pnas.1106420108

Chen, X., Hui, L., Soliman, M. L., & Geiger, J. D. (2014). Altered Cholesterol Intracellular Trafficking and the Development of Pathological Hallmarks of Sporadic AD. *J Parkinsons Dis Alzheimers Dis, 1*(1).

Chen, Y., Beffert, U., Ertunc, M., Tang, T. S., Kavalali, E. T., Bezprozvanny, I., & Herz, J. (2005). Reelin modulates NMDA receptor activity in cortical neurons. *J Neurosci, 25*(36), 8209-8216. doi:10.1523/JNEUROSCI.1951-05.2005

Chen, Y., Durakoglugil, M. S., Xian, X., & Herz, J. (2010). ApoE4 reduces glutamate receptor function and synaptic plasticity by selectively impairing ApoE receptor recycling. *Proc Natl Acad Sci U S A, 107*(26), 12011-12016. doi:10.1073/pnas.0914984107

Cook, D. G., Leverenz, J. B., McMillan, P. J., Kulstad, J. J., Ericksen, S., Roth, R. A., . . . Craft, S. (2003). Reduced hippocampal insulin-degrading enzyme in late-onset Alzheimer's disease is associated with the apolipoprotein E-epsilon4 allele. *Am J Pathol, 162*(1), 313-319. doi:10.1016/s0002-9440(10)63822-9

Corder, E. H., Saunders, A. M., Risch, N. J., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Jr., . . . et al. (1994). Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet, 7*(2), 180-184. doi:10.1038/ng0694-180

Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., . . . Pericak-Vance, M. A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science, 261*(5123), 921-923. doi:10.1126/science.8346443

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

Costa-Mattioli, M., Sossin, W. S., Klann, E., & Sonenberg, N. (2009). Translational control of long-lasting synaptic plasticity and memory. *Neuron, 61*(1), 10-26. doi:10.1016/j.neuron.2008.10.055

Cuesto, G., Enriquez-Barreto, L., Carames, C., Cantarero, M., Gasull, X., Sandi, C., . . . Morales, M. (2011). Phosphoinositide-3-kinase activation controls synaptogenesis and spinogenesis in hippocampal neurons. *J Neurosci, 31*(8), 2721-2733. doi:10.1523/JNEUROSCI.4477-10.2011

Cummings, J., Lee, G., Zhong, K., Fonseca, J., & Taghva, K. (2021). Alzheimer's disease drug development pipeline: 2021. *Alzheimers Dement (N Y), 7*(1), e12179. doi:10.1002/trc2.12179

D'Andrea, M. R., Nagele, R. G., Wang, H. Y., Peterson, P. A., & Lee, D. H. (2001). Evidence that neurones accumulating amyloid can undergo lysis to form amyloid plaques in Alzheimer's disease. *Histopathology, 38*(2), 120-134. doi:10.1046/j.1365-2559.2001.01082.x

Davidsson, P., & Blennow, K. (1998). Neurochemical dissection of synaptic pathology in Alzheimer's disease. *Int Psychogeriatr, 10*(1), 11-23. doi:10.1017/s1041610298005110

Deane, R., Sagare, A., Hamm, K., Parisi, M., Lane, S., Finn, M. B., . . . Zlokovic, B. V. (2008). apoE isoform-specific disruption of amyloid beta peptide clearance from mouse brain. *J Clin Invest, 118*(12), 4002-4013. doi:10.1172/JCI36663

DeKosky, S. T., & Scheff, S. W. (1990). Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Ann Neurol, 27*(5), 457-464. doi:10.1002/ana.410270502

DeKroon, R. M., & Armati, P. J. (2001). The endosomal trafficking of apolipoprotein E3 and E4 in cultured human brain neurons and astrocytes. *Neurobiol Dis, 8*(1), 78-89. doi:10.1006/nbdi.2000.0362

DeTure, M. A., & Dickson, D. W. (2019). The neuropathological diagnosis of Alzheimer's disease. *Mol Neurodegener, 14*(1), 32. doi:10.1186/s13024-019-0333-5

Dickson, T. C., & Vickers, J. C. (2001). The morphological phenotype of beta-amyloid plaques and associated neuritic changes in Alzheimer's disease. *Neuroscience, 105*(1), 99-107. doi:10.1016/s0306-4522(01)00169-5

Ding, Q., Markesbery, W. R., Chen, Q., Li, F., & Keller, J. N. (2005). Ribosome dysfunction is an early event in Alzheimer's disease. *J Neurosci, 25*(40), 9171-9175. doi:10.1523/JNEUROSCI.3040-05.2005

Dong, L. M., & Weisgraber, K. H. (1996). Human apolipoprotein E4 domain interaction. Arginine 61 and glutamic acid 255 interact to direct the preference for very low density lipoproteins. *J Biol Chem, 271*(32), 19053-19057. doi:10.1074/jbc.271.32.19053

Dumanis, S. B., Tesoriero, J. A., Babus, L. W., Nguyen, M. T., Trotter, J. H., Ladu, M. J., . . . Hoe, H. S. (2009). ApoE4 decreases spine density and dendritic complexity in cortical neurons in vivo. *J Neurosci, 29*(48), 15317-15322. doi:10.1523/JNEUROSCI.4026-09.2009

Eitan, E., Suire, C., Zhang, S., & Mattson, M. P. (2016). Impact of lysosome status on extracellular vesicle content and release. *Ageing Res Rev, 32*, 65-74. doi:10.1016/j.arr.2016.05.001

Elder, M. K., Erdjument-Bromage, H., Oliveira, M. M., Mamcarz, M., Neubert, T. A., & Klann, E. (2021). Age-dependent shift in the de novo proteome accompanies pathogenesis in an Alzheimer's disease mouse model. *Commun Biol, 4*(1), 823. doi:10.1038/s42003-021-02324-6

Esbjorner, E. K., Chan, F., Rees, E., Erdelyi, M., Luheshi, L. M., Bertoncini, C. W., . . . Kaminski Schierle, G. S. (2014). Direct observations of amyloid beta self-assembly in live cells provide insights into differences in the kinetics of Abeta(1-40) and Abeta(1-42) aggregation. *Chem Biol, 21*(6), 732-742. doi:10.1016/j.chembiol.2014.03.014

Esch, F. S., Keim, P. S., Beattie, E. C., Blacher, R. W., Culwell, A. R., Oltersdorf, T., . . . Ward, P. J. (1990). Cleavage of amyloid beta peptide during constitutive processing of its precursor. *Science, 248*(4959), 1122-1124. doi:10.1126/science.2111583

Fagan, A. M., Watson, M., Parsadanian, M., Bales, K. R., Paul, S. M., & Holtzman, D. M. (2002). Human and murine ApoE markedly alters A beta metabolism before and after plaque formation in a mouse model of Alzheimer's disease. *Neurobiol Dis, 9*(3), 305-318. doi:10.1006/nbdi.2002.0483

Farfel, J. M., Yu, L., De Jager, P. L., Schneider, J. A., & Bennett, D. A. (2016). Association of APOE with tau-tangle pathology with and without beta-amyloid. *Neurobiol Aging, 37*, 19-25. doi:10.1016/j.neurobiolaging.2015.09.011

Farrer, L. A., Cupples, L. A., Haines, J. L., Hyman, B., Kukull, W. A., Mayeux, R., . . . van Duijn, C. M. (1997). Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA, 278*(16), 1349-1356.

