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AMYLOID PRECURSOR PROTEIN WITH THE ALZHEIMER'S DISEASE 670/671 MUTATION

ANIMAL AND CELLULAR MODELS

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Stockholm 2008

Published by Karolinska Institutet. Printed by Larserics Digital Print AB.

ISBN 978-91-7409-169-4

Success is the ability to go from one failure to another with no loss of enthusiasm.

Sir Winston Churchill

ABSTRACT

The amyloid precursor protein (APP) and it's derivatives play a key role in the pathogenesis of Alzheimer's disease (AD), which is characterized by the presence of multiple aggregates of an APP proteolytic product, Aβ, and tau protein in the brains of affected patients. Clinically the disease is manifested by a progressive loss of memory and executive functions. Several mutations within APP or in proteins involved in APP processing cause an inherited form of AD. Our goal was to recapitulate the features of the disease in a rat model. We have established a transgenic rat expressing human APP with the so called "Swedish" mutation (APPswe). All human carriers of this mutation develop AD with an onset of clinical manifestation between the age of 44 and 61 years.

The **first paper** of this thesis describes the generation of the Tg6590 APPswe transgenic rat. A cDNA construct carrying human APPswe and an ubiquitin promotor was injected into the pronucleus of rat oocytes. After confirming the expression of the human protein in the transgenic founder, the rat offspring were breed to homozygosity. The Tg6590 rat line shows mainly neuronal APPswe expression, with the highest levels found in the cortex, hippocampus and cerebellum. APPswe is processed in the rat brain, as it's secreted fragments can be found in the cerebrospinal fluid. Homozygous Tg6590 rats begin to show Aβ accumulation, mainly in the cerebral blood vessels, starting from 15 months of age. At 11 months of age, the Aβ peptide levels are elevated by 65% in the hippocampus and by 40% in the cortex of transgenic animals, as compared to control animals (paper II).

In **paper II** the animals were characterized further by behavioral testing and magnetic resonance imaging (MRI) of the brain. At the age of 9 months, but possibly even earlier, the Tg6590 male rats show inferior spatial memory (assessed by Morris water maze) and altered spontaneous behavior (measured in open-field test), as compared to control animals. We have not detected any gross degeneration of the hippocampus or cortex of the 9 months old rats by MRI, but preliminary results suggest diminution of the cortex thickness in older animals.

Since destabilization of calcium homeostasis has been implied as one of the proximal events leading to neuronal degeneration in AD, in the last two papers we focus on calcium signaling in primary hippocampal neurons derived from heterozygous Tg6590 rats. Cytosolic free calcium levels ($[Ca^{2+}]_i$) were imaged by confocal microscopy using the fluorescent dye fluo-3AM. In **paper III** we demonstrate that transgenic hippocampal cultures show increased frequency but unaltered amplitude of spontaneous $[Ca^{2+}]_i$ oscillations as compared to wild-type neurons. The altered calcium signaling in transgenic neurons seems unlikely to be due to modulation of the N-methyl-D-aspartate or nicotinic neurotransmitter systems, nor to depend on secreted APP derivatives, suggesting that either the full-length (non-processed) APP protein or intracellular APP derivatives are responsible for this effect.

In **paper IV** we show, that transgenic neurons have increased basal $[Ca^{2+}]_i$ and altered response to hyperosmotic stress, but no perturbations in endoplasmic reticulum calcium loading. Increased osmolarity can be encountered by neurons during diabetic hyperglycemia or after ischemic stroke, which in their turn are associated with an increased risk of developing AD in later life. We found that the altered response to hypersomotic stress could involve aberrant activation of L-type calcium channels, since transgenic neurons showed significantly greater sensitivity to the L-type calcium channel antagonist, nimodipine.

In summary, we have demonstrated that APPswe induces complex alterations of calcium homeostasis in hippocampal neurons, which might at least partly be responsible for the memory deficits seen in the Tg6590 rats. We believe that the Tg6590 rat is a suitable model of early AD and should prove useful for testing new therapeutic strategies aimed at improving memory in AD patients.

LIST OF PUBLICATIONS

The thesis is based on the following articles, which will be referred to in the text by their roman numerals:

I. Folkesson R., Malkiewicz K., **Kloskowska E**., Nilsson T., Popova E., Bogdanovic N., Ganten U., Ganten D., Bader M., Winblad B., Benedikz E., 2007. A transgenic rat expressing human APP with the Swedish Alzheimer's disease mutation. *Biochem Biophys Res Commun*. 358(3), 777-782.

II. **Kloskowska E**., Pham T.M., Nilsson T., Zhu S., Öberg J., Pedersen L.Ø., Pedersen J.T., Malkiewicz K., Winblad B., Folkesson R., Benedikz E., 2008. Memory impairment in the Tg6590 transgenic rat model of Alzheimer's disease. *Submitted manuscript*.

III. **Kloskowska E**., Malkiewicz K., Winblad B., Benedikz E., Bruton J.D., 2008. APPswe mutation increases the frequency of spontaneous Ca^{2+}-oscillations in rat hippocampal neurons. *Neurosci Lett*. 436(2), 250-254.

IV. **Kloskowska E**., Bruton J.D., Winblad B., Benedikz E., 2008. The APP670/671 mutation alters calcium signaling and response to hyperosmotic stress in rat primary hippocampal neurons. *Neurosci Lett*. Advanced online publication: 2008 Aug 22.

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LIST OF ABBREVIATIONS

Aβ	Amyloid-β
Aβ*56	Aβ oligomer (most probably composed of 12 Aβ monomers)
ADAM	A disintegrin and metalloprotease enzyme
AICD	APP intracellular domain
AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
APL-1	*C. elegans* protein related to APP
APLP1 and APLP2	Amyloid precursor-like proteins 1 and 2
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
APP695, APP751, and APP770	695-, 751- and 770-amino-acid long APP isoforms
APP-BP1	APP binding protein 1
APPL	*Drosophila* APP-like protein
APPswe	APP with the K670N/M671L "Swedish" mutation
APPwt	Wild-type APP
BACE1 and 2	β-site APP cleaving enzymes 1 and 2
BRI	A membrane protein associated with British familial dementia
C105	Carboxyl-terminal APP fragment of 105 amino acids
C99	β-secretase cleaved APP carboxyl-terminal fragment
$[Ca^{2+}]_i$	Cytosolic free calcium concentration
CALHM1	Calcium homeostasis modulator 1
Ca_V	Voltage-gated L-type calcium channels
cDNA	Complementary DNA
CSF	Cerebrospinal fluid
EDTA	Ethylenediaminetetraacetic acid chelating agent
EGTA	Ethylene glycol tetraacetic acid chelating agent
ER	Endoplasmic reticulum
ERK	Regulated protein kinase
F	Fluorescence intensity value
FAD	Familial alzheimer's disease
Fisher's PLSD	Fisher's protected least significant difference
Fluo-3 AM	Fluo-3 acetoxymethyl
fMRI	Functional MRI

GSK3	Glycogen synthase kinase-3
HEK293	Human embryonic kidney cell line
HRP	Horse-radish peroxidase
IP_3	Triphosphoinositol
IP_3R	IP_3 receptor
JNK	c-jun N-terminal kinase
KPI	Kunitz-type serine protease inhibitor
LTD	Long term depression
LTP	Long term potentiation
MCI	Mild cognitive impairment
MRI	Magnetic resonance imaging
mTOR	Mammalian target of rapamycin kinase
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide
NF-κB	Nuclear transcription factor κ-B
NFT	Neurofibrillary tangles
NgR	Nogo-66 receptor
NMDA	N-methyl D-aspartate
NMDAR	NMDA receptor
PET	Positron emission tomography
PHF	Paired helical fragments (of protein tau)
PIB	Pittsburgh compound-B
Pin1	Prolyl isomerase
PP1 and PP2A	Protein phosphatase 1 and 2A
PS1 and PS2	Presenilin 1 and 2
RARE	Rapid acquisition with relaxation enhancement
RT-PCR	Reverse transcription-coupled PCR
RyR	Ryanodine receptor
sAPPα and sAPPβ	α- and β-secretase cleaved secreted APP fragments
SERCA	Sarco-endoplasmic reticulum calcium ATPase
siRNA	Small interfering RNA
sorLA/LR11	Sorting receptor, responsible for regulating APP trafficking
TACE	Tumor necrosis factor-α converting enzyme
v/v or v/w	Volume/volume or volume/weight
2D	Two-dimensional
3D	Three-dimensional

INTRODUCTION

Alzheimer's Disease – basic facts

Alzheimer's disease (AD) is a neurodegenerative disorder, which leads to the death of the patient within approximately 10 years from clinical diagnosis. It is the cause of 50-70% of all dementias in the elderly population (Fratiglioni and Rocca, 2001) and affects an estimated 10% of people over the age of 65. Unfortunately, there is currently no effective treatment acting on the underlying pathogenic process, although some symptomatic relief and slowing of the progression of symptoms is achievable. This is frightening since the disease process attacks memory and consciousness, and ultimately selfhood, the basics of what we consider human. The ultimate goal is to detect the pathological processes leading to brain deterioration before lasting damage is done, and to be able to prevent them. So far, the earliest events initiating the pathological cascade of AD are still a mystery.

Characteristics of the disease and its progression

The disease is characterized by progressive cognitive decline, where memory of recent facts, spatial orientation, attention and executive functions are ones of the first affected, followed by speech and behavioral problems which affect everyday life (Almkvist, 1996). Psychiatric symptoms such as apathy, anxiety, delusions or hallucinations are common, and so is weight loss (Piccininni et al., 2005, Reynish et al., 2001). In the final stages the patients lose the ability to control movement and are totally dependent on assistance from caregivers. The pathological changes in the brain, which define the disease, are abundant extracellular amyloid plaques (also called senile or neuritic plaques in their mature form) and intracellular neurofibrillary tangles (NFTs), accompanied by synaptic and neuronal loss and brain inflammation (Ball, 1977, Braak and Braak, 1991, Scheff et al., 2007, Schultzberg et al., 2007). The amyloid plaques are composed mainly of an aggregated 40 or 42 amino-acid long amyloid-β peptide (Aβ) derived from the amyloid precursor protein (APP), while neurofibrillary tangles consist of an aggregated form of hyperphosphorylated microtubule-associated protein, tau. Whereas the progression of neurofibrillary degeneration follows a defined pathway from the entorhinal cortex and hippocampal formation towards polymodal association areas and then primary cortical regions, the amyloid plaque distribution exhibits more inter-individual variation, especially during the early stages of the disease (Braak and Braak, 1991). Recent advances in imaging amyloid with the Pittsburgh compound-B (PIB) in living patients suggest however that amyloid plaques deposition is also sequential, first appearing in the brain's cingulate and frontal cortex areas, then progressing to the parietal and temporal cortex and caudate and finally to

occipital cortex and sensory-motor cortex (Klunk et al., 2005 presented at the 2005 Society for Neuroscience meeting).

Patients, who experience abnormal memory problems but their severity is not sufficient to fulfill the classification of dementia, are given the diagnosis of mild cognitive impairment (MCI). These patients tend to progress to clinically probable AD at a rate of 10-15% per year, show elevated levels of neurofibrillary tangle accumulation in the brain when studied postmortem, and also increased amyloid deposition as shown by positron emission tomography (PET) with the PIB tracer (Petersen, 2007, Markesbery et al., 2006, Forsberg et al., 2008).

Treatment of Alzheimer's disease

Some of the earliest affected neurons in the AD brain are cholinergic neurons of the basal forebrain (Davies and Maloney, 1976, Whitehouse et al., 1982). Since the loss of cholinergic function is thought to at least partly underlie the cognitive decline in AD, acetylcholinesterase inhibitors are commonly used for symptomatic treatment of the disease (Perry et al., 1978, Mega, 2000). These drugs (sold under the names: Donepezil, Rivastigmine, Galantamine and Tacrine), inhibit acetylcholine turnover and allow for longer action of this neurotransmitter on the remaining cholinergic neurons. Another currently used drug is Memantine, which acts through a different mechanism, by modulating the activity of the glutamatergic N-methyl D-aspartate receptor (NMDAR) as excessive NMDAR activation and resulting excitotoxicity has also been implied in the pathogenesis of the disease (Henneberry et al., 1989, Robinson and Keating, 2006). On average, these drugs delay worsening of symptoms for 6 to 12 months for about half of the people who take them (www.alz.org).

A pleiotropy of different approaches aimed at developing better treatment strategies for AD are under research. In the past few years much effort and hope has been put into immunization approaches, aiming at solubilizing the amyloid plaques found in the brains of AD patients by means of Aβ-specific antibodies. Initial Aβ vaccination experiments performed in APP transgenic mice were very promising, as both a decrease in amyloid aggregation and memory improvement was observed (Schenk et al., 1999, Janus et al., 2000, Morgan et al., 2000). However, the first analogous trials in human patients had to be halted after some of the patients developed signs of meningoencephalitis (Senior, 2002, Gilman et al., 2005). Also, the reports of increased incidence of vascular microhemorrhages in transgenic mice subjected to Aβ immunotherapy (Pfeifer et al., 2002, Wilcock et al., 2007) suggests that more caution should be taken with regards to the effects of immunization on cerebral amyloid angiopathy, which is a frequent feature in AD patients (Thal et al., 2008). All in all, although Aβ immunization remains a hot topic, as has been recently demonstrated during the 2008 International

Conference on Alzheimer's Disease in Chicago, there are reports showing no symptological improvement in AD patients subjected to such a therapy (Holmes et al., 2008) suggesting it might be too early for optimism that anti-Aβ vaccination will be "the cure" for Alzheimer's disease.

Familial Alzheimer's disease genes

Fewer than 5% of AD cases are caused by a known mutation in a single gene (www.alz.org) and are inherited in an autosomal dominant manner (familial Alzheimer's disease, FAD). The main difference between FAD and the more common sporadic AD is the age of onset. Whereas FAD usually strikes before the age of 65 years (early-onset), the sporadic forms typically occur after the age of 65 years (late-onset). Since FAD and sporadic AD share clinical and pathophysiological characteristics, it is likely that there is also a substantial overlap at the etiological level.

Mutations in three genes have been associated with FAD: the amyloid precursor protein (APP) on chromosome 21, presenilin 1 (PS1) on chromosome 14 and presenilin 2 (PS2) on chromosome 1. Sequential cleavage of APP by β- and γ-secretases releases the Aβ peptide, which is the main component of amyloid plaques and is considered to be neurotoxic. Presenilins are central components of the atypical aspartyl protease complex responsible for the γ-secretase cleavage of APP. Unlike the presenilins, no FAD-causing mutations have so far been identified in the primary β-secretase, β-site APP cleaving enzyme BACE1 (Vassar et al, 1999).

To date, 22 different missense mutations causative of early-onset AD or cerebral amyloid angiopathy have been described in APP, whereas more than 170 mutations have been found in the presenilin genes (http://www.molgen.ua.ac.be/ADMutations). Almost all of the FAD-linked mutations alter the processing of APP, leading to an overproduction of total Aβ or increased ratio of Aβ42 to Aβ40 levels, Aβ42 being the more pathogenic form and more prone to aggregation. Some mutations, found within the Aβ sequence itself like the so called Dutch APP mutation, do not affect the processing of APP but accelerate the aggregation of Aβ to fibrils (Levy et al., 2006). Mutations in PS1 are responsible for the most aggressive forms of FAD, with the age of onset between 30 and 65 years (Golan et al., 2007, Sherrington et al., 1995). However the presenilins most probably act also via Aβ-independent mechanisms, either through the loss of function towards other substrates of the γ-secretase complex or by direct involvement in intracellular calcium homeostasis (also discussed in the chapter on Calcium and AD, Calcium signaling downstream of AD-associated pathology).