Feringa, F. M., & van der Kant, R. (2021). Cholesterol and Alzheimer's Disease; From Risk Genes to Pathological Effects. *Front Aging Neurosci, 13*, 690372. doi:10.3389/fnagi.2021.690372

Fernandez-Garcia, S., Orlandi, J. G., Garcia-Diaz Barriga, G. A., Rodriguez, M. J., Masana, M., Soriano, J., & Alberch, J. (2020). Deficits in coordinated neuronal activity and network topology are striatal hallmarks in Huntington's disease. *BMC Biol, 18*(1), 58. doi:10.1186/s12915-020-00794-4

Ferreira, T. A., Blackman, A. V., Oyrer, J., Jayabal, S., Chung, A. J., Watt, A. J., . . . van Meyel, D. J. (2014). Neuronal morphometry directly from bitmap images. *Nat Methods, 11*(10), 982-984. doi:10.1038/nmeth.3125

Filippini, N., MacIntosh, B. J., Hough, M. G., Goodwin, G. M., Frisoni, G. B., Smith, S. M., . . . Mackay, C. E. (2009). Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. *Proc Natl Acad Sci U S A, 106*(17), 7209-7214. doi:10.1073/pnas.0811879106

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res, 12*(3), 189-198. doi:10.1016/0022-3956(75)90026-6

Freitas, S., Simoes, M. R., Alves, L., & Santana, I. (2013). Montreal cognitive assessment: validation study for mild cognitive impairment and Alzheimer disease. *Alzheimer Dis Assoc Disord, 27*(1), 37-43. doi:10.1097/WAD.0b013e3182420bfe

Galanis, C., Fellenz, M., Becker, D., Bold, C., Lichtenthaler, S. F., Muller, U. C., . . . Vlachos, A. (2021). Amyloid-Beta Mediates Homeostatic Synaptic Plasticity. *J Neurosci, 41*(24), 5157-5172. doi:10.1523/JNEUROSCI.1820-20.2021

Gao, S., Hendrie, H. C., Hall, K. S., & Hui, S. (1998). The relationships between age, sex, and the incidence of dementia and Alzheimer disease: a meta-analysis. *Arch Gen Psychiatry, 55*(9), 809-815. doi:10.1001/archpsyc.55.9.809

Gatz, M., Reynolds, C. A., Fratiglioni, L., Johansson, B., Mortimer, J. A., Berg, S., . . . Pedersen, N. L. (2006). Role of genes and environments for explaining Alzheimer disease. *Arch Gen Psychiatry, 63*(2), 168-174. doi:10.1001/archpsyc.63.2.168

Giau, V. V., Senanarong, V., Bagyinszky, E., An, S. S. A., & Kim, S. (2019). Analysis of 50 Neurodegenerative Genes in Clinically Diagnosed Early-Onset Alzheimer's Disease. *Int J Mol Sci, 20*(6). doi:10.3390/ijms20061514

Gilbert, J., Shu, S., Yang, X., Lu, Y., Zhu, L. Q., & Man, H. Y. (2016). beta-Amyloid triggers aberrant over-scaling of homeostatic synaptic plasticity. *Acta Neuropathol Commun, 4*(1), 131. doi:10.1186/s40478-016-0398-0

Ginsberg, S. D., Mufson, E. J., Counts, S. E., Wuu, J., Alldred, M. J., Nixon, R. A., & Che, S. (2010). Regional selectivity of rab5 and rab7 protein upregulation in mild cognitive impairment and Alzheimer's disease. *J Alzheimers Dis, 22*(2), 631-639. doi:10.3233/JAD-2010-101080

Giri, M., Zhang, M., & Lu, Y. (2016). Genes associated with Alzheimer's disease: an overview and current status. *Clin Interv Aging, 11*, 665-681. doi:10.2147/CIA.S105769

Goate, A., Chartier-Harlin, M. C., Mullan, M., Brown, J., Crawford, F., Fidani, L., . . . et al. (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature, 349*(6311), 704-706. doi:10.1038/349704a0

Gong, J. S., Kobayashi, M., Hayashi, H., Zou, K., Sawamura, N., Fujita, S. C., . . . Michikawa, M. (2002). Apolipoprotein E (ApoE) isoform-dependent lipid release from astrocytes prepared from human ApoE3 and ApoE4 knock-in mice. *J Biol Chem, 277*(33), 29919-29926. doi:10.1074/jbc.M203934200

Goritz, C., Mauch, D. H., & Pfrieger, F. W. (2005). Multiple mechanisms mediate cholesterol-induced synaptogenesis in a CNS neuron. *Mol Cell Neurosci, 29*(2), 190-201. doi:10.1016/j.mcn.2005.02.006

Gouras, G. K., Tsai, J., Naslund, J., Vincent, B., Edgar, M., Checler, F., . . . Relkin, N. R. (2000). Intraneuronal Abeta42 accumulation in human brain. *Am J Pathol, 156*(1), 15-20. doi:10.1016/s0002-9440(10)64700-1

Grubb, M. S., & Burrone, J. (2010). Activity-dependent relocation of the axon initial segment fine-tunes neuronal excitability. *Nature, 465*(7301), 1070-1074. doi:10.1038/nature09160

Haass, C., & Selkoe, D. J. (1993). Cellular processing of beta-amyloid precursor protein and the genesis of amyloid beta-peptide. *Cell, 75*(6), 1039-1042. doi:10.1016/0092-8674(93)90312-e

Harris, F. M., Brecht, W. J., Xu, Q., Tesseur, I., Kekonius, L., Wyss-Coray, T., . . . Huang, Y. (2003). Carboxyl-terminal-truncated apolipoprotein E4 causes Alzheimer's disease-like neurodegeneration and behavioral deficits in transgenic mice. *Proc Natl Acad Sci U S A, 100*(19), 10966-10971. doi:10.1073/pnas.1434398100

He, X., Cooley, K., Chung, C. H., Dashti, N., & Tang, J. (2007). Apolipoprotein receptor 2 and X11 alpha/beta mediate apolipoprotein E-induced endocytosis of amyloid-beta precursor protein and beta-secretase, leading to amyloid-beta production. *J Neurosci, 27*(15), 4052-4060. doi:10.1523/JNEUROSCI.3993-06.2007

Heeren, J., Grewal, T., Laatsch, A., Becker, N., Rinninger, F., Rye, K. A., & Beisiegel, U. (2004). Impaired recycling of apolipoprotein E4 is associated with intracellular cholesterol accumulation. *J Biol Chem, 279*(53), 55483-55492. doi:10.1074/jbc.M409324200

Heeren, J., Weber, W., & Beisiegel, U. (1999). Intracellular processing of endocytosed triglyceride-rich lipoproteins comprises both recycling and degradation. *J Cell Sci, 112 (Pt 3)*, 349-359.