Recently, increased dosage of APP due to mutations in the APP promotor or gene duplication have also been associated with AD (Theuns et al., 2006a, Sleegers et al., 2006). Individuals with Down's syndrome (trisomy of chromosome 21, the same chromosome which carries the APP gene) who survive to middle age almost always show some degree of AD-related neuropathology and most develop clinical features of dementia (Margallo-Lana et al., 2007).

Taken together, the data collected from studies on FAD-causing mutations points towards a central role for APP and/or its cleavage products in the pathogenesis of AD.

Risk factors

The main risk factor for developing AD is age, as is shown by the sharp increase in prevalence of dementias with increasing age (Figure 1). Even very old age is however by no means inevitably linked with dementia, as recently shown by the example of a 115 year old woman showing virtually no pathological changes in the brain. Her cognitive performance before death was above the average for healthy 60-75 year old adults (den Dunnen et al., 2008).

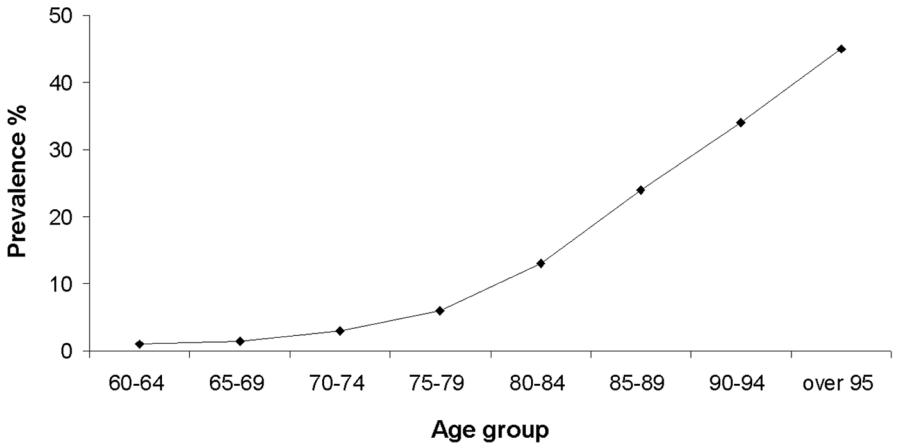

Figure 1. World-wide prevalence of age-dependent dementias in different age groups. Based on Fratiglioni and Rocca, 2001. Alzheimer's disease accounts for 50-70% of the dementias.

Generally, women develop the disease more often, which might be at least partly explained by their longer life-span. Also, older women have been shown to have a higher incidence of hypertension, hyperlipidemia, and diabetes, conditions which by themselves are associated with an increased risk of developing AD or other forms of dementia (Azad et al., 2007). Moreover, it

has been shown that female APP transgenic mice have more pronounced accumulation of extracellular Aβ and accumulate higher levels of different APP derivatives (the APP carboxyterminal fragment C99 and Aβ40) compared to age-matched male animals, suggesting gender differences in APP processing (Sturchler-Pierrat and Staufenbiel, 2000, Callahan et al., 2001, Schäfer et al., 2007). However, one study found no significant effect of gender on the prevalence of AD in human patients, when the groups were stratified by age year for year (Hebert et al., 2001), implying that the relationship between gender and risk of dementia should be more thoroughly studied.

Contrary to the early-onset FAD cases, late-onset AD is believed to be a multi-genetic disorder with many genes involved in modulating the individual susceptibility to the disease. The main known single gene polymorphism directly coupled to increased risk of late onset sporadic AD is in the apolipoprotein E (ApoE) gene. The ApoE protein is involved in lipid transport and is found in the general population in 3 different isoforms (ε2, ε3 and ε4), giving rise to six genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4 and ε4/ε4). The ε4 allele increases the risk of late-onset sporadic AD and reduces the mean age of onset in FAD, whereas the ε2 allele is protective (Corder et al., 1993, Corder et al., 1994, Sorbi et al., 1995, Bogdanovic et al., 2002, Pastor et al., 2003, Wijsman et al., 2005). The role of ApoE in AD pathology is likely related to the isoform-specific interactions of this protein with APP or the Aβ peptide and its influence on clearance, aggregation or conformation of Aβ. A direct influence of ApoE on tau phosphorylation state, synaptic repair processes or intracellular signaling has also been suggested (Cedazo-Mínguez, 2007).

In addition to hypertension and diabetes, which have already been mentioned, stroke, head injury and chronic inflammatory conditions are also associated with increased risk of developing AD (Fratiglioni et al., 2007, Van Den Heuvel et al., 2007, Schultzberg et al., 2007). The Swedish HARMONY study showing lack of concordance in 41% of monozygotic twins, and up to 7 years differences in age of onset in those monozygotic twins where both twins developed the disease, points towards a substantial environmental influence in AD (Gatz et al., 2005). The environmental risk factors may include exposure to metals, of which the most important may be Pb, but also air pollution, high fat and high calorie diet (obesity) or deficiency in vitamins B6, B12 and folic acid (Dosunmu et al., 2007). A good control of blood pressure both in middle and late life, caloric restriction, maintaining adequate brain exercise throughout life, as well as an active and socially integrated lifestyle in old age might be good preventive strategies against dementia (Fratiglioni et al., 2007).

Alzheimer's disease hypotheses

The coexistence of two degenerating processes in AD, namely amyloid deposition and tau aggregation, had for long divided the scientific world into advocates of the primary role of Aβ (jokingly dubbed "baptists") versus neurofibrillary lesions ("tauists") in the pathogenesis of the disease. The most prominent AD theory is the "amyloid cascade hypothesis" proposed first in 1991 by John Hardy and David Allsop and so named by Hardy and Gerry Higgins a year later (Hardy and Allsop, 1991, Hardy and Higgins, 1992). The original theory stated that since Aβ is the main component of senile plaques and is neurotoxic to neurons, then Aβ deposition must be solely responsible for initiating the pathological cascade leading to neurofibrillary aggregations and eventually neuronal death. Since then, the theory has been revised, also by Hardy himself, and today it is the soluble Aβ oligomers, instead of the plaques, which are considered to be the toxic forms (Hardy, 2006). Also it is now increasingly accepted, that the Aβ itself, and in particular the less fibrillogenic Aβ40 form, might have important physiological roles in the cell (discussed below, in the chapter on APP). An interesting new candidate which has emerged as a possible neurotoxic factor is a 56kDa soluble Aβ oligomer (named Aβ*56), most probably composed of 12 Aβ monomers. The Aβ*56 is proposed to be responsible for memory deficits in the APP transgenic Tg2576 AD mouse model and was also shown to induce reversible memory impairment when injected into healthy young rats (Lesné et al., 2006). If it is indeed the transitional state between monomeric Aβ and amyloid plaques which is responsible for initiating the AD cascade, then pharmacological approaches aimed at solubilizing the amyloid plaques already present in the affected patients might in fact worsen the disease in the patient (Holmes et al., 2008, Martins et al., 2008).

In contrast to the well defined "amyloid cascade hypothesis" no equally uniform "tau hypothesis" was formulated until quite recently. The idea that tau pathology was pivotal in the AD process came from the findings that neurofibrillary tangles correlate better with the progression of dementia (Arriagada et al., 1992). Also, the brain regions where tangles are first observed (the entorhinal cortex and hippocampus formation) are critical for memory consolidation, which is affected early in AD. Tau phosphorylation has been shown to decrease the binding of tau to microtubules and thus was proposed to lead to destabilization of the neural cytoskeleton and thereby neuronal function (Lindwall and Cole, 1984, Lee and Trojanowski, 1992). Hyperphosphorylated tau was also shown to exert a direct neurotoxic effect on cells by inducing apoptotic cell death (Fath et al., 2002). In 2005, Trojanowski and Lee formulated the modern "tau hypothesis of AD neurodegeneration" which states that disruption of axonal transport induced by microtubule disassembly is responsible for the dysfunction and death of neurons in AD.

Currently, it is widely accepted that APP/Aβ pathology lies upstream of tau pathology. Expression of a mutated APP is enough to trigger AD in its carriers, whereas no mutations in tau have been associated with AD. Tau mutations are however involved in other neurodegenerative diseases, like frontotemporal dementia (FTD), progressive supranuclear palsy (PSP), Pick's disease, or myotonic dystrophy (DM), but the tau aggregates found in these diseases have different characteristics than those found in AD (Delacourte, 2006). It is also interesting, that APP or APP/PS transgenic animal models of AD develop memory deficits in the absence of neurofibrillary pathology, whereas in triple transgenic mice carrying mutations in the tau, APP and PS genes amyloid deposition precedes tau pathology (McGowan et al., 2006, Oddo et al., 2003a). However, without the introduction of mutated tau, neither amyloid nor mutated APP alone are able to induce tangle pathology in a rodent model (Götz et al., 2001).

The complexity of the disease process is also reflected by the number of parallel theories trying to explain its course. The seemingly independent distribution of amyloid plaques and neurofibrillary tangles in the cerebral cortex and hippocampus of human patients has even led to the suggestion that the two might be distinct phenomena in AD or induced by a third signal transduction pathway independently affecting both amyloid aggregation and tau phosphorylation (Armstrong, 2005, Mudher and Lovestone, 2002).

The "calcium hypothesis of brain aging and Alzheimer's disease" was first proposed and later revised by Khachaturian in 1989. At that time Khachaturian speculated that cellular mechanisms which regulate the homeostasis of cytosolic free calcium ions ($[Ca^{2+}]_i$) play a critical role in brain aging, and that altered $[Ca^{2+}]_i$ homeostasis may account for many of the neurodegenerative changes seen in aging and aged-related diseases. Since then a substantial amount of data has been collected indicating that disturbed calcium signaling is in fact an early event in AD pathogenesis (LaFerla, 2002 and discussed further in the chapter on Calcium and AD).

The search for a pathway which initiates the pathological chain of events in sporadic AD has lead to the formulation of "The GSK3 hypothesis" (Hooper et al., 2008). Glycogen synthase kinase-3 (GSK3) is a serine/threonine kinase involved in a variety of vital cellular functions. GSK3 substrates include tau and cAMP responsive element-binding protein involved in memory modulation. The kinase has also been shown to affect the γ-secretase processing of APP by direct interaction with the presenilins and to promote pro-inflammatory responses by activating the production of various cytokines. "The GSK3 hypothesis" proposes that over-activation of GSK3 induces memory impairment, tau hyper-phosphorylation, increased β-amyloid production and local plaque-associated microglial-mediated inflammatory responses

both in sporadic AD and in FAD cases. Based on the similarity of pathologies in the doubly transgenic mice, APP-V717IxTau-P301L and Tau-P301LxGSK-3β, others have argued that it is rather Aβ that activates GSK3β to induce tau pathology (Muyllaert et al., 2006, Terwel et al., 2008). Mice overexpressing GSK3β alone show memory deficits, neuronal death and tau hyperphosphorylation, but no tau fibrillization (Hernández et al., 2002). No amyloid pathology was reported in them either.

The identification of a subpopulation of vulnerable neurons in the AD brain, which have re-entered into the cell cycle phase has lead to the formulation of the "mitotic hypothesis" and "two-hit hypothesis" stating that aberrant mitotic signaling by itself or together with oxidative insults initiate the pathological AD cascade (Davies, 2006, Zhu et al., 2007). Oxidative stress is intimately connected with mitochondrial function, and mitochondrial dysfunction induced by Aβ accumulation has been proposed to play a key role in AD (Anandatheerthavarada and Devi, 2007) and even resulted in its own "mitochondrial cascade hypothesis of sporadic AD" (Swerdlow and Khan, 2004).

Chronic inflammation, dysfunctional lysosomal system, involvement of herpes simplex virus infection and other pathways have also been implicated in the disease mechanisms (Schultzberg et al., 2007, Nixon and Cataldo, 2006, Itzhaki and Wozniak, 2008, Steen et al., 2005, Erol, 2008). These theories are not necessary mutually exclusive, but more work remains to be done to elucidate the exact relationships between the different cellular pathways involved in the complex pathological AD cascade.

The Amyloid Precursor Protein - expression and processing

The amyloid precursor protein (APP) is a type I integral membrane protein, with one transmembrane-spanning region, a large luminal domain and a short cytoplasmic domain (Figure 2). It is expressed in most but not all mammalian tissues and belongs to a larger protein family, which also includes the mammalian APP like protein 1 (APLP1) and 2 (APLP2). The APL-1 in *C. elegans* and APPL in *Drosophila* also share great homology as well as some functional conservation with the mammalian APP family. APLP2 shares the ubiquitous expression of APP, whereas APLP1 is expressed primarily in the nervous system (Lorent et al., 1995, Sisodia et al., 1996).

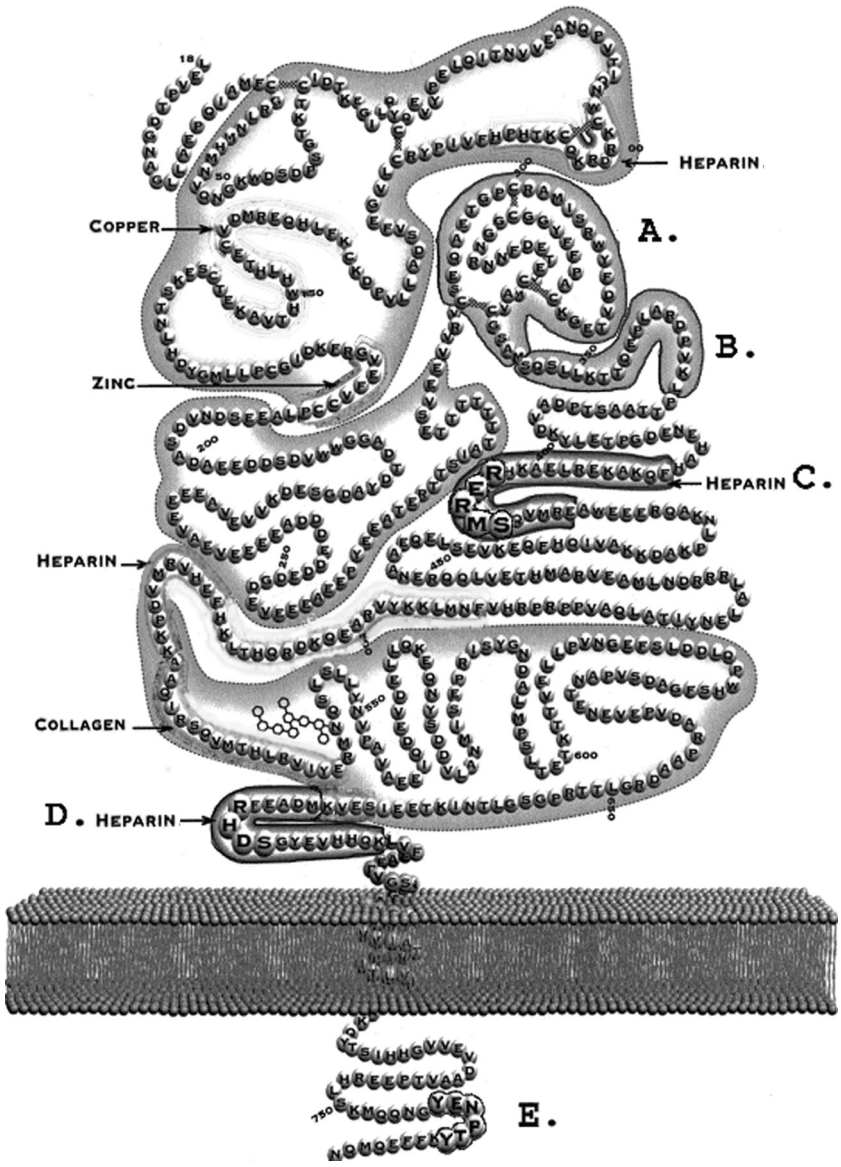

Figure 2. The APP protein (APP770 isoform). **A.** exon 7 - KPI domain (absent in APP695), **B.** exon 8 (absent in APP695 and APP751), **C**. Neurotrophic domain with RERMS sequence (amino acids 403–407), **D.** 17-aminoacid C-terminal domain of sAPPα (absent in sAPPβ) with adhesion-related RHDS sequence (amino acids 676–679), **E.** The YENPTY motif in the cytoplasmic domain of APP; the site of interactions with numerous intracellular proteins (adapted from Turner et al., 2003. Prog Neurobiol. 70(1), 1-32).

The human APP gene is located on chromosome 21. Alternative RNA splicing generates several protein isoforms, ranging from 365 to 770 amino acids. The major Aβ containing proteins are 695, 751 and 770 amino acids long (APP695, APP751 and APP770). No other APP family member shares any sequence similarity in the Aβ region. APP695, which is expressed predominantly in neurons, lacks a Kunitz-type serine protease inhibitor (KPI) domain, found in the extracellular part of the longer APP751 and APP770 isoforms (Sisodia et al., 1993). The KPI-lacking isoform accounts however for less that 14% of total brain APP protein, since glial cells and meninges express higher levels of the KPI-containing isoforms (Van Nostrand et al., 1991).

APP is constitutively transported to the cell surface, and undergoes extensive post-translational modifications during transit, such as N- and O-glycosylation, phosphorylation and tyrosine sulphation (Walter and Haass, 1999, Liu et al., 1999). In neurons, full-length APP moves to the axon terminals by means of fast anterograde transport (Koo et al., 1990).

APP is normally processed in the cell by two alternative processing pathways (Figure 3). Sequential cleavage by α- and γ-secretases leads to the shedding of a soluble APP ectodomain (sAPPα), a short p3 peptide and the APP intracellular domain AICD (non-amyloidogenic pathway), whereas cleavage by β- and γ-secretases gives rise to sAPPβ, Aβ and AICD (amyloidogenic pathway). A substantial proportion of APP in neurons is processed via the amyloidogenic pathway, whereas the non-amyloidogenic pathway dominates in other cells (Zhao et al., 1996, Esch et al., 1990, Sisodia et al., 1990). Several zinc metalloproteinases TACE/ADAM17, ADAM9, ADAM10 and MCD9 can cleave APP at the α-secretase site within the Aβ domain (Allinson et al., 2003). The major neuronal β-secretase is a transmembrane aspartyl protease BACE1 (Thinakaran and Koo, 2008). Different reports suggest that BACE2, a BACE1 homologue, cleaves APP either at the α-, β- and/or a novel τ-secretase site within the Aβ sequence (Farzan et al., 2000, Fluhrer et al., 2002, Sun and Song, 2006). The γ-secretase complex, responsible for an intramembranous cleavage of APP, consists of at least presenilin, nicastrin, anterior pharynx defective (APH1) and presenilin enhancer (PEN2), but possibly also additional modulatory molecules (Spasic and Annaert, 2008).

There is evidence that the different APP derivatives are generated in separate cellular compartments. Whereas the α-secretase cleavage seems to occur predominantly at the plasma membrane, the β-secretase processing occurs mainly in the recycling compartments of the late trans-Golgi network (Ikezu et al., 1998, Sisodia, 1992, Koo and Squazzo, 1994, Small and Gandy, 2006). The presence of the γ-secretase activity, on the other hand, has been indicated in multiple compartments including the plasma membrane, endoplasmic reticulum (ER), ER-golgi intermediate compartment, Golgi- and trans-Golgi network, endosomes as well as mitochondria

(Thinakaran and Koo, 2008, Selivanova et al., 2007, Hansson et al., 2004). Even though Aβ and sAPPβ seem to be produced mostly intracellularly, they are also released by cells into the extracellular milieu (Seubert et al., 1992, Sennvik et al., 2000).

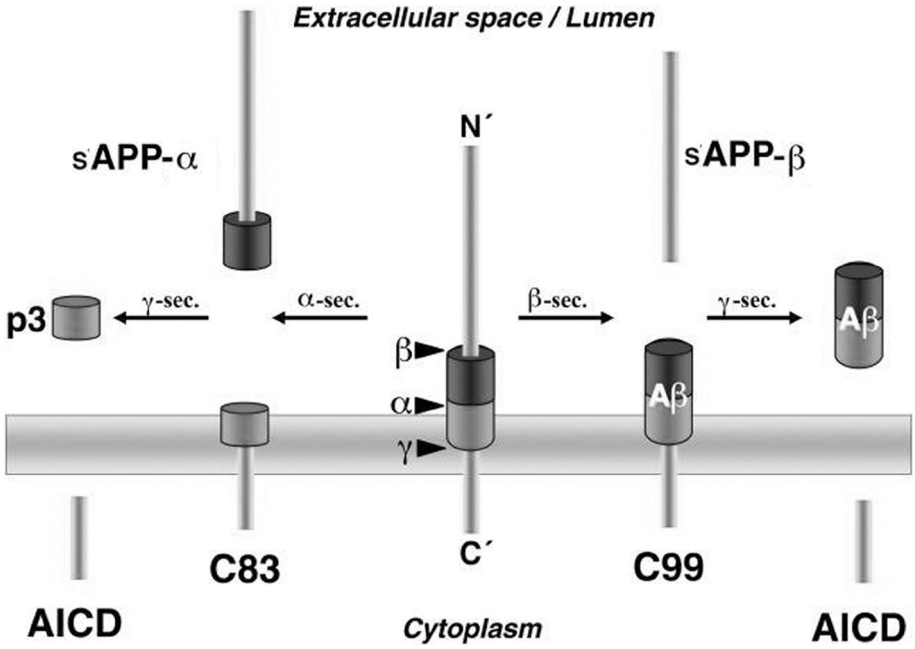

Figure 3. Processing of APP by α-, β- and γ-secretases (from Kaether and Haass, 2004, J Cell Biol. 167(5): 809–812).

The sorting of APP either to the cell membrane or recycling into trans-Golgi network might be regulated by the sorLA/LR11 protein receptor together with the cytosolic adaptors GGA and PACS-1 (Schmidt et al., 2007b). SorLA/LR11 has also been genetically linked with AD (Rogaeva et al., 2007). Multiple other mechanisms affect the processing of APP. These include: phosphorylation of APP at the threonine residue 668 (Thr668) by cyclin-dependent protein kinase 5 (cdk5), c-jun N-terminal kinase (JNK) or glycogen synthase kinase-3β (GSK3 β); as well as APP interactions with intra- and extracellular binding partners including the prolyl isomerase Pin1, X11, Fe65, Nogo-66 receptor (NgR) or ApoE (Suzuki and Nakaya, 2008, Tang and Liou, 2007, Cedazo-Mínguez, 2007). Other intracellular molecules such as Munc-13-1, protein phosphatases 1 and 2A (PP1 and PP2A), protein kinase C (PKC), extracellular signaled regulated protein kinase (ERK) or rho kinase (ROCK) have also been proposed to affect APP trafficking and/or processing (Caporaso et al., 1992, da Cruz e Silva et al., 1995, Liu et al., 2003, Rossner et al., 2004, Tang and Liou, 2007).

The APP protein is capable of forming homo- and hetero-dimers with other APP family members, both in a cis- and trans- (intercellular) fashion (Scheuermann et al., 2001, Soba et al., 2005). APP homodimerization has been shown to increase the production of Aβ, whereas destabilization of APP dimerization via the transmembrane GxxxA motif increases the Aβ42/Aβ40 ratio (Scheuermann et al., 2001, Gorman et al., 2008), suggesting that inhibition of APP dimerization favors the production of Aβ42 monomers. Interestingly many of the FAD-linked mutations in APP are localized to the transmembrane domain.

The APP holoprotein - function and binding partners

The resemblance of full-length APP to a cell-surface receptor, and reports that the protein, as well as its insect homologue, interact with the G-protein G_o has spurted the search for a functionally active APP ligand (Kang et al., 1987, Nishimoto et al., 1993, Swanson et al., 2005). The APP ectodomain binds to extracellular matrix proteins such as heparin, collagen, reelin or fibulin-1, as well as itself and the other APP family members, as already mentioned (Beher et al., 1996, Small et al., 1994, Hoe et al., 2006, Ohsawa et al., 2001, Scheuermann et al., 2001, Soba et al., 2005). A recently discovered APP binding protein, the axonal glycoprotein TAG1, induces AICD release and activation of Fe65 signaling (Ma et al., 2008), whereas APP's interactions with the secreted glycoprotein F-spondin or membrane-bound NgR affect Aβ production (Ho and Südhof, 2004, Park et al., 2006).

Aβ itself has also been shown to bind to membrane-bound APP and this binding is suggested to mediate Aβ toxicity to hippocampal neurons via APP-receptor like activation of the G_o protein (Lorenzo et al., 2000, Sola Vigo et al., 2008). Other proteins interacting with the extracellular domain of APP have also been identified (see Table 1), but the concept that APP is indeed a functional cell surface receptor activated by an extracellular ligand remains to be definitely proven.

The fact that APP expression is upregulated during neuronal maturation, differentiation and synaptogenesis (Hung et al., 1992, Moya et al., 1994, Murray and Igwe, 2003), as well as after brain injury (Ciallella et al., 2002, Olsson et al., 2004) suggests a functional role for the protein in determining neuronal development and in neuroprotection. Full-length APP has been shown to play a critical role for the proper migration of cortical neuronal progenitor cells in the developing brain (Young-Pearse et al., 2007). It has also been proposed to have a role in cell adhesion, via its interactions with the extracellular matrix, and axonal transport of membrane-associated cargo (Breen et al., 1991, Kamal et al., 2001). Most scientific attention however has focused on the physiological, and above all the pathological, roles of the different APP derivatives.

Table 1. APP interacting proteins

APP ectodomain	**Reference**
collagen	Beher et al., 1996
heparin	Small et al., 1994
ApoE	Haas et al., 1998, also reviewed in Hoe and Rebeck, 2008
Fibulin-1	Ohsawa et al., 2001
APP	Scheuermann et al., 2001, Soba et al., 2005
activated high molecular weight kininogen	Das et al., 2002
F-spondin	Ho and Südhof, 2004
SorLa	Andersen et al., 2005
LRP	Bu et al., 2006
NgR (Nogo-66 receptor)	Park et al., 2006
Reelin	Hoe et al., 2006
serum albumin, actin and human Collapsin Response Mediator Protein-2 (hCRMP-2) as well as two novel proteins of 41 and 63kDa	Pawlik et al., 2007
ATP synthase	Schmidt et al., 2007a
Integrin-β 1	Young-Pearse et al., 2008
TAG1	Ma et al., 2008
Contactin 4	Osterfield et al., 2008
NgCAM	Osterfield et al., 2008
APP transmembrane domain	
Notch	Fassa et al., 2005
APP	Gorman et al., 2008

Continued on next page.

Table 1. APP interacting proteins (continued from previous page)

APP cytoplasmic domain	Reference
G_o (GTP-binding protein)	Nishimoto et al., 1993
Fe65	Fiore et al., 1995
X11	Borg et al., 1996
APP-BP1 (APP binding protein 1)	Chow et al., 1996
clathrin	Marquez-Sterling et al., 1997
PAT1 (protein interacting with APP tail 1) – a microtubule interacting protein	Zheng et al., 1998
mDab1 (mammalian disabled-1)	Howell et al., 1999
14-3-3g protein	Horie et al., 1999
kinesin light chain, KLC	Kamal et al., 2000
Jip (c-Jun N-terminal kinase interacting protein)	Matsuda et al., 2001, Scheinfeld et al. 2002
Abl – non-receptor tyrosine kinase	Zambrano et al., 2001
Shc adaptor protein	Tarr et al., 2002
Numb and Numb-like proteins	Roncarati et al., 2002
PAK3	McPhie et al., 2003
hARD1 (human homologue of yeast amino-terminal acetyltransferase ARD1)	Asaumi et al., 2005
flotillin-1	Chen et al., 2006
FKBP12 (immunophilin with a peptidyl-prolyl cis-trans isomerase (PPIase) activity)	Liu et al., 2006
Pin1 (prolyl isomerase)	Pastorino et al., 2006
Calnuc	Lin et al., 2007
GRB2 (growth factor receptor-bound protein 2) adaptor protein	Nizzari et al., 2007
Homer2 and Homer3	Parisiadou et al., 2008

Secreted sAPPα and sAPPβ

The α-secretase cleaved APP fragment, sAPPα, seems to play a particularly important function in the brain, where it is involved in neurogenesis, neurotrophic and neuroprotective actions, synaptogenesis and in early memory formation (Roch et al., 1994, Small et al., 1999, Cheng et al., 2002, Caillé et al., 2004, Bour et al., 2004). Antibodies against endogenous sAPP reduce long term potentiation (LTP) in adult rat dentate gyrus, whereas infusion of recombinant sAPPα produces the opposite effect (Taylor et al., 2008). The trophic and memory potentiating effects of sAPP are thought to be mediated by the RERMS sequence (amino acids 328-332 of APP695, see Figure 2), which is also present in sAPPβ (Ninomiya et al., 1993, Roch et al., 1994, Jin et al., 1994). The trophic effects of sAPPβ, however, are about 100 times weaker than that of sAPPα (Furukawa et al., 1996b), indicating that the 16 amino-acid long C-terminal fragment of sAPPα also plays an important functional role either directly or by facilitating the binding of sAPPα to other effector molecules. This heparin-binding domain has been shown to be responsible for protecting hippocampal neurons against excitotoxicity, Aβ toxicity, and glucose deprivation as well as attenuating increases in intraneuronal calcium levels in response to glutamate (Furukawa et al., 1996a).

sAPP also has growth promoting activities in a variety of cells (Ninomiya et al., 1993, Popp et al., 1996, Pietrzik et al., 1998). The trophic function of sAPPα was also confirmed in our own experiments, in which the human embryonic kidney cell line HEK293 transfected with sAPP695α showed enhanced survival in serum-free cell medium, as compared to untransfected or vector-transfected cells (un-published data). The postulated participation in signal transduction mechanisms, as well as the fact that sAPPα has been shown to bind to cell surfaces, suggests the existence of a cell membrane receptor for sAPPα (Hoffmann et al., 1999). This putative receptor has not been identified thus far; however it seems possible that sAPPα binds to APP itself based on the transdimerization capabilities of the APP family and on the inability of sAPPα to stimulate neurite elongation in the absence of cellular APP expression (Soba et al., 2005, Young-Pearse et al., 2008). Young-Pearse et al. have proposed that the soluble derivate modulates APP function by interfering with the interactions of the full-length protein and its binding partners, in this case integrin-β-1 (Young-Pearse et al., 2008). Such competitive binding may also act in the opposite direction, as fibulin-1 interaction with the amino-terminal APP domain has been shown to block sAPP-dependent proliferation of primary cultured rat neural stem cells (Ohsawa et al., 2001).

Amyloid-β

Since the discovery that Aβ is the main components of the AD amyloid plaques, most attention has been focused on dissecting the pathological role of this peptide. Excessive amounts (μM range) of Aβ have indeed been shown to be toxic to neurons (Pike et al., 1991, Furukawa et al., 1996b, Sola Vigo et al., 2008). However, Aβ is also present in the cerebrospinal fluid and plasma of healthy individuals throughout life (Seubert et al., 1992, Giedraitis et al., 2007) and is secreted by neurons in response to activity (Kamenetz et al., 2003, Cirrito et al., 2005). Kamenetz et al. speculated that activity-dependent secretion of Aβ might be part of a negative feedback loop directed at downregulating excitatory synaptic transmission. More recently, work from different laboratories indicates that low concentrations of Aβ (in the pM-nM range) enhances LTP and memory formation (Morley et al., 2008, Arancio et al., 2008, Mathews et al., 2008, and also Wu et al., 1995).