Heinsinger, N. M., Gachechiladze, M. A., & Rebeck, G. W. (2016). Apolipoprotein E Genotype Affects Size of ApoE Complexes in Cerebrospinal Fluid. *J Neuropathol Exp Neurol, 75*(10), 918-924. doi:10.1093/jnen/nlw067

Heise, C., Gardoni, F., Culotta, L., di Luca, M., Verpelli, C., & Sala, C. (2014). Elongation factor-2 phosphorylation in dendrites and the regulation of dendritic mRNA translation in neurons. *Front Cell Neurosci, 8*, 35. doi:10.3389/fncel.2014.00035

Henningfield, C. M., Arreola, M. A., Soni, N., Spangenberg, E. E., & Green, K. N. (2021). Microglia-specific ApoE knock-out does not alter Alzheimer's disease plaque pathogenesis or gene expression. *Glia*. doi:10.1002/glia.24105

Hoe, H. S., Harris, D. C., & Rebeck, G. W. (2005). Multiple pathways of apolipoprotein E signaling in primary neurons. *J Neurochem, 93*(1), 145-155. doi:10.1111/j.1471-4159.2004.03007.x

Holtzman, D. M., Bales, K. R., Tenkova, T., Fagan, A. M., Parsadanian, M., Sartorius, L. J., . . . Paul, S. M. (2000). Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A, 97*(6), 2892-2897. doi:10.1073/pnas.050004797

Hu, Y. B., Dammer, E. B., Ren, R. J., & Wang, G. (2015). The endosomal-lysosomal system: from acidification and cargo sorting to neurodegeneration. *Transl Neurodegener, 4*, 18. doi:10.1186/s40035-015-0041-1

Huang, Y., Liu, X. Q., Wyss-Coray, T., Brecht, W. J., Sanan, D. A., & Mahley, R. W. (2001). Apolipoprotein E fragments present in Alzheimer's disease brains induce neurofibrillary tangle-like intracellular inclusions in neurons. *Proc Natl Acad Sci U S A, 98*(15), 8838-8843. doi:10.1073/pnas.151254698

Huang, Y. A., Zhou, B., Wernig, M., & Sudhof, T. C. (2017). ApoE2, ApoE3, and ApoE4 Differentially Stimulate APP Transcription and Abeta Secretion. *Cell, 168*(3), 427-441 e421. doi:10.1016/j.cell.2016.12.044

Huynh, T. V., Liao, F., Francis, C. M., Robinson, G. O., Serrano, J. R., Jiang, H., . . . Holtzman, D. M. (2017). Age-Dependent Effects of apoE Reduction Using Antisense Oligonucleotides in a Model of beta-amyloidosis. *Neuron, 96*(5), 1013-1023 e1014. doi:10.1016/j.neuron.2017.11.014

Ikeda, K., Akiyama, H., Arai, T., Sahara, N., Mori, H., Usami, M., . . . Takahashi, H. (1997). A subset of senile dementia with high incidence of the apolipoprotein E epsilon2 allele. *Ann Neurol, 41*(5), 693-695. doi:10.1002/ana.410410522

Ikonen, E. (2008). Cellular cholesterol trafficking and compartmentalization. *Nat Rev Mol Cell Biol, 9*(2), 125-138. doi:10.1038/nrm2336

Ioannou, M. S., Jackson, J., Sheu, S. H., Chang, C. L., Weigel, A. V., Liu, H., . . . Liu, Z. (2019). Neuron-Astrocyte Metabolic Coupling Protects against Activity-Induced Fatty Acid Toxicity. *Cell, 177*(6), 1522-1535 e1514. doi:10.1016/j.cell.2019.04.001

Iwatsubo, T., Odaka, A., Suzuki, N., Mizusawa, H., Nukina, N., & Ihara, Y. (1994). Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). *Neuron, 13*(1), 45-53. doi:10.1016/0896-6273(94)90458-8

Jarrett, J. T., Berger, E. P., & Lansbury, P. T., Jr. (1993). The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochemistry, 32*(18), 4693-4697. doi:10.1021/bi00069a001

Ji, Z. S., Miranda, R. D., Newhouse, Y. M., Weisgraber, K. H., Huang, Y., & Mahley, R. W. (2002). Apolipoprotein E4 potentiates amyloid beta peptide-induced lysosomal leakage and apoptosis in neuronal cells. *J Biol Chem, 277*(24), 21821-21828. doi:10.1074/jbc.M112109200

Ji, Z. S., Mullendorff, K., Cheng, I. H., Miranda, R. D., Huang, Y., & Mahley, R. W. (2006). Reactivity of apolipoprotein E4 and amyloid beta peptide: lysosomal stability and neurodegeneration. *J Biol Chem, 281*(5), 2683-2692. doi:10.1074/jbc.M506646200

Jiang, Y., Sato, Y., Im, E., Berg, M., Bordi, M., Darji, S., . . . Nixon, R. A. (2019). Lysosomal Dysfunction in Down Syndrome Is APP-Dependent and Mediated by APP-betaCTF (C99). *J Neurosci, 39*(27), 5255-5268. doi:10.1523/JNEUROSCI.0578-19.2019

Jin, L. W., Shie, F. S., Maezawa, I., Vincent, I., & Bird, T. (2004). Intracellular accumulation of amyloidogenic fragments of amyloid-beta precursor protein in neurons with Niemann-Pick type C defects is associated with endosomal abnormalities. *Am J Pathol, 164*(3), 975-985. doi:10.1016/s0002-9440(10)63185-9

Jones, L., Holmans, P. A., Hamshere, M. L., Harold, D., Moskvina, V., Ivanov, D., . . . Williams, J. (2010). Genetic evidence implicates the immune system and cholesterol metabolism in the aetiology of Alzheimer's disease. *PLoS One, 5*(11), e13950. doi:10.1371/journal.pone.0013950

Jovic, M., Sharma, M., Rahajeng, J., & Caplan, S. (2010). The early endosome: a busy sorting station for proteins at the crossroads. *Histol Histopathol, 25*(1), 99-112. doi:10.14670/HH-25.99

Kanekiyo, T., Xu, H., & Bu, G. (2014). ApoE and Abeta in Alzheimer's disease: accidental encounters or partners? *Neuron, 81*(4), 740-754. doi:10.1016/j.neuron.2014.01.045

Keren-Shaul, H., Spinrad, A., Weiner, A., Matcovitch-Natan, O., Dvir-Szternfeld, R., Ulland, T. K., . . . Amit, I. (2017). A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. *Cell, 169*(7), 1276-1290 e1217. doi:10.1016/j.cell.2017.05.018

Klein, R. C., Acheson, S. K., Mace, B. E., Sullivan, P. M., & Moore, S. D. (2014). Altered neurotransmission in the lateral amygdala in aged human apoE4 targeted replacement mice. *Neurobiol Aging, 35*(9), 2046-2052. doi:10.1016/j.neurobiolaging.2014.02.019

Klunk, W. E., Engler, H., Nordberg, A., Wang, Y., Blomqvist, G., Holt, D. P., . . . Langstrom, B. (2004). Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann Neurol, 55*(3), 306-319. doi:10.1002/ana.20009

Knobloch, M., Konietzko, U., Krebs, D. C., & Nitsch, R. M. (2007). Intracellular Abeta and cognitive deficits precede beta-amyloid deposition in transgenic arcAbeta mice. *Neurobiol Aging, 28*(9), 1297-1306. doi:10.1016/j.neurobiolaging.2006.06.019

Knoferle, J., Yoon, S. Y., Walker, D., Leung, L., Gillespie, A. K., Tong, L. M., . . . Huang, Y. (2014). Apolipoprotein E4 produced in GABAergic interneurons causes learning and memory deficits in mice. *J Neurosci, 34*(42), 14069-14078. doi:10.1523/JNEUROSCI.2281-14.2014

Knopman, D. S., Jones, D. T., & Greicius, M. D. (2021). Failure to demonstrate efficacy of aducanumab: An analysis of the EMERGE and ENGAGE trials as reported by Biogen, December 2019. *Alzheimers Dement, 17*(4), 696-701. doi:10.1002/alz.12213

Koffie, R. M., Hashimoto, T., Tai, H. C., Kay, K. R., Serrano-Pozo, A., Joyner, D., . . . Spires-Jones, T. L. (2012). Apolipoprotein E4 effects in Alzheimer's disease are mediated by synaptotoxic oligomeric amyloid-beta. *Brain, 135*(Pt 7), 2155-2168. doi:10.1093/brain/aws127

Koffie, R. M., Meyer-Luehmann, M., Hashimoto, T., Adams, K. W., Mielke, M. L., Garcia-Alloza, M., . . . Spires-Jones, T. L. (2009). Oligomeric amyloid beta associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. *Proc Natl Acad Sci U S A, 106*(10), 4012-4017. doi:10.1073/pnas.0811698106