Echeverria et al. (2005) showed that whereas endogenous Aβ might be involved in cAMP response element-directed gene expression, micromolar levels of extracellular fibrillar Aβ block the same gene expression pathway induced by potassium and forskolin. Such a concentration dependent effect is in line with the general law of hormesis, indicating that the same substance which shows stimulating effects at low doses can have inhibitory actions at higher doses, e.g. as demonstrated for the N-methyl-D-aspartate (NMDA) receptor antagonist, Memantine, used for treatment of AD, on spatial memory in rats (Calabrese, 2008, Wise and Lichtman, 2007).

Aβ40 has also been shown to induce neuronal differentiation in rat primary neural progenitor cells, in contrast to Aβ42, which promotes glial differentiation in these cells (Chen and Dong, 2008). Normally, Aβ40 constitutes about 90% of all secreted Aβ, but the Aβ42/Aβ40 ratio is increased in cells harboring FAD-linked mutations in the presenilins, or APP mutations in the vicinity of the γ-secretase cleavage site (Suzuki et al., 1994, Pinnix et al., 2001, Walker et al., 2005, Theuns et al., 2006b).

Certainly, the Aβ peptide will prove very interesting for future research, since its action seem to depend not only on the concentration and the isoform (40 or 42 amino acid long or even shorter species), but also the aggregation state (monomers, dimers, globulomeres etc.) or perhaps even the site of production (at the synapses or intracellularly in ER, mitochondria or Golgi).

APP intracellular domain – AICD

The resemblance of APP to another class I membrane receptor, namely Notch, has raised speculations that APP might have an analogous cellular signaling function. The intracellular domain of APP interacts with numerous proteins (Table 1 and reviewed in Suzuki and Nakaya, 2008) including the Fe65 and X11 adaptor proteins binding to the YENPTY motif (amino acids 682-687 of APP695).

The Fe65/AICD complex together with the histone acetyltransferase Tip60 is translocated to the nucleus (Kimberly et al., 2001, Cao and Sudhof, 2001, Kinoshita et al., 2002), whereas interaction with the X11α adaptor protein seems to arrest AICD within the cytosol (von Rotz et al., 2004). In a reporter gene assay, cells transfected with AICD showed activation of reporter gene transcription and this transcription was enhanced by co-transfection with Fe65 (Cao and Sudhof 2001). Conversely, overexpression of X11α or X11β, displayed an inhibitory effect on AICD-mediated gene trans-activation (Biederer et al., 2002). Cells and transgenic mice overexpressing AICD/APP showed increased levels of KAI1, GSK-3β, APP, BACE, TIP60, Fe65 and neprilysin (Baek et al., 2002, Ryan and Pimplikar, 2005, von Rotz et al., 2004, Pardossi-Piquard et al., 2005). On the other hand in a different set of experiments, neither pharmacological inhibition of AICD generation, nor APP/APPL2 double knock-out or PS double knock-out mice showed differences in the levels of KAI1, GSK-3β, APP and neprilysin proteins (Hebert et al., 2006), leaving the question of any putative transcriptional role of AICD still open.

APP family member knock-outs

Given the pleiotropy of functions ascribed to APP and its derivatives it came as a surprise that APP knock-out (APP-KO) mice were viable and fertile (Zheng et al., 1995, Li et al., 1996). More detailed studies of the APP-KO mice revealed that the animals exhibited reduced body and brain weight, decreased viability and impaired neurite outgrowth of hippocampal neurons, agenesis of the corpus callosum, muscular weakness with altered locomotor activity, hyper-sensitivity to kainate-induced seizures, as well as impaired spatial learning and LTP, among others (reviewed in Anliker and Müller, 2006). Interestingly, most of these abnormalities in mice could be rescued by introducing the secreted ectodomain of APP by a knock-in approach, supporting the view that this part of APP is vital for brain function, but is also responsible for several other physiological roles of APP (Ring et al., 2007). Double and triple APP/APLP knock-out animals on the other hand died shortly after birth, with the exception of the $APP^{-/-}/APLP1^{-/-}$ mice, which showed no major abnormalities (Heber et al., 2000). These results

suggest that the APP family proteins have at least partly redundant functions, and that the APLP2 is the most essential for post-natal viability. The function of the APP protein family is not fully conserved between the different species. Whereas deletion of the single APL-1 gene in *C. elegans* is lethal at early larval stages, APPL-deficient *Drosophila* flies show only mild behavioral deficits, which can be saved by transfection with the human APP gene (Hornsten et al., 2007, Luo et al., 1992).

The APPswe mutation

Most of the known pathogenic mutations in APP lie in the vicinity of the β-secretase or γ-secretase cleavage sites, which lie between residues 671 and 672 and approximately at residues 712-714 respectively (according to APP770 numbering). The effect of these mutations is an increased ratio of Aβ42/Aβ40 or increased total Aβ (reviewed in Theuns et al., 2006b). Mutations like the E693Q "Dutch" or E693K "Arctic" mutation, which lie within the Aβ sequence, result in decreased total Aβ, but altered processing or aggregation properties of the Aβ peptide. The Dutch APP mutation causes hereditary cerebral hemorrhage with amyloidosis of Dutch type, whereas the Arctic APP mutation is the cause of an Alzheimer-type dementia characterized by atypical amyloid plaques with a ringlike structure (Bornebroek et al., 2003, Nilsberth et al., 2001, Basun et al., 2008).

The so called "Swedish" double K670N/M671L mutation (APPswe), has been shown to give a three- to eightfold increase in total Aβ production without changing the Aβ42/Aβ40 ratio (Citron et al., 1992, Cai et al., 1993, Citron et al., 1994). Human carriers of the APPswe mutation develop Alzheimer's disease in an autosomal dominant manner at an average age of 53 (age span: 44 – 61 years) (Axelman et al., 1994). It has also been shown, that the processing of APPswe may occur in different cellular compartments than that of wild-type APP (APPwt). Whereas APPwt is re-internalized from the cell surface prior to γ-secretase cleavage, the APPswe can be cleaved independently of the internalization process, before the protein reaches the cell surface (Haass et al., 1995, Essalmani et al., 1996, Thinakaran et al., 1996, Steinhilb et al., 2002). Such altered processing might in consequence leave less substrate available for α-secretase cleavage, and decreased levels of the neuroprotective sAPPα could add to the pathogenic effect of this mutation. APPswe bearing cells have indeed been shown to secrete less sAPPα as compared to APPwt expressing cells (Thinakaran et al., 1996). APPswe is the most frequently used mutation (alone or with other mutations) in APP transgenic models of AD (Table 2, and discussed further in the chapter: Animal models of AD).

Animal models of AD

Transgenic animals have proven very valuable in dissecting the mechanisms involved in AD pathology, even though not all features of the disease have been successfully replicated in them (Table 2). The first mouse models of AD appeared over ten years ago (Games et al., 1995, Hsiao et al., 1996). The PDAPP model, expressing APP with the V717F mutation with 10-fold higher expression levels over endogenous APP, develops extracellular diffuse and neuritic plaques in the hippocampus, cortex and limbic areas beginning at the age of 6-8 months (Games et al., 1995, Masliah et al., 1996). These mice develop age-related memory problems with no neuronal loss (Irizarry et al., 1997b, Chen et al., 2000, German et al., 2005).

The APPswe mutation was used for the first time in the Tg2576 mouse model. These mice express 5.5 times higher APP levels over endogenous APP and develop diffuse and neuritic plaques in the hippocampus, cortex, subiculum and cerebellum beginning at about 9-11 months of age. The Tg2576 exhibit age-related memory deficits starting at the age of 6-8 months and impaired induction of LTP in the hippocampus, in the absence of global synaptic or neuronal loss (Hsiao et al., 1996, Chapman et al., 1999, Irizarry et al., 1997a, Westerman, 2002).

The above mentioned mouse models continue to be some of the most extensively used in the AD research field, but more APP-based mouse models have followed (the complete list is continually updated on: http://www.alzforum.org/res/com/tra/default.asp). Of these, only the APPswe expressing APP23 mice have been reported to exhibit neuronal loss (about 14%) in the C1 of hippocampus, but not in the cortex (Calhoun et al., 1998). Introducing an extra mutation, either in APP or in one of the presenilin genes, can accelerate plaque formation (Table 2). One of the more recent mouse models, expressing both APPswe and the very aggressive PS1 L166P mutation, which causes FAD in early adulthood (Moehlmann et al., 2002), shows neuritic plaques, abundant hippocampal CA1 neuron loss, severe axonopathy, motor dysfunction and memory deterioration from the age of 6 months (reviewed in Bayer and Wirths, 2008). Expression of a mutated presenilin alone is nevertheless not enough to cause amyloid pathology in a mouse model. This might be due to the fact that the rodent Aβ sequence differs from human in 3 amino acids and in N-terminal processing, which might affect the secondary structure and solubility of this peptide (Johnstone et al., 2003, Jankowsky et al., 2007).

Table 2. Selected rodent models of Alzheimer's disease

Mouse AD models	Transgene and promoter	Amyloid pathology age of onset (months)	P-tau / NFT / Cell loss	Memory impairment age of onset (months)	References
PDAPP	APPind PDGFβ	6-9	Yes / No / No	13-15	Games et al., 1995, Masliah et al., 1996, Irizarry et al., 1997b, Chen et al., 2000, German et al., 2005
Tg2576	APP695swe Hamster PrP	9-11	Yes / No / No	6-8	Hsiao et al., 1996, Irizarry et al., 1997a, Chapman et al., 1999, Westerman et al., 2002.
APP23	APP751swe Murine Thy1	6	Yes / No / Yes (CA1)	3	Sturchler-Pierrat et al., 1997, Calhoun et al., 1998, Van Dam et al., 2003
TgCRND8	APPswe/ind Syrian hamster PrP	2-3	Yes / No / Nr	3	Chishti et al., 2001, Dudal et al., 2004, Bellucci et al., 2007
PSAPP (Tg2576 + PS1)	APP695swe and PS1 M146L Hamster PrP, PDGFβ	3-6	Yes / Nr / Minor	3-6	Holcomb et al., 1998, Holcomb et al., 1999 Takeuchi et al., 2000.
APP/PS1	APP751swe and PS1 L166P Murine Thy1.2	2	Yes / No / Yes	6	Radde et al., 2006, Bayer and Wirths, 2008
TAPP (Tg2576 x JNPL3)	APP695swe, 4R tau P301L Hamster PrP, Murine PrP	9-11	Yes / Yes / Nr	6	Lewis et al., 2001
3xTg-AD	APPswe, PS1 Finn, tau P301L Hamster PrP, Murine PrP, Murine Thy1 (PS1 knock in)	6	Yes / Yes / Nr	4	Oddo et al., 2003a, Oddo et al., 2003b, Billings et al., 2005

Table 2. Selected rodent models of Alzheimer's disease

Rat AD models	Transgene and promoter	Amyloid pathology age of onset (months)	P-tau / NFT / Cell loss	Memory impairment age of onset (months)	References
TgAPPswe	APPswe PDGF	No	Nr / Nr / Nr	Attenuated memory decline	Ruiz-Opazo et al., 2004
UKUR28	APP751swe/ind PDGF	No	Nr / Nr / Nr	Nr	Echeverria et al., 2004a
UKUR25	APP751 swe/ind and hPS1 Finn PDGF	No	Yes / Nr / Nr	16 (mild impairment)	Echeverria et al., 2004a, Echeverria et al., 2004b
Tg6590	APPswe Ubiquitin-C	15 *	Unsure / No / Nr	9 (or earlier)	Folkesson et al., 2007, Kloskowska et al., 2008
Tg478	APP695 swe rat synapsin I	No	Nr / Nr / Nr	Nr	Flood et al., 2007
Tg1116	APP swe/lon (APP exons 6-9) PDGFβ	Nr	Nr / Nr / Nr	Nr	Flood et al., 2007
Tg478/Tg1116/ Tg11587	APP swe/lon and hPS1 Finn	9	Nr / Nr / Nr	7	Flood et al., 2007, Liu et al., 2008
APP21 and APP31	APP695 swe/ind Ubiquitin-C	Nr	Nr / Nr / Nr	Nr	Agca et al., 2008

APPswe = APP with the "Swedish" K670N/M671L mutation; APPind = V717F "Indiana" mutation; APPlon = APP with the V717I "London" mutation; APP exons 6-9 allowing for alternative splicing to 695, 751 and 770 isoforms); PS1 Finn = presenilin 1 with the M146L Finnish mutation; PDGF - platelet-derived growth factor; PrP = prion promoter; Thy1 = Thymocyte differentiation antigen 1 promoter; P-tau = phosphorylated tau immunoreactivity; Nr = not reported

* Mainly Aβ deposition in the cerebral blood vessels.

Several models expressing mutated tau have also been developed. Although no tau mutations have been reported in AD patients, they do cause other dementia disorders like fronto-temporal dementia associated with chromosome 17 (FTDP-17), proving that tau dysfunction can cause memory deterioration and neuronal loss on its own. Data from these models have allowed for a better understanding of the biophysical and pathological properties of tau polymers in dementia (Brandt et al., 2005, García-Sierra et al., 2008). The only rodent tau-based model relevant for AD is the rat developed by Novak's group (Zilka et al., 2006). This transgenic rat expresses a truncated form of the human tau protein (truncated at amino acid positions 151–391), which is found in the brains of sporadic AD patients. Work on this rat has showed that the truncated tau can drive neurofibrillary aggregation and decrease the life span of the animals without causing any neuronal loss in the hippocampus or brain stem (Zilka et al., 2006, Koson et al., 2008).

More recently, a triple transgenic mouse line (3xTg-AD) expressing mutated APP, presenilin and tau has been described (Oddo et al., 2003a). These mice develop both amyloid plaques and neurofibrillary tangles, and the amyloid accumulation precedes tau pathology. Whereas the 3xTg-AD can prove useful in pharmacological research aimed at eliminating both the tau tangle and amyloid aggregation, it might be less suitable in evaluating approaches aimed at the processes initiating the pathological AD cascade, and less informative about the basic mechanisms of the disease.

The differential contribution of Apolipoprotein E alleles in the pathogenesis of the disease has also been verified in transgenic mice. Crossing the PDAPP or Tg2576 mice with ApoE knockout mice results in strongly reduced plaque pathology, and in the PDAPP mice also induces a redistribution of Aβ deposition (Bales et al., 1997, Holtzman et al., 2000, Irizarry et al., 2000). When ApoE3 or ApoE4 is re-introduced into these animals the dense Aβ neuritic plaques re-appear, with the AD risk-factor ApoE4 allele inducing a 10-fold higher plaque density as compared to ApoE3 (Holtzman et al., 2000). On the other hand, overexpression of the ApoE2 in the PDAPP and Tg2576 reduces amyloid-induced dendritic spine loss in the hippocampus of these animals, emphasizing the AD-protective effect of this allele (Lanz et al., 2003).

Whereas transgenic mice have been easier to create, rats are the preferred species by behavioral scientists and physiologists (Abbott, 2004). Several single- and multitransgenic rat models of AD have emerged in the last few years offering a promising new era for AD pharmacological research. Being available for a much shorter time, the rat models are not yet as well characterized as the mouse lines with respect to the pathology and memory deterioration (Table 2 and paper II in this thesis).

Besides the transgenic technique, perhaps worth mentioning is a novel approach to model AD in animal models, where virus mediated gene transfer is used to introduce APPswe or Aβ fragments selectively into the animal's hippocampus. APPswe transfected rats have shown Aβ42 immunoreactivity in the vicinity to the injection sites but no plaques nor signs of neurotoxicity up to 15 months post-transfection (Gong et al., 2006). Nevertheless the animals have impaired memory retention in the probe phase of Morris water maze task.

In another rat strain cDNAs encoding APPswe or a fusion between human Aβ (40, 42 or both) and a transmembrane protein BRI, which is involved in amyloid deposition in British and Danish familial dementia, have been virally introduced into the hippocampus of adult animals (Lawlor et al., 2007). Of the four transfected groups only the BRI-(Aβ42) animals have showed diffuse plaque-like structures in the hippocampus three months post-infusion, but had no impairment in the open-field or water maze tests. BRI-(Aβ40+42) infused animals on the other hand, exhibited no extracellular Aβ depositions but showed altered behavior both in the open-field test and in the Morris water maze. Surprisingly, no behavioral differences were observed between APPswe infused and control animals. These results as well as our own data demonstrate that rat models can show memory deterioration in the absence of, or long before, amyloid deposition.

Last year, ScienceDaily reported the interesting news of a transgenic pig, which could act as a novel model of AD disease (Jorgensen et al., 2007). No information about the nature of the transgene was given and no further data about this animal has been reported thus far. If the breeding of these animals is successful, it could be a large step forward for AD research, due to the higher similarity between humans and pigs, as compared to rodents.

Calcium and its role in the cell

Calcium is an important intracellular messenger in the brain, being essential for neuronal development, synaptic transmission and plasticity, and the regulation of various metabolic pathways (reviewed in Berridge et al., 2003). At rest, cytosolic free calcium levels ($[Ca^{2+}]_i$) in neurons are maintained at 50-300 nM, and rise rapidly to the low micromolar range upon electrical or receptor-mediated activation. Most of the intracellular Ca^{2+} ions are bound to various calcium-binding proteins, or stored within specialized stores (see next paragraph). Extracellular calcium concentrations are several magnitudes higher, about 2 mM. Calcium ions can enter the cell via a myriad of channels including voltage-gated calcium channels and various ligand-gated channels, such as glutamate or acetylcholinic receptors (LaFerla, 2002, Berridge et al., 2003).

The main calcium store in neurons is the endoplasmic reticulum (ER), where calcium concentrations reach 100-500 μM. Calcium can be released from the ER through the inositol-1,4,5-trisphosphate receptors (IP_3Rs) or ryanodine receptors (RyRs). The IP_3R pathway is initiated by activation of G proteins on the cell surface, which induce phospholipase C (PLC) to cleave phosphatidylinositol-4,5-biphosphate to diacylglycerol and IP_3 (LaFerla, 2002). Resting $[Ca^{2+}]_i$ is controlled by means of a variety of calcium-buffering systems such as calbindin, calretinin or parvalbumin, as well as active uptake of cytosolic Ca^{2+} ions by the sarco ER Ca^{2+}-ATPase (SERCA) and the mitochondrial uniporter, together with extrusion of Ca^{2+} ions across the plasma membrane by the plasma membrane Ca^{2+}-ATPases (PMCA), the Na^+/Ca^{2+} exchanger (NCX) (Berridge et al., 2003).

Transient $[Ca^{2+}]_i$ rises can induce different cellular effects, depending on their spatial distribution (local or global $[Ca^{2+}]_i$ increases) and temporal aspects (lasting seconds or minutes). Intracellular signaling can also be mediated by $[Ca^{2+}]_i$ oscillations of different frequency and amplitude (reviewed in Berridge, 1997, Berridge et al., 2003). Synchronized $[Ca^{2+}]_i$ oscillations, driven by cellular depolarizations, have been described in many types of networked neurons in vitro, in the absence of external stimuli (Murphy et al., 1992, Bacci et al., 1999, Tang et al., 2003, Ruscheweyh and Sandkühler, 2005). They are believed to represent neuronal interactions and are thought to play an important role in synaptic plasticity and information processing. The frequency and amplitude of $[Ca^{2+}]_i$ oscillations has been shown to regulate gene expression, neuronal axon outgrowth and long distance wiring within the developing cortex (Dolmetsch et al., 1998, Li et al., 1998, Tang et al., 2003, Garaschuk et al., 2000). The oscillations have been shown to be driven by calcium entering the cell from the extracellular space, via NMDARs and voltage-activated channels, followed by Ca^{2+}-induced Ca^{2+} release from the endoplasmic reticulum (Bacci et al., 1999, Dravid and Murray, 2004). Increased $[Ca^{2+}]_i$ is rapidly restored to

baseline levels by calcium buffering systems, and active calcium removal (Berridge et al., 2003, Hernández-SanMiguel et al., 2006, Ishii et al., 2006). These synchronized neuronal firings might play an important role not only during physiological but also during pathological events such as epileptic seizures (Nadkarni and Jung, 2003). Aberrant epileptiform neuronal activity has also been described in AD patients and AD mouse models overexpressing human mutated APP (Lozsadi and Larner, 2006, Palop et al., 2007).

Calcium signaling upstream of AD-associated pathology

Given the pivotal role of calcium in cellular signaling, it is not surprising that any alterations in calcium homeostasis can have a profound effect on the cells well-being and ultimately fate. Calcium dysregulation might lie upstream of AD pathology, as proposed by the "calcium hypothesis of brain aging and Alzheimer's disease", and indeed cytosolic calcium levels have been suggested to control APP processing. The effects of calcium might depend on the source of this ion. Increasing $[Ca^{2+}]_i$ by treating cells with the calcium ionophore A23187 or by depolarization, leading to influx of Ca^{2+} from the extracellular space, leads to increased production of Aβ, and perhaps more so of intraneuronal Aβ42 species (Querfurth and Selkoe, 1994, Pierrot et al., 2004). On the other hand, treating cells with the endoplasmic reticulum SERCA pump inhibitor, thapsigargin, results in increased $[Ca^{2+}]_i$ due to inhibition of Ca^{2+} re-uptake into the ER, and increases sAPPα secretion (Buxbaum et al., 1994). Interestingly, in this last study, 10 nM of thapsigargin induced a parallel increase in Aβ secretion, whereas 20 nM of the same drug depressed Aβ secretion.

A recent study has demonstrated that a polymorphism in CALHM1, a putative novel component of a yet uncharacterized Ca^{2+}-channel family, is associated with an increased risk of late onset AD (Dreses-Werringloer et al., 2008). The protein is expressed in the hippocampus where it forms a trans-membrane calcium channel and affects $[Ca^{2+}]_i$ homeostasis. Its expression has been shown to negatively control intracellular Aβ levels, favoring the non-amyloidogenic APP processing pathway.

Aβ aggregation itself and fibril formation can be accelerated by 100 μM Ca^{2+}; that is levels much lower than normally present in the extracellular space (Isaacs et al., 2006). Likewise, depolarization-induced Ca^{2+} influx potentiates tau phosphorylation (Pierrot et al., 2006). Since abnormal tau hyperphosphorylation has been shown to promote the self-assembly of tau into PHF tangles, this suggests a possible involvement of calcium dysregulation in tau fibrillary aggregation (Lindwall and Cole, 1984, Alonso et al., 2001).

Calcium signaling downstream of AD-associated pathology

Aberrant Ca^{2+} homeostasis is also induced by many of the factors associated with AD. For example, every FAD-associated mutation in PS1 and PS2 that has been studied so far, as well as presenilin deficiency, disrupts calcium signaling (reviewed in LaFerla, 2002). The presenilins seem to specifically affect ER-mediated Ca^{2+} responses by inducing overloading of these intracellular Ca^{2+} stores. At least some of these effects, resembling Ca^{2+} alterations in APP deficient cells, can be reversed by reintroduction of AICD expression (Leissring et al., 2002). However, presenilins affect not only APP cleavage and Aβ production, but also seem to be directly involved in controlling the ER Ca^{2+} levels, independently of their γ-secretase activity. Wild-type presenilins have been shown to form divalent-cation-permeable ion channels in lipid membranes, which allow for passive leakage of excessive Ca^{2+} ions from the ER (Tu et al., 2006). Disruption of this function by FAD-linked mutations leads to uncontrolled accumulation of Ca^{2+} in the ER. Recently, the SERCA pump activity has been shown to be physiologically regulated by the presenilins (Green et al., 2008). Whereas increased SERCA activity or expression results in higher Aβ40 production, pharmacological inhibition or siRNA knockdown of SERCA expression lowers Aβ40 and Aβ42 levels. Other calcium-related proteins, with which presenilins interact directly and possibly modulate calcium homeostasis include: calsenilin, calmyrin, μ-calpain or sorcin, the RyR channel modulator (LaFerla, 2002).

ApoE has also been demonstrated to alter intracellular Ca^{2+} levels. External application of ApoE4 (100 nM) to cultured hippocampal or cortical neurons can increase $[Ca^{2+}]_i$ by 70% and the Ca^{2+} influx has been shown to mediate the neurotoxic effect of this allele (Veinbergs et al., 2002). ApoE activates the P/Q type Ca^{2+}-channels, with the ε4 isoform leading to highest and ε2 to lowest increases in $[Ca^{2+}]_i$ and Aβ seems to additionally potentiate this effect (Ohm et al., 2001). ApoE4 induced increase in $[Ca^{2+}]_i$ also seems to involve Ca^{2+} influx via the L-type calcium channels, NMDA receptors and ER RyR-channels (Ohkubo et al., 2001).

APP and calcium

The relationship between calcium and APP is reciprocal. Not only does calcium homeostasis affect APP processing, but also virtually every important derivative of this protein (the secreted ectodomain, Aβ, β-carboxyterminal fragments and AICD) have been shown to modify Ca^{2+} signaling. Likewise, cells carrying mutations in APP exhibit altered Ca^{2+} homeostasis. Cultured fibroblasts from individuals with the APPswe mutation show reduced bombesin-induced increase in $[Ca^{2+}]_i$, as compared to fibroblasts from control individuals and are less sensitive to low concentrations of bradykinin (Gibson et al., 1997). Both of these substances stimulate the release of Ca^{2+} from intracellular calcium stores. Wild-type APP can also induce alterations in

Ca^{2+} signaling. Cortical neuronal cells from a mouse trisomy 16 model, resembling the human trisomy 21 (Down's syndrome), show increased basal $[Ca^{2+}]_i$ and enhanced calcium response to glutamate, NMDA, AMPA and kainate stimulation (Cárdenas et al., 1999). Knocking-down APP by the siRNA antisense technique, to levels comparable to those found in normal mice, normalizes the Ca^{2+} responses to these neurotransmitters in cells derived from this mouse model (Rojas et al., 2008). In another experiment, performed in wild-type cultured mouse cortical neurons, activation of endogenous mouse APP by an antibody directed to its extracellular domain has been shown to elevate $[Ca^{2+}]_i$ by the release of calcium from intracellular stores and the induction of extracellular calcium entry through store-operated Ca^{2+}-channels (Bouron et al., 2004).

Overexpression of wild-type human APP751 in postnatal type II skeletal muscles of a transgenic mouse line elevated resting $[Ca^{2+}]_i$ and increased membrane depolarization in a subset of dissociated muscle fibres (Moussa et al., 2006). The accumulation of Aβ in muscle fibers and resulting calcium dyshomeostasis has been proposed to underlie the aberrant muscle weakness seen in these animals with increasing age (Moussa et al., 2006).

Externally applied Aβ, or the expression of carboxyterminal APP fragments (C99 or C105) have been shown to affect the function of multiple membrane proteins (reviewed in Kourie, 2001). The resulting increase in $[Ca^{2+}]_i$ can involve release of Ca^{2+} from intracellular stores and influx from extracellular space, as well as perturbation of Ca^{2+} removal from the cytosol. Aβ has been shown to stimulate IP_3 production, directly affect the function and expression of RyRs, interfere with the normal function of plasma membrane Ca^{2+} channels and ionotropic receptors (including voltage-gated calcium and potassium channels, the NMDA receptor and the nicotinic receptor), and inhibit ion pumps and exchangers (Wang et al., 2000, Kourie, 2001, Molnár et al., 2004, Supnet et al., 2006). Such a wide range of effects of a single peptide might be due to its ability to insert into lipid bilayers and induce membrane lipid peroxidation, which would affect the function of membrane proteins (Butterfield et al., 2002). Aβ itself is also capable of forming transmembrane ion-permeable channels (reviewed in Kagan et al., 2002).

Whereas Aβ-containing fragments tend to increase intracellular calcium, which leads to cell death, the secreted APP can protect against Aβ- or glutamate-induced toxicity and normalize cytosolic calcium levels (Mattson et al., 1992, Kim et al., 2000, Mattson et al., 1993, Goodman and Mattson, 1994). The stabilizing effects of sAPPα have been proposed to involve activation of the charybdotoxin-sensitive potassium channels (leading to membrane hyperpolarization) and/or NF-κB signaling pathway (Furukawa et al., 1996a, Guo et al., 1998).

Cells with reduced AICD levels due to γ-secretase inhibition or increased AICD degradation and cells lacking APP have reduced endoplasmic reticulum [Ca^{2+}] but elevated resting cytosolic [Ca^{2+}], suggesting that AICD plays a physiological role in regulating intracellular calcium signaling (Leissring et al., 2002, Hamid et al., 2007). However, these findings from HEK293, human neuroglioma cells (H4), mouse fibroblasts or primary mouse astrocytes could not be replicated in human salivary epithelial cells, when γ-secretase activity was inhibited, suggesting that there might be differences in the effects of AICD in different tissues (Oh and Turner, 2006).

AIMS OF THE STUDY

APP is a key molecule in the pathogenesis of Alzheimer's disease. The overall aim of this thesis was to study the etiology of AD using a mutated APP, causative of the disease in a Swedish family.

The first two papers describe the generation and characteristics of our Tg6590 APPswe transgenic rat. The specific aim of this part of the project was to create an animal model of the disease, which would be suitable for studying the course of the disease and helpful in pharmacological AD research. The last two papers focus on the molecular pathways involved in APPswe pathogenesis. By studying the alterations in calcium signaling in hippocampal neurons expressing APPswe, we hoped to find a cellular mechanism which might also be relevant for sporadic AD and which would help us to understand the disease better.

RESULTS AND DISCUSSION

APPswe transgenic rats

At the time when we started working on our transgenic rats, there were no other rat models of AD within the research field. The available mouse models had proven very useful in recapitulating many aspects of the disease, but there was a need for a model better suited for more advanced studies, such as serial CSF sampling, electrophysiology, neuroimaging or complex behavioral testing. The rat fulfills these criteria, due to the mere fact that it is a bigger and more intelligent animal than the mouse, as well as a flexible learner (Abbott, 2004).

Paper I describes the generation of our APPswe transgenic rat. The rat was generated by pronuclear injection of a cDNA construct carrying the human APP695 with the double "Swedish" mutation. We chose the APP695 sequence as it is the major APP isoform expressed by neurons. The transgene expression is driven by the ubiquitin (UbC) promotor, which shows a ubiquitous expression pattern similar to the human APP promotor.

Expression of the APP protein was confirmed in two fertile lines named Tg6590 and Tg6601. The latter had low APP overexpression levels and we were never able to breed it to homozygosity. The Tg6590 rat line shows mainly neuronal APP expression, with highest levels in the cortex, hippocampus and cerebellum, which is similar to the expression pattern found in the Tg2576 and PDAPP mice lines (Irizarry et al., 2001). This finding is interesting, since different promoters were used in each of these models (PrP in Tg2576 and PDGF in PDAPP mice).

Human APP is processed in the transgenic rat brain; with caudate putamen showing the highest levels of the α-secretase cleaved fragment and hippocampus showing the highest levels of the β-secretase cleaved fragment. Secreted human APP fragments are also found in the CSF of transgenic rats. Homozygous Tg6590 rats older than 15 months of age begin to show Aβ42 and fainter Aβ40 accumulation, mainly in the cerebral blood vessels. Only very rare diffuse Aβ42 immunoreactive plaques can be found in the deep cortical layers of these rats. At the time of writing paper I, we were not able to measure Aβ levels in our animals. However, we later showed in paper II that compared to control rats, the levels of soluble Aβ40 and Aβ42 were statistically significantly elevated by more than 65% in the hippocampus and more than 40% in the cortex of transgenic animals.