Koldamova, R., Staufenbiel, M., & Lefterov, I. (2005). Lack of ABCA1 considerably decreases brain ApoE level and increases amyloid deposition in APP23 mice. *J Biol Chem, 280*(52), 43224-43235. doi:10.1074/jbc.M504513200

Krasemann, S., Madore, C., Cialic, R., Baufeld, C., Calcagno, N., El Fatimy, R., . . . Butovsky, O. (2017). The TREM2-APOE Pathway Drives the Transcriptional

Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. *Immunity, 47*(3), 566-581 e569. doi:10.1016/j.immuni.2017.08.008

Kuller, L. H., & Lopez, O. L. (2021). ENGAGE and EMERGE: Truth and consequences? *Alzheimers Dement, 17*(4), 692-695. doi:10.1002/alz.12286

Kute, P. M., Ramakrishna, S., Neelagandan, N., Chattarji, S., & Muddashetty, R. S. (2019). NMDAR mediated translation at the synapse is regulated by MOV10 and FMRP. *Mol Brain, 12*(1), 65. doi:10.1186/s13041-019-0473-0

LaFerla, F. M., Troncoso, J. C., Strickland, D. K., Kawas, C. H., & Jay, G. (1997). Neuronal cell death in Alzheimer's disease correlates with apoE uptake and intracellular Abeta stabilization. *J Clin Invest, 100*(2), 310-320. doi:10.1172/JCI119536

Lambert, J. C., Ibrahim-Verbaas, C. A., Harold, D., Naj, A. C., Sims, R., Bellenguez, C., . . . Amouyel, P. (2013). Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet, 45*(12), 1452-1458. doi:10.1038/ng.2802

Langui, D., Girardot, N., El Hachimi, K. H., Allinquant, B., Blanchard, V., Pradier, L., & Duyckaerts, C. (2004). Subcellular topography of neuronal Abeta peptide in APPxPS1 transgenic mice. *Am J Pathol, 165*(5), 1465-1477. doi:10.1016/s0002-9440(10)63405-0

Li, G., Bien-Ly, N., Andrews-Zwilling, Y., Xu, Q., Bernardo, A., Ring, K., . . . Huang, Y. (2009). GABAergic interneuron dysfunction impairs hippocampal neurogenesis in adult apolipoprotein E4 knockin mice. *Cell Stem Cell, 5*(6), 634-645. doi:10.1016/j.stem.2009.10.015

Li, J., Kanekiyo, T., Shinohara, M., Zhang, Y., LaDu, M. J., Xu, H., & Bu, G. (2012). Differential regulation of amyloid-beta endocytic trafficking and lysosomal degradation by apolipoprotein E isoforms. *J Biol Chem, 287*(53), 44593-44601. doi:10.1074/jbc.M112.420224

Li, S., Hong, S., Shepardson, N. E., Walsh, D. M., Shankar, G. M., & Selkoe, D. (2009). Soluble oligomers of amyloid Beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. *Neuron, 62*(6), 788-801. doi:10.1016/j.neuron.2009.05.012

Lin, Y. T., Seo, J., Gao, F., Feldman, H. M., Wen, H. L., Penney, J., . . . Tsai, L. H. (2018). APOE4 Causes Widespread Molecular and Cellular Alterations Associated with Alzheimer's Disease Phenotypes in Human iPSC-Derived Brain Cell Types. *Neuron, 98*(6), 1141-1154 e1147. doi:10.1016/j.neuron.2018.05.008

Lindwall, G., & Cole, R. D. (1984). Phosphorylation affects the ability of tau protein to promote microtubule assembly. *J Biol Chem, 259*(8), 5301-5305.

Liu, C. C., Liu, C. C., Kanekiyo, T., Xu, H., & Bu, G. (2013). Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol, 9*(2), 106-118. doi:10.1038/nrneurol.2012.263

Liu, C. C., Murray, M. E., Li, X., Zhao, N., Wang, N., Heckman, M. G., . . . Bu, G. (2021). APOE3-Jacksonville (V236E) variant reduces self-aggregation and risk of dementia. *Sci Transl Med, 13*(613), eabc9375. doi:10.1126/scitranslmed.abc9375

Liu, C. C., Zhao, N., Fu, Y., Wang, N., Linares, C., Tsai, C. W., & Bu, G. (2017). ApoE4 Accelerates Early Seeding of Amyloid Pathology. *Neuron, 96*(5), 1024-1032 e1023. doi:10.1016/j.neuron.2017.11.013

Liu, L., MacKenzie, K. R., Putluri, N., Maletic-Savatic, M., & Bellen, H. J. (2017). The Glia-Neuron Lactate Shuttle and Elevated ROS Promote Lipid Synthesis in Neurons and Lipid Droplet Accumulation in Glia via APOE/D. *Cell Metab, 26*(5), 719-737 e716. doi:10.1016/j.cmet.2017.08.024

Liu, M., Kuhel, D. G., Shen, L., Hui, D. Y., & Woods, S. C. (2012). Apolipoprotein E does not cross the blood-cerebrospinal fluid barrier, as revealed by an improved technique for sampling CSF from mice. *Am J Physiol Regul Integr Comp Physiol, 303*(9), R903-908. doi:10.1152/ajpregu.00219.2012

Ljungberg, M. C., Asuni, A., Pearce, J., Dayanandan, R., Marz, W., Hoffmann, M. M., . . . Lovestone, S. (2003). Apolipoprotein E (apoE) uptake and distribution in mammalian cell lines is dependent upon source of apoE and can be monitored in living cells. *Neurosci Lett, 341*(1), 69-73. doi:10.1016/s0304-3940(03)00064-8

Ljungberg, M. C., Dayanandan, R., Asuni, A., Rupniak, T. H., Anderton, B. H., & Lovestone, S. (2002). Truncated apoE forms tangle-like structures in a neuronal cell line. *Neuroreport, 13*(6), 867-870. doi:10.1097/00001756-200205070-00026

Ma, J., Yee, A., Brewer, H. B., Jr., Das, S., & Potter, H. (1994). Amyloid-associated proteins alpha 1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer beta-protein into filaments. *Nature, 372*(6501), 92-94. doi:10.1038/372092a0

Ma, Q., Zhao, Z., Sagare, A. P., Wu, Y., Wang, M., Owens, N. C., . . . Zlokovic, B. V. (2018). Blood-brain barrier-associated pericytes internalize and clear aggregated amyloid-beta42 by LRP1-dependent apolipoprotein E isoform-specific mechanism. *Mol Neurodegener, 13*(1), 57. doi:10.1186/s13024-018-0286-0

Ma, T., Trinh, M. A., Wexler, A. J., Bourbon, C., Gatti, E., Pierre, P., . . . Klann, E. (2013). Suppression of eIF2alpha kinases alleviates Alzheimer's disease-related plasticity and memory deficits. *Nat Neurosci, 16*(9), 1299-1305. doi:10.1038/nn.3486

Markus, A., Zhong, J., & Snider, W. D. (2002). Raf and akt mediate distinct aspects of sensory axon growth. *Neuron, 35*(1), 65-76. doi:10.1016/s0896-6273(02)00752-3

Mata, G., Cuesto, G., Heras, J., Morales, M., Romero, A., & Rubio, J. (2017). *SynapCountJ: A Validated Tool for Analyzing Synaptic Densities in Neurons*, Cham.