Although we originally reported higher levels of tau phosphorylation at the double serine 396 and 404 sites in the older transgenic animals, the differences between transgenic and control rats were not statistically significant, when additional animals were analyzed. This lack of significance is due to a surprisingly high heterogeneity in tau phosphorylation levels between the different animals within each group, which has been also observed in the rat study from Cuello's group, where the same 396/404 phosphorylation-specific PHF-1 antibody was used (Echeverria et al., 2004b). At present, we do not have any explanation for this finding, but intend to study a larger animal cohort and determine the distinctive localizations of tau phosphorylation.

Behavioral studies and brain MRI analysis of the Tg6590 rats

In paper II we continued the characterization of our homozygous transgenic male rats by behavioral testing and magnetic resonance imaging (MRI) of the brain. We had originally planned to assess differences between older transgenic and control animals, but due to unexpected loss of these aged animals, we had only a small group available for testing. Our preliminary data from the Morris water maze test showed spatial memory deficits in 14-months old transgenic males as compared to controls. The animals used in behavioral testing, were analyzed by MRI two months later. Due to further loss of animals, we had an even smaller group available for testing at the time. The MRI data suggested enlarged ventricles and reduced cortex thickness in the then 16-months old transgenics, but we cannot draw any general conclusions about brain atrophy in rats of this age before analyzing a larger cohort. The protocol used for the original MRI scans did not allow for a clear visualization of the hippocampus area, so we have not assessed the volume of this region.

We then proceeded with behavioral analysis of nine months old male rats by Morris water maze and open-field tests. As in the older group of animals, these younger transgenic rats showed inferior spatial memory when compared to control rats, both during the acquisition phase of the Morris water test, when the animal is supposed to learn and memorize the position of the hidden platform as well as during the retention phase testing how well the animal remembers the previous position of the platform. Since these tasks are thought to be hippocampal-dependent, our results suggest deterioration of hippocampal function in the transgenic animals. The hippocampus region is the first brain area to be affected in humans, and visuospatial memory problems can also be detected at an early stage of AD, already in MCI patients (Alescio-Lautier et al., 2007).

The transgenic rats showed altered behavior during the Morris water test in terms of the percent of time spent circling along the edges of the pool (thigmotaxis) instead of actively searching the

platform. Thigmotactic behavior has been interpreted differently by various researchers – as a measure of escape behavior, increased anxiety, behavioral inflexibility or inability to switch to an appropriate search strategy (Kallai et al., 2007).

The nine months old transgenics also behaved differently in the open-field test, designed to measure such behavioral responses as locomotor activity, hyperactivity, anxiety and exploratory behaviors. During the first 40 minutes of the test, the transgenic rats displayed significantly less rearing counts than control rats, whereas no differences were found in the total distance moved or time spent in the periphery versus the central area of the open-field. The differences between the transgenic and control rats diminished when the entire 60 minutes period of the test was analyzed, which is not surprising considering that the animals gradually became accustomed to the new surroundings. Rearing activity of rodents is thought to represent a way of orienting in a novel environment, and can also be interpreted as a measure of the animal's motivational state and general arousal level (Sadile, 1996). Taken together with the thigmotaxis behavior, we believe these results reflect deficits in attention in our transgenic rats, an impairment which is also characteristic for human patients during the early stages of AD (Levinoff et al., 2005, Alescio-Lautier et al., 2007). Such attention deficits could be brought on by default activation and/or deactivation of neuronal networks, as has been shown for MCI and AD patients subjected to a computer-based visual navigation task (Buckner et al., 2005, Drzezga et al., 2005).

Considering the altered behavioral patterns of the transgenic animals, and interesting results from the first MRI scans on older rats, we also performed MRI analysis on this younger rat group. This was done two months after behavioral testing, when the animals were 11 months old. For these measurements we used an improved MRI protocol, which gave a better resolution of the different brain regions and specifically the hippocampus region. Careful analysis of both the hippocampus and cortex did not reveal any measurable diminution of these structures, suggesting that brain atrophy might reach detectable levels only when the animals become older (16 months of age). Our results, together with a lack of neurofibrillary pathology, suggest that the Tg6590 rat is a model of early AD, rather than representing the later stages of the disease. Since rats are intelligent animals and very suitable for behavioral analysis, the Tg6590 should prove a good model for testing new therapeutic strategies aimed at improving memory in AD patients before the disease inflicts gross damage to the brain.

Our results from the rat study are consistent with the idea that early memory impairment in AD reflects a dysfunction of neuronal networks (Palop et al., 2006, Rowan et al., 2007), whereas the death of neurons is a later event. Soluble Aβ oligomers have emerged as toxic species responsible for inducing reversible memory loss in rodents (Lesné et al., 2006, and reviewed in

Catalano et al., 2006, Shankar et al., 2007) and therefore most probably also responsible for triggering the progression of dementia in human patients. Lesné et al. have pointed out a specific Aβ oligomer, named Aβ*56, which induces memory impairments in rats through transient physiological silencing, rather than permanent neuropathological destruction of neurons. It would be very interesting to see if our rats show the presence of this Aβ*56 in the brain.

Calcium signaling in primary hippocampal cultures derived from the Tg6590 transgenic rats

Destabilization of calcium homeostasis has been implied in AD, but not that many studies have concentrated on the effect of APP or FAD-causing mutations in APP on calcium signaling. Much more has been done on the presenilin FAD-causing mutations, or the effect of exogenous application of Aβ to cell cultures. While interesting, it is unclear if all of the results on Aβ are physiologically relevant, due to the high concentrations of the peptide often used in these experiments. Having established the transgenic rat model, we had the opportunity to study the effect of APPswe on calcium signaling in primary neurons derived from our rat. We chose to study hippocampal neurons, since hippocampus is critical for the early phases of learning and memory and is affected early in the course of AD. Without applying external stimulation, we observed some basic differences in calcium metabolism of transgenic and control neurons. These included increased frequency of spontaneous $[Ca^{2+}]_i$ oscillations and increased basal $[Ca^{2+}]_i$ in the APPswe transgenic neurons. These two findings could potentially be linked to each other, reflecting a mechanism by which the neuron copes with the increased calcium burden. For example, pharmacologically induced calcium oscillations have been shown to protect neurons from $[Ca^{2+}]_i$ increase after trauma (Geddes-Klein et al., 2006). Elevated $[Ca^{2+}]_i$ can induce the activation of calcium-activated enzymes, such as calpains, which has in fact been detected in the hippocampus of APPswe transgenic Tg2576 mice (Vaisid et al., 2007).

Increased frequency of spontaneous calcium oscillations in APPswe transgenic neurons

In order to decipher the molecular entities affecting the frequency of oscillations, we performed a series of experiments, in which we treated control cultures with conditioned medium (in which neurons had grown for 3 days) from transgenic cultures (and vice versa), or stimulated the cultures with agonists and antagonists of the nicotinic and NMDA receptors (paper III). We reasoned that since spontaneous $[Ca^{2+}]_i$ oscillations are thought to be induced by sequential Ca^{2+} influx through NMDA receptors and voltage gated Ca^{2+} channels, and Aβ has been shown to (over)activate the NMDARs (Bacci et al., 1999, Wu et al., 1995b, De Felice et al., 2007), then

this pathway might be responsible for the altered frequency of $[Ca^{2+}]$ oscillations in transgenic neurons. We found however no effect of conditioned medium from transgenic neurons on the frequency of spontaneous $[Ca^{2+}]_i$ oscillations in control neurons, indicating that secreted APP derivatives (sAPPα, sAPPβ or Aβ) did not mediate this effect. Since we waited 48 hours after application of conditioned medium, before performing calcium measurements, we cannot exclude a direct and transient effect of, for example, monomeric Aβ on the ionic channels mediating calcium fluxes. Another possible explanation would be that either full-length membrane-bound APP or intracellular APP derivatives (Aβ, p3 or AICD) were responsible for altering the frequency of $[Ca^{2+}]_i$ oscillations in our transgenic neurons. We were not able to measure Aβ levels in our primary cultures, but considering that the transgenic rats showed elevated levels of both the Aβ40 and Aβ42 species (measured after publication of paper III) and that we had increased APP expression in the transgenic primary cultures (27% more than in control cultures), we suspect that Aβ was also elevated in the transgenic primary neurons.

We found no major differences in the calcium responses of the transgenic and control neurons to direct stimulation with NMDA or the NMDAR antagonist ketamine. Nicotinic potentiation of NMDAR mediated currents (Yamazaki et al., 2006) or blockade of the nicotinic acetylcholine receptor α7 subtype by α-bungarotoxin had similar effects on the $[Ca^{2+}]_i$ oscillations in the transgenic and control neurons. This suggests that neither the NMDA nor the nicotinic pathways are involved in increasing the frequency of spontaneous $[Ca^{2+}]_i$ oscillations in the APPswe transgenic cultures. Recent work by Li et al. (2008) demonstrated that the frequency of spontaneous $[Ca^{2+}]_i$ oscillations could be modulated via the mammalian target of rapamycin kinase (mTOR) pathway without directly affecting the permeability of ion channels. mTOR is an atypical serine/threonine protein kinase involved in transcription, ubiquitin-dependent proteolysis, and microtubule and actin dynamics, all of which are crucial for neuronal development and long-term modification of synaptic strength (Jaworski and Sheng, 2006). In primary hippocampal neurons, rapamycin, the specific inhibitor of mTOR was shown to decrease the frequency of $[Ca^{2+}]_i$ oscillations, whereas insulin, the upstream activator of mTOR (mediated via Akt phosphorylation) was shown to increase the oscillation frequency (Li et al., 2008). Very high concentrations (20 μM) of externally added Aβ42 and to a lesser extent Aβ40, have been shown to inactivate mTOR signaling by decreasing phoshorylation of mTOR in murine Neuro-2A cells (Lafay-Chebassier et al., 2005). Interestingly, in cultured hippocampal neurons sAPPα activates the phosphatidylinositol-3-kinase (PI_3K)-Akt kinase signaling pathway acting upstream of mTOR (Cheng et al., 2002) and thus might have an opposing action to Aβ on mTOR signaling. We have however not tested whether the mTOR pathway is involved in the altered frequency of $[Ca^{2+}]_i$ oscillations in our transgenic neurons.

Altered response to hyperosmotic stress in APPswe transgenic neurons

In paper IV, we investigated the responses of our APPswe transgenic neurons to modest hyperosmotic stress induced by sucrose. A greater understanding of the adaptive responses to osmotically induced cell stress are important physiologically, since increased osmolarity can be encountered by neurons during such pathophysiological conditions as diabetic hyperglycemia or after ischemic stroke, which in their turn are associated with an increased risk of developing AD later in life (Haan, 2006, Honig et al., 2003).

We found that whereas mild hyperosmotic stress (50 mM sucrose) decreased the amplitude of spontaneous $[Ca^{2+}]_i$ oscillations in control hippocampal neurons, it led to increased amplitude in the transgenic neurons. Since we found no evidence for overloading of the major intracellular calcium store – the ER – in the transgenic neurons, we concluded that ER did not contribute to the altered response of APPswe transgenic neurons to hyperosmosity. This differential effect could however involve altered activation of L-type voltage gated calcium channels. Under basal isotonic conditions, we saw a small but significantly greater sensitivity of the transgenic neurons to low (100 nM) concentrations of the L-type channel antagonist, nimodipine. At higher concentrations of this drug the transgenic and control neurons responded similarly. After a prolonged (30 hours) hyperosmotic stress, the sensitivity to nimodipine increased in the transgenic neurons and differed significantly from the responses of control neurons at all nimodipine concentrations tested (100 nM, 1 μM and 10 μM). With the highest nimodipine concentration (10 μM) transgenic neurons pretreated with sucrose for 30 hours showed a marked increase in sensitivity to nimodipine as compared to non-pretreated neurons. Since other voltage gated channels, such as N-type calcium channels as well as potassium and sodium channels have also been shown to be blocked by higher concentrations (≥10 μM) of compounds of the dihydropyridines family (including nimodipine, nitrendipine and nifedipine), it is plausible that their function may also be altered in the APPswe transgenic neurons. Increased activation of L-type as well as non-L-type calcium channels has also been implicated to be partly responsible for the elevated resting $[Ca^{2+}]_i$ in cortical neurons derived from adult 3xTg-AD mice harboring the APPswe, tau P301L and PS1 M146V AD mutations (Lopez et al., 2008). Whereas the 3xTg-AD neurons also showed enhanced contribution of IP_3R channel mediated Ca^{2+} efflux from intracellular stores to their steady-state $[Ca^{2+}]_i$ levels, we did not observe any differences in the responses of our transgenic and control neurons to stimulation of either the IP_3 or ryanodine receptors of the ER. These results indicate that PS1 rather than APP is involved in altering the ER calcium homeostasis in 3xTg-AD neurons.

A very recent paper demonstrated that alternative RNA splicing can modulate voltage- and Ca^{2+}-dependent gating of $Ca_V1.4$ and $Ca_V1.3$ L-type Ca^{2+} channels (Singh et al., 2008), which

have been shown to possess a pacemaker function in neurons and ability to shape neuronal firing (Chan et al., 2007, Helton et al., 2005). A longer splice variant harbors a C-terminal modulator, which can also regulate the binding of calmodulin to the channel. The absence of this modulator domain facilitates activation of the channels at lower, subthreshold voltages. If APP can physically bind to this modulatory domain of the L-type channels, then we could expect this interaction to influence the function of the channels. A direct interaction of APP and L-type channels has been suggested, although not yet proven, at the recent Alzheimer's Association International Conference on AD in Chicago, July 26-31, 2008 (poster presentation by Susana Ferrao Santos and personal communication with Jean-Noel Octave). It remains to be determined whether and how the APPswe mutation affects this potential interaction.

To summarize, we have demonstrated that APPswe induces complex alterations of cellular calcium homeostasis in hippocampal neurons, which – we believe – are at least partly responsible for the memory deficits seen in the APPswe transgenic Tg6590 rats.

CONCLUSIONS AND FUTURE PERSPECTIVES

Alzheimer's disease is a frightening disorder, the more so that we are not yet capable of significantly delaying or stopping the progression of the disease. The insight that the earliest signs of memory deterioration might be more due to a dysfunction of synaptic networks, rather than a permanent loss of neurons (Palop et al., 2006) gives hope that one day we might be able to treat the disease better. Hopefully, if we can prevent the earliest synaptical disturbances, then secondary brain deterioration will also be halted. It is my belief that animal models showing memory impairment, but where the pathology is not pushed to the extremes, will prove most suitable for pharmacological research aimed at these earliest AD-related events.

Our APPswe transgenic rat Tg6590, described in papers I and II, shows behavioral alterations by nine months of age, if not earlier, followed by amyloid accumulation in the cerebral vessels and deep cortical layers after the age of 15 months. It seems plausible to conclude that the pathological abnormalities are a consequence of the elevated Aβ40 and Aβ42 levels, seen in the hippocampus and cortex of these animals. The model has not yet been fully characterized, as we need to determine the exact onset of memory deterioration and need more animals to perform more reliable assessment of tau-phosphorylation, MRI volumetry or synaptical and neuronal density in animals of different ages. Functional MRI (fMRI) or PET imaging would allow one to asses whether brain activity is altered before the onset of measurable memory impairment in these animals, as has been shown for human FAD-carriers (Mondadori et al., 2006). Since women are said to be at a greater risk of developing AD than men, it is also of importance to evaluate the cerebral pathology and performance of both male and female Tg6590 animals.