Mathias, J. L., & Burke, J. (2009). Cognitive functioning in Alzheimer's and vascular dementia: a meta-analysis. *Neuropsychology, 23*(4), 411-423. doi:10.1037/a0015384

Mattsson, N., Scholl, M., Strandberg, O., Smith, R., Palmqvist, S., Insel, P. S., . . . Hansson, O. (2017). (18)F-AV-1451 and CSF T-tau and P-tau as biomarkers in Alzheimer's disease. *EMBO Mol Med, 9*(9), 1212-1223. doi:10.15252/emmm.201707809

Mauch, D. H., Nagler, K., Schumacher, S., Goritz, C., Muller, E. C., Otto, A., & Pfrieger, F. W. (2001). CNS synaptogenesis promoted by glia-derived cholesterol. *Science, 294*(5545), 1354-1357. doi:10.1126/science.294.5545.1354

McGowan, E., Pickford, F., Kim, J., Onstead, L., Eriksen, J., Yu, C., . . . Golde, T. (2005). Abeta42 is essential for parenchymal and vascular amyloid deposition in mice. *Neuron, 47*(2), 191-199. doi:10.1016/j.neuron.2005.06.030

McKay, G. J., Silvestri, G., Chakravarthy, U., Dasari, S., Fritsche, L. G., Weber, B. H., . . . Patterson, C. C. (2011). Variations in apolipoprotein E frequency with age in a pooled analysis of a large group of older people. *Am J Epidemiol, 173*(12), 1357-1364. doi:10.1093/aje/kwr015

Medway, C. W., Abdul-Hay, S., Mims, T., Ma, L., Bisceglio, G., Zou, F., . . . Younkin, S. G. (2014). ApoE variant p.V236E is associated with markedly reduced risk of Alzheimer's disease. *Mol Neurodegener, 9*, 11. doi:10.1186/1750-1326-9-11

Meijering, E., Jacob, M., Sarria, J. C., Steiner, P., Hirling, H., & Unser, M. (2004). Design and validation of a tool for neurite tracing and analysis in fluorescence microscopy images. *Cytometry A, 58*(2), 167-176. doi:10.1002/cyto.a.20022

Miller, S. L., Fenstermacher, E., Bates, J., Blacker, D., Sperling, R. A., & Dickerson, B. C. (2008). Hippocampal activation in adults with mild cognitive impairment predicts subsequent cognitive decline. *J Neurol Neurosurg Psychiatry, 79*(6), 630-635. doi:10.1136/jnnp.2007.124149

Miners, J. S., Van Helmond, Z., Chalmers, K., Wilcock, G., Love, S., & Kehoe, P. G. (2006). Decreased expression and activity of neprilysin in Alzheimer disease are associated with cerebral amyloid angiopathy. *J Neuropathol Exp Neurol, 65*(10), 1012-1021. doi:10.1097/01.jnen.0000240463.87886.9a

Minkeviciene, R., Rheims, S., Dobszay, M. B., Zilberter, M., Hartikainen, J., Fulop, L., . . . Tanila, H. (2009). Amyloid beta-induced neuronal hyperexcitability triggers progressive epilepsy. *J Neurosci, 29*(11), 3453-3462. doi:10.1523/JNEUROSCI.5215-08.2009

Mochizuki, A., Tamaoka, A., Shimohata, A., Komatsuzaki, Y., & Shoji, S. (2000). Abeta42-positive non-pyramidal neurons around amyloid plaques in Alzheimer's disease. *Lancet, 355*(9197), 42-43. doi:10.1016/S0140-6736(99)04937-5

Moloney, C. M., Lowe, V. J., & Murray, M. E. (2021). Visualization of neurofibrillary tangle maturity in Alzheimer's disease: A clinicopathologic perspective for biomarker research. *Alzheimers Dement, 17*(9), 1554-1574. doi:10.1002/alz.12321

Monteilhet, C., Lachacinski, N., & Aggerbeck, L. P. (1993). Cytoplasmic and periplasmic production of human apolipoprotein E in Escherichia coli using natural and bacterial signal peptides. *Gene, 125*(2), 223-228. doi:10.1016/0378-1119(93)90333-x

Motter, R., Vigo-Pelfrey, C., Kholodenko, D., Barbour, R., Johnson-Wood, K., Galasko, D., . . . et al. (1995). Reduction of beta-amyloid peptide42 in the cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol, 38*(4), 643-648. doi:10.1002/ana.410380413

Mullan, M., Crawford, F., Axelman, K., Houlden, H., Lilius, L., Winblad, B., & Lannfelt, L. (1992). A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. *Nat Genet, 1*(5), 345-347. doi:10.1038/ng0892-345

Namba, Y., Tomonaga, M., Kawasaki, H., Otomo, E., & Ikeda, K. (1991). Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in

Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. *Brain Res, 541*(1), 163-166. doi:10.1016/0006-8993(91)91092-f

Nathan, B. P., Bellosta, S., Sanan, D. A., Weisgraber, K. H., Mahley, R. W., & Pitas, R. E. (1994). Differential effects of apolipoproteins E3 and E4 on neuronal growth in vitro. *Science, 264*(5160), 850-852. doi:10.1126/science.8171342

Neefjes, J., & van der Kant, R. (2014). Stuck in traffic: an emerging theme in diseases of the nervous system. *Trends Neurosci, 37*(2), 66-76. doi:10.1016/j.tins.2013.11.006

Nieweg, K., Schaller, H., & Pfrieger, F. W. (2009). Marked differences in cholesterol synthesis between neurons and glial cells from postnatal rats. *J Neurochem, 109*(1), 125-134. doi:10.1111/j.1471-4159.2009.05917.x

Nixon, R. A. (2004). Niemann-Pick Type C disease and Alzheimer's disease: the APP-endosome connection fattens up. *Am J Pathol, 164*(3), 757-761. doi:10.1016/S0002-9440(10)63163-X

Nuriel, T., Angulo, S. L., Khan, U., Ashok, A., Chen, Q., Figueroa, H. Y., . . . Duff, K. E. (2017a). Neuronal hyperactivity due to loss of inhibitory tone in APOE4 mice lacking Alzheimer's disease-like pathology. *Nat Commun, 8*(1), 1464. doi:10.1038/s41467-017-01444-0

Nuriel, T., Peng, K. Y., Ashok, A., Dillman, A. A., Figueroa, H. Y., Apuzzo, J., . . . Duff, K. E. (2017b). The Endosomal-Lysosomal Pathway Is Dysregulated by APOE4 Expression in Vivo. *Front Neurosci, 11*, 702. doi:10.3389/fnins.2017.00702

Oakley, H., Cole, S. L., Logan, S., Maus, E., Shao, P., Craft, J., . . . Vassar, R. (2006). Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J Neurosci, 26*(40), 10129-10140. doi:10.1523/JNEUROSCI.1202-06.2006

Orlandi, J. G., Fernández-García, S., Comella-Bolla, A., Masana, M., García-Díaz Barriga, G., Yaghoubi, M., … Soriano, J. (2017, November 12). NETCAL: An interactive platform for large-scale, NETwork and population dynamics analysis of CALcium imaging recordings (Version 7.0.0 Open Beta). Zenodo. http://doi.org/10.5281/zenodo.1119026. (2017).

Overly, C. C., & Hollenbeck, P. J. (1996). Dynamic organization of endocytic pathways in axons of cultured sympathetic neurons. *J Neurosci, 16*(19), 6056-6064.

Palmqvist, S., Janelidze, S., Stomrud, E., Zetterberg, H., Karl, J., Zink, K., . . . Hansson, O. (2019). Performance of Fully Automated Plasma Assays as Screening Tests for Alzheimer Disease-Related beta-Amyloid Status. *JAMA Neurol, 76*(9), 1060-1069. doi:10.1001/jamaneurol.2019.1632

Palmqvist, S., Zetterberg, H., Blennow, K., Vestberg, S., Andreasson, U., Brooks, D. J., . . . Hansson, O. (2014). Accuracy of brain amyloid detection in clinical practice using cerebrospinal fluid beta-amyloid 42: a cross-validation study against amyloid positron emission tomography. *JAMA Neurol, 71*(10), 1282-1289. doi:10.1001/jamaneurol.2014.1358

Palop, J. J., Chin, J., Roberson, E. D., Wang, J., Thwin, M. T., Bien-Ly, N., . . . Mucke, L. (2007). Aberrant excitatory neuronal activity and compensatory remodeling of

inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron, 55*(5), 697-711. doi:10.1016/j.neuron.2007.07.025

Perdigao, C., Barata, M. A., Araujo, M. N., Mirfakhar, F. S., Castanheira, J., & Guimas Almeida, C. (2020). Intracellular Trafficking Mechanisms of Synaptic Dysfunction in Alzheimer's Disease. *Front Cell Neurosci, 14*, 72. doi:10.3389/fncel.2020.00072

Petersen, R. C. (2001a). Mild Cognitive Impairment: Transition from Aging to Alzheimer's Disease. In *Alzheimer's Disease* (pp. 141-151).

Petersen, R. C., Stevens, J. C., Ganguli, M., Tangalos, E. G., Cummings, J. L., & DeKosky, S. T. (2001b). Practice parameter: early detection of dementia: mild cognitive impairment (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology, 56*(9), 1133-1142. doi:10.1212/wnl.56.9.1133

Pfrieger, F. W., & Barres, B. A. (1997). Synaptic efficacy enhanced by glial cells in vitro. *Science, 277*(5332), 1684-1687. doi:10.1126/science.277.5332.1684

Pitas, R. E., Boyles, J. K., Lee, S. H., Foss, D., & Mahley, R. W. (1987). Astrocytes synthesize apolipoprotein E and metabolize apolipoprotein E-containing lipoproteins. *Biochim Biophys Acta, 917*(1), 148-161. doi:10.1016/0005-2760(87)90295-5

Pohlkamp, T., Xian, X., Wong, C. H., Durakoglugil, M. S., Werthmann, G. C., Saido, T. C., . . . Herz, J. (2021). NHE6 depletion corrects ApoE4-mediated synaptic impairments and reduces amyloid plaque load. *Elife, 10*. doi:10.7554/eLife.72034

Qi, G., Mi, Y., Shi, X., Gu, H., Brinton, R. D., & Yin, F. (2021). ApoE4 Impairs Neuron-Astrocyte Coupling of Fatty Acid Metabolism. *Cell Rep, 34*(1), 108572. doi:10.1016/j.celrep.2020.108572

Quiroz, Y. T., Budson, A. E., Celone, K., Ruiz, A., Newmark, R., Castrillon, G., . . . Stern, C. E. (2010). Hippocampal hyperactivation in presymptomatic familial Alzheimer's disease. *Ann Neurol, 68*(6), 865-875. doi:10.1002/ana.22105

Raber, J., Huang, Y., & Ashford, J. W. (2004). ApoE genotype accounts for the vast majority of AD risk and AD pathology. *Neurobiol Aging, 25*(5), 641-650. doi:10.1016/j.neurobiolaging.2003.12.023

Rajendran, L., Honsho, M., Zahn, T. R., Keller, P., Geiger, K. D., Verkade, P., & Simons, K. (2006). Alzheimer's disease beta-amyloid peptides are released in association with exosomes. *Proc Natl Acad Sci U S A, 103*(30), 11172-11177. doi:10.1073/pnas.0603838103

Rall, S. C., Jr., Weisgraber, K. H., & Mahley, R. W. (1982). Human apolipoprotein E. The complete amino acid sequence. *J Biol Chem, 257*(8), 4171-4178.

Rawat, V., Wang, S., Sima, J., Bar, R., Liraz, O., Gundimeda, U., . . . Yassine, H. N. (2019). ApoE4 Alters ABCA1 Membrane Trafficking in Astrocytes. *J Neurosci, 39*(48), 9611-9622. doi:10.1523/JNEUROSCI.1400-19.2019

Rebeck, G. W., Reiter, J. S., Strickland, D. K., & Hyman, B. T. (1993). Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. *Neuron, 11*(4), 575-580. doi:10.1016/0896-6273(93)90070-8

Reddy, P. H., Mani, G., Park, B. S., Jacques, J., Murdoch, G., Whetsell, W., Jr., . . . Manczak, M. (2005). Differential loss of synaptic proteins in Alzheimer's disease:

implications for synaptic dysfunction. *J Alzheimers Dis, 7*(2), 103-117; discussion 173-180. doi:10.3233/jad-2005-7203

Rensen, P. C., Jong, M. C., van Vark, L. C., van der Boom, H., Hendriks, W. L., van Berkel, T. J., . . . Havekes, L. M. (2000). Apolipoprotein E is resistant to intracellular degradation in vitro and in vivo. Evidence for retroendocytosis. *J Biol Chem, 275*(12), 8564-8571. doi:10.1074/jbc.275.12.8564

Robert, J., Button, E. B., Yuen, B., Gilmour, M., Kang, K., Bahrabadi, A., . . . Wellington, C. L. (2017). Clearance of beta-amyloid is facilitated by apolipoprotein E and circulating high-density lipoproteins in bioengineered human vessels. *Elife, 6*. doi:10.7554/eLife.29595

Robinson, A. C., Davidson, Y. S., Roncaroli, F., Minshull, J., Tinkler, P., Horan, M. A., . . . Mann, D. M. A. (2020). Influence of APOE genotype in primary age-related tauopathy. *Acta Neuropathol Commun, 8*(1), 215. doi:10.1186/s40478-020-01095-1

Rodriguez, G. A., Burns, M. P., Weeber, E. J., & Rebeck, G. W. (2013). Young APOE4 targeted replacement mice exhibit poor spatial learning and memory, with reduced dendritic spine density in the medial entorhinal cortex. *Learn Mem, 20*(5), 256-266. doi:10.1101/lm.030031.112

Rogaev, E. I., Sherrington, R., Rogaeva, E. A., Levesque, G., Ikeda, M., Liang, Y., . . . et al. (1995). Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature, 376*(6543), 775-778. doi:10.1038/376775a0

Sannerud, R., Declerck, I., Peric, A., Raemaekers, T., Menendez, G., Zhou, L., . . . Annaert, W. (2011). ADP ribosylation factor 6 (ARF6) controls amyloid precursor protein (APP) processing by mediating the endosomal sorting of BACE1. *Proc Natl Acad Sci U S A, 108*(34), E559-568. doi:10.1073/pnas.1100745108

Sannerud, R., Esselens, C., Ejsmont, P., Mattera, R., Rochin, L., Tharkeshwar, A. K., . . . Annaert, W. (2016). Restricted Location of PSEN2/gamma-Secretase Determines Substrate Specificity and Generates an Intracellular Abeta Pool. *Cell, 166*(1), 193-208. doi:10.1016/j.cell.2016.05.020

Scheltens, P., Blennow, K., Breteler, M. M., de Strooper, B., Frisoni, G. B., Salloway, S., & Van der Flier, W. M. (2016). Alzheimer's disease. *Lancet, 388*(10043), 505-517. doi:10.1016/S0140-6736(15)01124-1

Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., . . . Younkin, S. (1996). Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med, 2*(8), 864-870. doi:10.1038/nm0896-864