To our knowledge the impact of APPswe alone on intracellular signaling has not been studied extensively thus far. Our findings, that modest overexpression of APPswe induces measurable alterations of calcium homeostasis in primary hippocampal neurons from our transgenic rats (paper III and IV), is of considerable significance for a better understanding of the pathogenetic mechanisms of this mutation. Whether or not the same signaling pathways are also involved in sporadic AD remains to be determined. However, considering that the overproduction of Aβ in our rat hippocampal cells is probably modest rather than robust, we believe that the observed signaling alterations are physiologically relevant and share similarity with the pathological mechanisms involved in sporadic AD. Our work still leaves many questions unanswered:

What is the exact mechanism underlying the increased frequency of spontaneous calcium oscillations in our APPswe primary hippocampal neurons?

- Is intracellular Aβ involved, and if so – which species?
- Does APP physically interact with L-type calcium channels and if so – does the APPswe mutation influence the binding?

Hopefully, these can be answered in the foreseeable future.

MATERIALS AND METHODS

Establishment of transgenic rats

Human APP695 containing the Swedish mutation (Mullan et al., 1992) was cloned into the pUBi1Z vector. The pUbi1Z vector was generated by replacing the CMV (cytomegalovirus) promoter in pcDNA3.1/Zeo with the human ubiquitin (UbC) promoter from pTEJ-8 (Johansen et al., 1990), and also removal of the ampicillin resistance in pcDNA3.1/Zeo.

The construct was sequenced and its integrity verified by tranfection into human HEK293 cells, followed by Western blotting for APP. The promoter and APP cDNA were excised from the vector and purified. The transgenic rats were generated by pronuclear injection using Sprague-Dawley rats. Founder animals were identified by PCR using genomic DNA extracted from the tail. Two sets of primers were used, one for amplifying the whole APP coding region using primers APP1,5'-GCGGCCGCATGCTGCCCGGTTTGGC-3'
and APP2, 5'-GGGCCCCTAGTTCTGCATCTGCTC-3' and the second to amplify parts of the promoter and APP using primers pUbC1, 5'-GTTGGCGAGTGTGTTTTGTGAAG-3' and APP3, 5'-AATCGATGTGGTTCTCTCTGTGGC-3'.

The presence of human APP mRNA was investigated by reverse transcription-coupled PCR (RT-PCR). Total RNA from the cortex of transgenic rats was isolated using RNeasy (Quiagen). The RT-PCR reaction was run using Qiagens OneStep RT-PCR Kit and APP primers (see above).

Tissue preparation and Western blotting

The left brain hemisphere was fixed in formalin, and the right dissected and stored at -80 °C until used. Brain tissues were homogenized in 20 volumes of phosphate-buffered saline (PBS) with 1% (w/v) CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate), 2.5 mM EDTA, 2.5 mM EGTA, 2 mM Na_3VO_4, 60 mM NaF, 6 mM glycerol 2-phosphate containing a protease inhibitor cocktail (1:400; Sigma-Aldrich). Homogenates were then gently mixed for 30 minutes at 4 °C, transferred to microcentrifuge tubes, and centrifuged for 90 min at 17,000×g to remove cellular debris. The supernatants were transferred to separate clean tubes, frozen on dry ice and stored at -70 °C.

Protein content was determined using bicinchoninic acid (BCA) protein assay (Pierce). Proteins were separated by electrophoresis on polyacrylamide gels and transferred onto nitrocellulose membranes. Blotted membranes were blocked with 5% w/v non-fat dry milk in tris-buffered saline (TBS) for 60 min and incubated overnight at 4 °C with indicated primary antibodies. The

membranes were washed in TBS-T (TBS with Tween) and incubated with HRP-conjugated secondary antibodies. After washing, antibody binding was visualized using ECL detection system (Amersham). Primary antibodies used in these studies were: mouse anti-human APP [6E10] (Signet, diluted 1:2000), mouse APP N-terminal binding MAB348 [clone 22C11] specific for both human and rat APP (Chemicon, diluted 1:1000), polyclonal rabbit APP C-terminal (Sigma, diluted 1:1000), anti-Neuronal Nuclei [Neu N] (Chemicon, 1:400); phospho-specific tau antibody PHF-1 (generously provided by P.Davies, 1:400); anti-total tau [tau-5] (Biosourse, 1:1000); phospho-specific tau antibody AT8 (Innogenetics, 1:1000); rabbit anti-phospho tau antibodies Tau ps396, ps404 and ps199 (BioSource, 1:1000) and polyclonal rabbit anti-synaptophysin (DakoCytomation, 1:4,000). The secondary antibodies from Amersham were diluted 1:2,500. Optical density of protein bands were quantified using ImageJ software.

Immunohistochemistry

4% formaldehyde-fixed tissues were embedded in paraffin, cut with a microtome to sections 6 μm thick, and mounted onto coated slides. Following deparaffinization, masked epitopes were exposed by treatment with formic acid. 30 minute-wash step in Dako protein block solution (DakoCytomation) was used to reduce non-specific staining. Sections were incubated with primary antibodies overnight and then with biotinylated secondary antibodies for 30 minutes. Biotin-labeled tissue was further processed using ABC Elite HRP reagents (Vector laboratories) and was developed with a solution of hydrogen peroxide (0.003%) and diaminobenzidine (0.02%). Sections from AD patients and controls were used as positive and negative controls respectively, to confirm specificity of the immunohistochemical Aβ42 and Aβ40 staining. Before mounting the sections, they were counter stained with haematoxylin or Congo red.

Aβ quantification

The frozen brains were homogenized (Heidolph instruments- DIAX 100) in 10 volumes (w/v) of 0.2% diethylamine (DEA, Sigma-Aldrich 38,645-6) containing 50 mM NaCl (pH 10) and protease inhibitors (Roche Germany, 11 697 498), AEBSF (Calbiochem 101500), and 0.5% NP-40 (Calbiochem 492016). Samples were incubated 30 min on ice after brief sonication (Bandelin Sonopuls HD 2070), then centrifuged at 20000g, (Ole Dich Microcentrifuge 157.MP.RF) at 4°C for 30 min. The resulting supernatant was retained as the soluble fraction and neutralized by addition of 10% 0.5 M Tris/HCl, pH 6.8. Samples were diluted 1:1 in 8 M Urea (AppliChem A1049, 9025), incubated 30 min at ice and diluted five-fold before Aβ40 or Aβ42 Enzyme-Linked ImmunoSorbent Assay (ELISA) analysis (Wako 294-62501) following manufactures instructions.

Behavioral studies - spontaneous open-field test

Nine months old animals were used for the assessment of spontaneous behavioral activity. Test apparatus consisted of 4 square gray PVC arenas, 70 x 70 x 60 cm each. The rats were placed individually in the center of the arena, and their movements were recorded for 60 min using the Ethovision automated video tracking system (Noldus, The Netherlands). Behavioral parameters that were measured included locomotor activity (distance moved and mean velocity) and vertical activity defined as rearing (standing on hind limbs with the forelimbs in the air or against the wall of the arena) in peripheral and central zones of the field. At the end of each test session the arena was cleaned with 70 % ethanol and water.

Morris water-maze test

Spatial memory was tested in 9 and 14 months old animals. The rats were required to learn the location of a hidden platform by referring to visual cues (consisting of several wall posters approximately 50-75cm in size) placed around the room (Morris, 1984). The circular pool (gray PVC) was 140 cm in diameter and 50 cm in height. Water temperature was maintained at 21±2°C. A plastic transparent platform (9 x 9 cm) was placed approximately 0.7 cm below the water surface and 10 cm from the edge of the pool. One day before start of the experiment, the animals were habituated to the apparatus by being given a trial swim for 60 sec. Rats were transferred to the testing room in a non-transparent cage to avoid visual orientation prior to release into the pool. Release points were balanced across 4 symmetrical positions on the pool perimeter. The acquisition phase, during which the position of the hidden platform remained fixed, lasted 5 days (4 days in the 14 month old rats). 4 trials of 60 sec length with 30 sec inter trial intervals were given per day. Rats that did not find the platform within 60 seconds were placed on the platform and allowed to stay there for 30 sec to assist their learning. To check retention of spatial learning, probe trials were performed on the 5^{th} and 6^{th} day of the experiment (only on the 5^{th} for 14 month old rats). The platform was removed and the animal allowed to swim for 60 sec before being removed from the pool. A visual cue test, with the platform placed visibly above the water surface, was carried out on day 7. All parameters assessed in this test were recorded by an automated video-tracking system (Ethovision, Noldus, Netherlands).

Magnetic resonance imaging

The same animals, which were earlier used for behavioral testing, were later analyzed by magnetic resonance imaging: those which were tested for behavior at 9 months of age, were MRI scanned at 11 months of age, and those which were tested for behavior at 14 months of age were MRI scanned at 16 months of age.

MRI examination of 16 months old rats were performed using a 4.7 T magnet with a horizontal bore (Bruker Biospec Avance 47/40, Bruker, Karlsruhe, Germany) equipped with a 12 cm inner diameter self-shielded gradient system (max. gradient strength 200mTm^{-1}). A circular resonator (Bruker, Karlsruhe, Germany) with an inner diameter of 72 mm was used for excitation and signal detection. Structural images were obtained producing an axial multi slice package consisting of 21 continuous slices through the brain, using spin echo sequences with rapid acquisition with relaxation enhancement (RARE) (Wimmer et al., 1986). The parameters were adjusted as follows: TR 2500 ms, TE 37.4 ms, RARE-factor 8, matrix size 256x256, slice thickness 1 mm, FOV 4 mm and 16 averages.

MRI examination of the 11 months old animals was performed using a 4.7 T, 40 mm bore horizontal magnet (Bruker Biospec Avance 47/40, Bruker, Karlsruhe, Germany) fitted with a 12 cm inner diameter self-shielded gradient system (maximum gradient strength 200 mT/m). A volume coil (Bruker) with 72 mm inner diameter was used for excitation and signal detection. 3D images were obtained using inversion recovery (IR) spin echo sequence with RARE. The parameters were: repetition time (TR) 2.5667 ms, echo time (TE) 8.9 ms, RARE-factor 8 with RARE-maximum 4, inversion delay 500 ms, matrix size 64 x 64 x 128 and 2 averages. Total acquisition time was 1 h 28 min. Field of view (FOV) for the 3D was 1.2 x 2.2 x 3 cm. Image reconstruction resulted in a resolution of 0.19 x 0.34 x 0.23 mm in dorso-ventral, left-right and rostro-caudal directions, respectively.

Rats were anesthetized with 1.5-2.0% isoflurane in air delivered via a mouth piece allowing spontaneous respiration. The rats were then positioned in supine position and the head fixed to an acrylic rig. Body temperature was recorded and maintained at approximately 37 °C using a MRI-compatible air temperature control system.

MRI volumetric analysis

2D images from 16 months old animals were analyzed using ImageJ 1.37V software (http://rsb.info.nih.gov/ij/). The area of the cortex and whole brain were manually delineated on 3 slices approximately -1.3, -3.3 and -5.3 from Bregma point and the areas were calculated by the ImageJ program. The measurements were performed twice and the mean value was used for estimating the cortex:brain ratio. The hippocampus was difficult to distinguish in these images and was therefore not measured.

3D images from 11 months old animals were analyzed using Amira 3.0 software (Mercury Computer Systems, GmbH). The different brain structures were segmented in accordance with the G. Paxinos and C. Watson atlas (The Rat Brain in Stereotaxic Coordinates). Structure volumes were estimated using stereological quantification based on Cavalieri's principle and

point counting (reviewed in Mayhew, 1992). Choice of the first slice used for quantification was semi-random, as it was always the MRI slice where the given structure was first visible (in the direction from anterior to posterior for coronal slices and from dorsal to ventral for horizontal slices). The lateral ventricles were measured in each second contiguous coronal slice. Brain and cortex volumes were calculated from a total of 16 coronal slices (every other slice) between approximately +1.6 and -5.3 from Bregma point, characterized by continuous corpus callosum. Hippocampi were measured in all slices depicting it. The borders of the hippocampus were checked on both horizontal and coronal slices to ensure accuracy and calculations from both orthogonal planes were averaged. The total volumes were calculated by multiplying the estimated area by known slice thickness (0.23 mm). All measurements were performed twice and the mean value calculated. The calculated volumes were divided by the brain volume of each animal, yielding a ratio that is adjusted to the animal's brain size.

Preparation of primary cultures for calcium measurements

Primary hippocampal cultures were prepared from day E17 embryos of wild-type (control) and transgenic heterozygotic Sprague-Dawley rats expressing the human APP695 isoform with the "Swedish" mutation. Hippocampi from embryos of 1-2 mothers were mixed together. Mechanically dispersed cells were plated on poly-D-lysine (Sigma Aldrich) coated 3.5 cm glass bottom Petri dishes (Mattek Corporation) at a density of 450,000 cells/dish and cultured for 12-14 days at 37 °C and 5% CO_2. Serum-free Neurobasal™ medium was supplemented with 2% B27 (serum-free medium supplement enhancing survival of neurons *in vitro)*, 0.5 mM L-glutamine and 100 units/ml penicillin and streptomycin (Invitrogen). Half of the medium was replaced with fresh medium every 3-4 days. Conditioned medium was collected after 3 days of incubation.

Calcium imaging

Cells were loaded with 3-4 μM fluo-3AM in culture medium for 30 minutes at 37 °C in 5% CO_2. Plates were then transferred to Zeiss LSM 510 Meta scanning laser confocal microscope equipped with Plan-Neofluar 40x/1.3 Oil DIC objective (Zeiss) and washed for 5 minutes in standard HHSS buffer containing: 20 mM HEPES (4-(2-hydroxyethyl)-1-piperazinee-thanesulfonic acid), 137 mM NaCl, 1.3 mM $CaCl_2$, 0.4 $MgSO_4$, 0.5 mM $MgCl_2$, 5 mM KCl, 0.4 mM KH_2PO_4, 0.6 mM Na_2HPO_4, 3 mM $NaHCO_3$, 5.6 mM glucose, 10 μM glycine, pH 7.45) before Ca^{2+} imaging. All Ca^{2+} measurements were performed at room temperature (24 -28 °C) in HHSS buffer alone or with the different drugs. Plates with transgenic and control cultures were measured alternately to minimize any temperature-dependent variations during the day. The perfusion rate was constant during the whole course of the experiment and set to 1.3-1.4 ml/min. HHSS supplemented with 2 mM EGTA was used as a "Ca^{2+}-free" buffer. Changes in intracellular Ca^{2+} concentrations ($[Ca^{2+}]i$) from the soma region were followed at an

acquisition speed of 4 images per second. Fluo-3 was illuminated with 488 nm light and the emitted light was collected through a 505-570 nm filter. Laser power was set to 0.5-1% to minimize photobleaching and phototoxicity.

Analysis of calcium imaging data

Cells were defined as neurons based on the abolition of spontaneous activity by 1 μM tetrodotoxin (TTX, a Na^+ channel antagonist which blocks the propagation of action potentials), or by 2 mM EGTA ("calcium-free buffer"). All spontaneously active neurons within the imaging field were analyzed. Calcium fluxes were followed for 2 minutes in 1-3 different imaging fields per plate. $[Ca^{2+}]_i$ oscillations of each neuron were represented as relative changes in fluo-3 fluorescence intensity (F/F_{mean}), where F_{mean} is the mean intensity for the neuron during the 2 min control period. Ca^{2+} spikes were defined as rapid increases (< 1 s) in F/F_{mean} equal to or larger than 20% of F_{mean}. Neurons with a basal F level higher than half of the maximum capacity of the system were excluded from the analysis. The peak calcium levels of these cells was above the maximum measuring capacity of the confocal system and/or showed F/F_{mean} increases of less than 20%. Since the frequency of oscillations was a common feature for the neuronal network within the analyzed field and not a characteristic of single neurons, we compared mean frequencies per imaging field from all 2 min measuring periods.