Schmechel, D. E., Saunders, A. M., Strittmatter, W. J., Crain, B. J., Hulette, C. M., Joo, S. H., . . . Roses, A. D. (1993). Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci U S A, 90*(20), 9649-9653. doi:10.1073/pnas.90.20.9649

Schonfeld, P., & Reiser, G. (2013). Why does brain metabolism not favor burning of fatty acids to provide energy? Reflections on disadvantages of the use of free fatty acids as

fuel for brain. *J Cereb Blood Flow Metab, 33*(10), 1493-1499. doi:10.1038/jcbfm.2013.128

Schonfeld, P., & Reiser, G. (2017). Brain energy metabolism spurns fatty acids as fuel due to their inherent mitotoxicity and potential capacity to unleash neurodegeneration. *Neurochem Int, 109*, 68-77. doi:10.1016/j.neuint.2017.03.018

Schwartzentruber, J., Cooper, S., Liu, J. Z., Barrio-Hernandez, I., Bello, E., Kumasaka, N., . . . Bassett, A. (2021). Genome-wide meta-analysis, fine-mapping and integrative prioritization implicate new Alzheimer's disease risk genes. *Nat Genet, 53*(3), 392-402. doi:10.1038/s41588-020-00776-w

Selkoe, D. J., & Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med, 8*(6), 595-608. doi:10.15252/emmm.201606210

Serrano-Pozo, A., Frosch, M. P., Masliah, E., & Hyman, B. T. (2011). Neuropathological alterations in Alzheimer disease. *Cold Spring Harb Perspect Med, 1*(1), a006189. doi:10.1101/cshperspect.a006189

Sevigny, J., Chiao, P., Bussiere, T., Weinreb, P. H., Williams, L., Maier, M., . . . Sandrock, A. (2016). The antibody aducanumab reduces Abeta plaques in Alzheimer's disease. *Nature, 537*(7618), 50-56. doi:10.1038/nature19323

Sherrington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., Ikeda, M., . . . St George-Hyslop, P. H. (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature, 375*(6534), 754-760. doi:10.1038/375754a0

Shi, Y., Yamada, K., Liddelow, S. A., Smith, S. T., Zhao, L., Luo, W., . . . Holtzman, D. M. (2017). ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. *Nature, 549*(7673), 523-527. doi:10.1038/nature24016

Shie, F. S., LeBoeuf, R. C., & Jin, L. W. (2003). Early intraneuronal Abeta deposition in the hippocampus of APP transgenic mice. *Neuroreport, 14*(1), 123-129. doi:10.1097/01.wnr.0000051151.87269.7d

Shinohara, M., Kanekiyo, T., Tachibana, M., Kurti, A., Shinohara, M., Fu, Y., . . . Bu, G. (2020). APOE2 is associated with longevity independent of Alzheimer's disease. *Elife, 9*. doi:10.7554/eLife.62199

Shore, V. G., & Shore, B. (1973). Heterogeneity of human plasma very low density lipoproteins. Separation of species differing in protein components. *Biochemistry, 12*(3), 502-507. doi:10.1021/bi00727a022

Sisodia, S. S., Koo, E. H., Beyreuther, K., Unterbeck, A., & Price, D. L. (1990). Evidence that beta-amyloid protein in Alzheimer's disease is not derived by normal processing. *Science, 248*(4954), 492-495. doi:10.1126/science.1691865

Strittmatter, W. J., Saunders, A. M., Schmechel, D., Pericak-Vance, M., Enghild, J., Salvesen, G. S., & Roses, A. D. (1993). Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A, 90*(5), 1977-1981. doi:10.1073/pnas.90.5.1977

Sultana, R., Perluigi, M., & Butterfield, D. A. (2013). Lipid peroxidation triggers neurodegeneration: a redox proteomics view into the Alzheimer disease brain. *Free Radic Biol Med, 62*, 157-169. doi:10.1016/j.freeradbiomed.2012.09.027

Sutton, M. A., & Schuman, E. M. (2006). Dendritic protein synthesis, synaptic plasticity, and memory. *Cell, 127*(1), 49-58. doi:10.1016/j.cell.2006.09.014

Sutton, M. A., Wall, N. R., Aakalu, G. N., & Schuman, E. M. (2004). Regulation of dendritic protein synthesis by miniature synaptic events. *Science, 304*(5679), 1979-1983. doi:10.1126/science.1096202

Tabira, T., Chui, D. H., & Kuroda, S. (2002). Significance of intracellular Abeta42 accumulation in Alzheimer's disease. *Front Biosci, 7*, a44-49. doi:10.2741/tabira

Takahashi, R. H., Almeida, C. G., Kearney, P. F., Yu, F., Lin, M. T., Milner, T. A., & Gouras, G. K. (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., . . . Gouras, G. K. (2002). Intraneuronal Alzheimer abeta42 accumulates in multivesicular bodies and is associated with synaptic pathology. *Am J Pathol, 161*(5), 1869-1879. doi:10.1016/s0002-9440(10)64463-x

Takami, M., Nagashima, Y., Sano, Y., Ishihara, S., Morishima-Kawashima, M., Funamoto, S., & Ihara, Y. (2009). gamma-Secretase: successive tripeptide and tetrapeptide release from the transmembrane domain of beta-carboxyl terminal fragment. *J Neurosci, 29*(41), 13042-13052. doi:10.1523/JNEUROSCI.2362-09.2009

Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., . . . Katzman, R. (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

Tesseur, I., Van Dorpe, J., Spittaels, K., Van den Haute, C., Moechars, D., & Van Leuven, F. (2000). Expression of human apolipoprotein E4 in neurons causes hyperphosphorylation of protein tau in the brains of transgenic mice. *Am J Pathol, 156*(3), 951-964. doi:10.1016/S0002-9440(10)64963-2

Thal, D. R., Capetillo-Zarate, E., Del Tredici, K., & Braak, H. (2006). The development of amyloid beta protein deposits in the aged brain. *Sci Aging Knowledge Environ, 2006*(6), re1. doi:10.1126/sageke.2006.6.re1

Thinakaran, G., Teplow, D. B., Siman, R., Greenberg, B., & Sisodia, S. S. (1996). Metabolism of the "Swedish" amyloid precursor protein variant in neuro2a (N2a) cells. Evidence that cleavage at the "beta-secretase" site occurs in the golgi apparatus. *J Biol Chem, 271*(16), 9390-9397. doi:10.1074/jbc.271.16.9390

Tiraboschi, P., Hansen, L. A., Masliah, E., Alford, M., Thal, L. J., & Corey-Bloom, J. (2004). Impact of APOE genotype on neuropathologic and neurochemical markers of Alzheimer disease. *Neurology, 62*(11), 1977-1983. doi:10.1212/01.wnl.0000128091.92139.0f

Trommer, B. L., Shah, C., Yun, S. H., Gamkrelidze, G., Pasternak, E. S., Ye, G. L., . . . LaDu, M. J. (2004). ApoE isoform affects LTP in human targeted replacement mice. *Neuroreport, 15*(17), 2655-2658. doi:10.1097/00001756-200412030-00020

Turrigiano, G. (2012). Homeostatic synaptic plasticity: local and global mechanisms for stabilizing neuronal function. *Cold Spring Harb Perspect Biol, 4*(1), a005736. doi:10.1101/cshperspect.a005736

Turrigiano, G. G. (2008). The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell, 135*(3), 422-435. doi:10.1016/j.cell.2008.10.008

Turrigiano, G. G., Leslie, K. R., Desai, N. S., Rutherford, L. C., & Nelson, S. B. (1998). Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature, 391*(6670), 892-896. doi:10.1038/36103

Utermann, G. (1975). Isolation and partial characterization of an arginine-rich apolipoprotein from human plasma very-low-density lipoproteins: apolipoprotein E. *Hoppe Seylers Z Physiol Chem, 356*(7), 1113-1121. doi:10.1515/bchm2.1975.356.2.1113

Van Acker, Z. P., Bretou, M., & Annaert, W. (2019). Endo-lysosomal dysregulations and late-onset Alzheimer's disease: impact of genetic risk factors. *Mol Neurodegener, 14*(1), 20. doi:10.1186/s13024-019-0323-7

van der Kant, R., & Goldstein, L. S. (2015). Cellular functions of the amyloid precursor protein from development to dementia. *Dev Cell, 32*(4), 502-515. doi:10.1016/j.devcel.2015.01.022

van Niel, G., Bergam, P., Di Cicco, A., Hurbain, I., Lo Cicero, A., Dingli, F., . . . Raposo, G. (2015). Apolipoprotein E Regulates Amyloid Formation within Endosomes of Pigment Cells. *Cell Rep, 13*(1), 43-51. doi:10.1016/j.celrep.2015.08.057

Vassar, R., Bennett, B. D., Babu-Khan, S., Kahn, S., Mendiaz, E. A., Denis, P., . . . Citron, M. (1999). Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science, 286*(5440), 735-741. doi:10.1126/science.286.5440.735

Vossel, K. A., Beagle, A. J., Rabinovici, G. D., Shu, H., Lee, S. E., Naasan, G., . . . Mucke, L. (2013). Seizures and epileptiform activity in the early stages of Alzheimer disease. *JAMA Neurol, 70*(9), 1158-1166. doi:10.1001/jamaneurol.2013.136

Wahrle, S. E., Jiang, H., Parsadanian, M., Hartman, R. E., Bales, K. R., Paul, S. M., & Holtzman, D. M. (2005). Deletion of Abca1 increases Abeta deposition in the PDAPP transgenic mouse model of Alzheimer disease. *J Biol Chem, 280*(52), 43236-43242. doi:10.1074/jbc.M508780200

Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., . . . Selkoe, D. J. (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, C., Najm, R., Xu, Q., Jeong, D. E., Walker, D., Balestra, M. E., . . . Huang, Y. (2018). Gain of toxic apolipoprotein E4 effects in human iPSC-derived neurons is ameliorated by a small-molecule structure corrector. *Nat Med, 24*(5), 647-657. doi:10.1038/s41591-018-0004-z

Wang, C., Xiong, M., Gratuze, M., Bao, X., Shi, Y., Andhey, P. S., . . . Holtzman, D. M. (2021). Selective removal of astrocytic APOE4 strongly protects against tau-mediated neurodegeneration and decreases synaptic phagocytosis by microglia. *Neuron, 109*(10), 1657-1674 e1657. doi:10.1016/j.neuron.2021.03.024

Wang, H., Kulas, J. A., Wang, C., Holtzman, D. M., Ferris, H. A., & Hansen, S. B. (2021). Regulation of beta-amyloid production in neurons by astrocyte-derived cholesterol. *Proc Natl Acad Sci U S A, 118*(33). doi:10.1073/pnas.2102191118

Weeber, E. J., Beffert, U., Jones, C., Christian, J. M., Forster, E., Sweatt, J. D., & Herz, J. (2002). Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. *J Biol Chem, 277*(42), 39944-39952. doi:10.1074/jbc.M205147200

Wefelmeyer, W., Puhl, C. J., & Burrone, J. (2016). Homeostatic Plasticity of Subcellular Neuronal Structures: From Inputs to Outputs. *Trends Neurosci, 39*(10), 656-667. doi:10.1016/j.tins.2016.08.004

Weingarten, M. D., Lockwood, A. H., Hwo, S. Y., & Kirschner, M. W. (1975). A protein factor essential for microtubule assembly. *Proc Natl Acad Sci U S A, 72*(5), 1858-1862. doi:10.1073/pnas.72.5.1858

Weisgraber, K. H. (1994). Apolipoprotein E: structure-function relationships. *Adv Protein Chem, 45*, 249-302. doi:10.1016/s0065-3233(08)60642-7

Weisgraber, K. H., Rall, S. C., Jr., & Mahley, R. W. (1981). Human E apoprotein heterogeneity. Cysteine-arginine interchanges in the amino acid sequence of the apo-E isoforms. *J Biol Chem, 256*(17), 9077-9083.

Willen, K., Edgar, J. R., Hasegawa, T., Tanaka, N., Futter, C. E., & Gouras, G. K. (2017). Abeta accumulation causes MVB enlargement and is modelled by dominant negative VPS4A. *Mol Neurodegener, 12*(1), 61. doi:10.1186/s13024-017-0203-y

Wolfe, M. S. (2012). Processive proteolysis by gamma-secretase and the mechanism of Alzheimer's disease. *Biol Chem, 393*(9), 899-905. doi:10.1515/hsz-2012-0140

Xian, X., Pohlkamp, T., Durakoglugil, M. S., Wong, C. H., Beck, J. K., Lane-Donovan, C., . . . Herz, J. (2018). Reversal of ApoE4-induced recycling block as a novel prevention approach for Alzheimer's disease. *Elife, 7*. doi:10.7554/eLife.40048

Xu, Q., Bernardo, A., Walker, D., Kanegawa, T., Mahley, R. W., & Huang, Y. (2006). Profile and regulation of apolipoprotein E (ApoE) expression in the CNS in mice with targeting of green fluorescent protein gene to the ApoE locus. *J Neurosci, 26*(19), 4985-4994. doi:10.1523/JNEUROSCI.5476-05.2006

Yamaguchi, H., Hirai, S., Morimatsu, M., Shoji, M., & Harigaya, Y. (1988). Diffuse type of senile plaques in the brains of Alzheimer-type dementia. *Acta Neuropathol, 77*(2), 113-119. doi:10.1007/BF00687420

Yamazaki, Y., Zhao, N., Caulfield, T. R., Liu, C. C., & Bu, G. (2019). Apolipoprotein E and Alzheimer disease: pathobiology and targeting strategies. *Nat Rev Neurol, 15*(9), 501-518. doi:10.1038/s41582-019-0228-7

Yiannopoulou, K. G., & Papageorgiou, S. G. (2020). Current and Future Treatments in Alzheimer Disease: An Update. *J Cent Nerv Syst Dis, 12*, 1179573520907397. doi:10.1177/1179573520907397

Yu, J. T., Tan, L., & Hardy, J. (2014). Apolipoprotein E in Alzheimer's disease: an update. *Annu Rev Neurosci, 37*, 79-100. doi:10.1146/annurev-neuro-071013-014300

Zhao, N., Liu, C. C., Van Ingelgom, A. J., Linares, C., Kurti, A., Knight, J. A., . . . Bu, G. (2018). APOE epsilon2 is associated with increased tau pathology in primary tauopathy. *Nat Commun, 9*(1), 4388. doi:10.1038/s41467-018-06783-0

Zhao, N., Liu, C. C., Van Ingelgom, A. J., Martens, Y. A., Linares, C., Knight, J. A., . . . Bu, G. (2017). Apolipoprotein E4 Impairs Neuronal Insulin Signaling by Trapping

Insulin Receptor in the Endosomes. *Neuron, 96*(1), 115-129 e115. doi:10.1016/j.neuron.2017.09.003

Zhao, W., Dumanis, S. B., Tamboli, I. Y., Rodriguez, G. A., Jo Ladu, M., Moussa, C. E., & William Rebeck, G. (2014). Human APOE genotype affects intraneuronal Abeta1-42 accumulation in a lentiviral gene transfer model. *Hum Mol Genet, 23*(5), 1365-1375. doi:10.1093/hmg/ddt525