For NMDA treatment (paper III), F_{mean} was calculated for the 2 min period while NMDA was present in the bathing solution. The amplitude and frequency of oscillations were calculated during a 100 sec period, excluding the rise and fall period as our program could not handle these. A spike limit was set to Δ10% of F_{mean}, as smaller spikes were difficult to distinguish from background noise. The loss of fluo-3 intensity due to photo-bleaching resulted in 7 ± 3.5% drop in amplitude of oscillations in untreated cells per 10 minutes, and was corrected for in the 10 minutes treatment with sucrose (paper IV), by adding 7% to the value obtained for each cell.

Intracellular free Ca^{2+} concentration (paper IV) was determined using the formula:

$$[Ca^{2+}]_i = K_d \times (F-F_{min}) / (F_{max}-F).$$

The dissociation constant K_d for Fluo-3 was taken as 390 nM, as indicated by the manufacturer protocol. Maximal absorbance (F_{max}) of each cell was obtained by lysing cells in high calcium Locke solution (100 mM $CaCl_2$ with 1% V/V TritonX). Background fluorescence (F_{min}) was obtained from an area without cells. Baseline of oscillations was assessed by taking the mean of 50 lowest values during the 2 minute measuring period (equivalent to 16 seconds runtime). Baseline for initial $[Ca^{2+}]$ rise during caffeine treatment was calculated as mean of 10 lowest values (equivalent to 3 seconds runtime) during first 20 seconds after introduction of caffeine.

Analysis and quantification of Ca^{2+} oscillations was performed using MATLAB software with a code written by JK System AB, Stockholm.

MTT cell viability assay

Cell viability (paper IV) was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay, which measures the capacity of mitochondria to convert MTT salt (yellow) to formazan (purple). 22,500 cells were plated in 100 µl medium per well in 96-well plates. The same medium, culturing procedures and cell concentration were used as for calcium measurements. On day 12, half of the medium was replaced with fresh medium with or without 100 mM sucrose resulting in 50 mM final sucrose concentration. 30 hours later 50 µl medium with 0.9 mg/ml of MTT salt was added per well (0.3 mg/ml final MTT concentration) and the incubation was continued for 3 hours. Cells were solubilized in 100 µl DMSO (dimetylsulfoxid) per well and the absorbance was measured in a plate reader at 570 nm.

Statistical analysis

Paper I and II. Body weights and results from behavioral tests were analyzed by repeated measures analysis of variance (ANOVA) using Statview software. Where the ANOVAs showed significant main effects, multiple comparisons were made using either Fisher's PLSD post hoc test, or Tukey's pairwise multiple comparison test. MRI brain structure data was analyzed using ANOVA. Non-parametric Mann–Whitney U tests were used for comparing protein levels. The level of statistical significance was set at $p<0.05$. All data are expressed as mean values ± standard error of the mean (SEM).

Paper III and IV. Statistical analysis of differences between the calcium responses of transgenic and control neurons was performed using a one-way Anova, and a paired t-test was used when comparing the responses of individual neurons before and during treatment.

All experiments were performed in accordance with ethical permission from Stockholm South Ethical Committee.

ACKNOWLEDGEMENTS

I would like to thank all my supervisors: **Eirikur Benedikz, Bengt Winblad** and **Ronnie Folkesson** for accepting me as a PhD student in the very interesting Alzheimer research field. I am grateful for the financial support I have received and for the helpful discussions about my work and general life (as well as worthwhile places to visit in Iceland!). At times it seamed a struggle, but you had not left me alone with it, after all.

A very special thanks to my co-author **Joseph Bruton**, for your belief in my work and ideas, and your help when I actually needed it the most. I really appreciated the scientific discussions we had, even when we did not always agree on our points of view!

Thank you **Eirikur**, **Joseph** and **Dorota** for your valuable comments on this thesis!

I am most grateful to the former members of our lab group: **Tanja** and **Kasia**, for our long discussions on everything from science to life, and for your encouragement when times seamed the toughest for me. **Kasia** – you have also become a very dear private friend. Thanks to you **Tanja** I have visited the beautiful land, Macedonia.

I would also like to wish all the best to all my past and present fellow PhD students: **Alexandra, Anna B, Anna S, Anna Ö, Anne, Annelie, Annika, Atique, Beata, Benoush, Bogdan, Camilla H, Camilla N, Catharina, Cecilla, Daniel, Dorota, Erik, Halinder, Hanna, Hedvig, Helen, Linda, Jenny, Jinghua, Ji-Yeun, Johanna, Maria, Mircea, Nagat, Nahid, Nodi, Sara, Stefan, Susanne, Tina, Wen-Lin, Zhigou** and **Åsa**. Looking back I wish I had taken more active part in the social life at our department. I will always remember the fun and serious chats we had with all my past and present office mates and during our conferences (not to forget the 2004 PhD student skiing conference!).

All the senior scientists and all the people working at Neurotec/NVS and administration – the place wouldn't have been itself without you.

I am grateful for the wonderful friends I have outside the lab who always listened when I needed to talk and who made me forget about everything and just have fun! Most thanks to the "ladies club": **Ania, Beata, Iwona, Iza,** and **Magda**! You are fantastic!

A great thanks to my family (you are also included **Hania**!), and especially my mother for always being there, when I didn't have time to take care of everyday matters myself. **Kristoffer**, you have actually made my two last papers possible, thanks to your calcium oscillations analysis program you have made for me when I started to creep the walls! You have also helped with finding financial solutions, when they were most needed.

My son **Francis** – kochanie. I often wished I had more time for you in the past years!

Finally – I am grateful for the financial support without which this work would have been impossible to perform. The generous supporters include: the Gun and Bertil Stohnes Foundation, Loo and Hans Osterman Foundation, Åke Wibergs Foundation, Magnus Bergwalls Foundation, Knut och Alice Wallenbergs Stiftelse, Alzheimerfonden, Gamla Tjänarinnor Foundation, Stiftelsen Dementia, Swedish Animal Welfare Agency, Swedish Brain Power and Swedish Research Council and JK System AB.

REFERENCES

Abbott A., 2004. Laboratory animals: the Renaissance rat. Nature. 428(6982), 464-466.

Agca C., Fritz J.J., Walker L.C., Levey A.I., Chan A.W., Lah J.J., Agca Y., 2008. Development of transgenic rats producing human β-amyloid precursor protein as a model for Alzheimer's disease: transgene and endogenous APP genes are regulated tissue-specifically. BMC Neurosci. 2008, 9:28.

Alescio-Lautier B., Michel B.F., Herrera C., Elahmadi A., Chambon C., Touzet C., Paban V., 2007. Visual and visuospatial short-term memory in mild cognitive impairment and Alzheimer disease: role of attention. Neuropsychologia. 45(8), 1948-1860.

Allinson T.M., Parkin E.T., Turner A.J., Hooper N.M., 2003. ADAMs family members as amyloid precursor protein α-secretases. J Neurosci Res. 74(3), 342-352.

Almkvist O., 1996. Neuropsychological features of early Alzheimer's disease: preclinical and clinical stages. Acta Neurol Scand Suppl.165, 63-71.

Alonso A., Zaidi T., Novak M., Grundke-Iqbal I., Iqbal K., 2001. Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. Proc Natl Acad Sci U S A. 98(12), 6923-6928.

Anandatheerthavarada H.K., Devi L., 2007. Amyloid precursor protein and mitochondrial dysfunction in Alzheimer's disease. Neuroscientist. 3(6), 626-638.

Andersen O.M., Reiche J., Schmidt V., Gotthardt M., Spoelgen R., Behlke J., von Arnim C.A., Breiderhoff T., Jansen P., Wu X., Bales K.R., Cappai R., Masters C.L., Gliemann J., Mufson E.J., Hyman B.T., Paul S.M., Nykjaer A., Willnow T.E., 2005. Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. Proc Natl Acad Sci., USA. 102, 13461-13466.

Anliker B., Müller U., 2006. The functions of mammalian amyloid precursor protein and related amyloid precursor-like proteins. Neurodegener Dis. 3(4-5), 239-246.

Arancio O., Puzzo D., Privitera L., Leznik E., Fa' M., Staniszewski A., Palmeri A., 2008. Aβ peptide is a positive modulator of synaptic plasticity and memory. Oral presentation at the 2008 ICAD conference in Chicago, abstract no. O4-06-03.

Armstrong R.A., 2005. Is there a spatial association between senile plaques and neurofibrillary tangles in Alzheimer's disease? Folia Neuropathol.43(3), 133-138.

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.

Asaumi M., Iijima K., Sumioka A., Iijima-Ando K., Kirino Y., Nakaya T., Suzuki T., 2005. Interaction of N-terminal acetyltransferase with the cytoplasmic domain of β-amyloid precursor protein and its effect on Aβ secretion. J Biochem. 137(2), 147-155.

Axelman K., Basun H., Winblad B., Lannfelt L., 1994. A large Swedish family with Alzheimer's disease with a codon 670/671 amyloid precursor protein mutation. A clinical and genealogical investigation. Arch Neurol. 51(12), 1193-1197.

Azad N.A., Al Bugami M., Loy-English I., 2007. Gender differences in dementia risk factors. Gend Med. 4(2), 120-129.

Bacci A., Verderio C., Pravettoni E., Matteoli M., 1999. Synaptic and intrinsic mechanisms shape synchronous oscillations in hippocampal neurons in culture. Eur J Neurosci. 11(2), 389-397.

Baek S.H., Ohgi K.A., Rose D.W., Koo E.H., Glass C.K., Rosenfeld M.G., 2002. Exchange of N-CoR corepressor and Tip60 coactivator complexes links gene expression by NF-κB and β-amyloid precursor protein. Cell 110: 55–67.

Bales K.R., Verina T., Dodel R.C., Du Y., Altstiel L., Bender M., Hyslop P., Johnstone E.M., Little S.P., Cummins D.J., Piccardo P., Ghetti B., Paul S.M., 1997. Lack of apolipoprotein E dramatically reduces Aβ peptide deposition. Nat Genet. 17(3), 263-264.

Ball M.J., 1977. Neuronal loss, neurofibrillary tangles and granulovacuolar degeneration in the hippocampus with ageing and dementia. A quantitative study. Acta Neuropathol. 37(2), 111-118.

Basun H., Bogdanovic N., Ingelsson M., Almkvist O., Näslund J., Axelman K., Bird T.D., Nochlin D., Schellenberg G.D., Wahlund L.O., Lannfelt L., 2008. Clinical and neuropathological features of the arctic APP gene mutation causing early-onset Alzheimer disease. Arch Neurol. 65(4), 499-505.

Bayer T.A., Wirths O., 2008. Review on the APP/PS1KI mouse model: intraneuronal Aβ accumulation triggers axonopathy, neuron loss and working memory impairment. Genes Brain Behav. 7 Suppl 1:6-11.

Beher D., Hesse L., Masters C.L., Multhaup G., 1996. Regulation of amyloid protein precursor (APP) binding to collagen and mapping of the binding sites on APP and collagen type I. J Biol Chem. 271(3), 1613-1620.

Bellucci A., Rosi M.C., Grossi C., Fiorentini A., Luccarini I., Casamenti F., 2007. Abnormal processing of tau in the brain of aged TgCRND8 mice. Neurobiol Dis. 27(3), 328-338.

Berridge M.J., 1997. The AM and FM of calcium signalling. Nature. 386(6627), 759-760.

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

Biederer T., Cao X., Südhof T.C., Liu X., 2002. Regulation of APP-dependent transcription complexes by Mint/X11s: differential functions of Mint isoforms. J Neurosci. 22(17), 7340-7351.

Billings L.M., Oddo S., Green K.N., McGaugh J.L., LaFerla F.M., 2005. Intraneuronal Aβ causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. Neuron. 45(5), 675-688.

Bogdanovic N., Corder E., Lannfelt L., Winblad B., 2002. APOE polymorphism and clinical duration determine regional neuropathology in Swedish APP(670, 671) mutation carriers: implications for late-onset Alzheimer's disease. J Cell Mol Med. 6(2), 199-214.

Borg J.P., Ooi J., Levy E., Margolis B., 1996. The phosphotyrosine interaction domains of X11 and FE65 bind to distinct sites on the YENPTY motif of amyloid precursor protein. Mol Cell Biol 16, 6229–6241.

Bornebroek M., De Jonghe C., Haan J., Kumar-Singh S., Younkin S., Roos R., Van Broeckhoven C., 2003. Hereditary cerebral hemorrhage with amyloidosis Dutch type (AβPP 693): decreased plasma Aβ42 concentration. Neurobiol Dis. 14(3), 619-623.

Bour A, Little S, Dodart JC, Kelche C, Mathis C., 2004. A secreted form of the β-amyloid precursor protein (sAPP695) improves spatial recognition memory in OF1 mice. Neurobiol Learn Mem. 81(1), 27-38.

Bouron A., Mbebi C., Loeffler J.P., De Waard M., 2004. The β-amyloid precursor protein controls a store-operated Ca^{2+} entry in cortical neurons. Eur J Neurosci. 20(8), 2071-2078.

Braak H., Braak E., 1991. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. 82(4), 239-259.

Brandt R., Hundelt M., Shahani N., 2005. Tau alteration and neuronal degeneration in tauopathies: mechanisms and models. Biochim Biophys Acta. 1739(2-3), 331-354.

Breen K.C., Bruce M., Anderton B.H., 1991. β amyloid precursor protein mediates neuronal cell-cell and cell-surface adhesion. J Neurosci Res. 28(1), 90-100.

Bu G., Cam J., Zerbinatti C., 2006. LRP in Aβ production and metabolism. Ann N.Y. Acad Sci. 1086, 35-53.

Buckner R.L., Snyder A.Z., Shannon B.J., LaRossa G., Sachs R., Fotenos A.F., Sheline Y.I., Klunk W.E., Mathis C.A., Morris J.C., Mintun M.A., 2005. Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. J Neurosci. 25(34), 7709-7717.

Butterfield D.A., Castegna A., Lauderback C.M., Drake J., 2002. Evidence that amyloid β-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death. Neurobiol Aging. 23(5), 655-664.

Buxbaum J.D., Ruefli A.A., Parker C.A., Cypess A.M., Greengard P., 1994. Calcium regulates processing of the Alzheimer amyloid protein precursor in a protein kinase C-independent manner. Proc Natl Acad Sci U S A. 91(10), 4489-4493.

Cai X.D., Golde T.E., Younkin S.G., 1993. Release of excess amyloid β protein from a mutant amyloid β protein precursor. Science. 259(5094), 514-516.

Caillé I, Allinquant B, Dupont E, Bouillot C, Langer A, Müller U, Prochiantz A., 2004. Soluble form of amyloid precursor protein regulates proliferation of progenitors in the adult subventricular zone. Development. 131(9), 2173-2181.

Calabrese E.J., 2008. Hormesis and medicine. Br J Clin Pharmacol. Advance online publication : 2008 Jun 28.

Calhoun M.E,. Wiederhold K.H., Abramowski D., Phinney A.L., Probst A., Sturchler-Pierrat C., Staufenbiel M., Sommer B., Jucker M., 1998. Neuron loss in APP transgenic mice. Nature. 395(6704), 755-756.

Callahan M.J., Lipinski W.J., Bian F., Durham R.A., Pack A., Walker L.C., 2001. Augmented senile plaque load in aged female β-amyloid precursor protein-transgenic mice. Am J Pathol. 158(3), 1173-1177.

Cao X., Sudhof T.C., 2001. A transcriptionally active complex of APP with Fe65 and histone acetyltransferase Tip60. Science 293: 115–120.

Cárdenas A.M., Rodríguez M.P., Cortés M.P., Alvarez R.M., Wei W., Rapoport S.I., Shimahara T., Caviedes R., Caviedes P., 1999. Calcium signals in cell lines derived from the cerebral cortex of normal and trisomy 16 mice. Neuroreport. 10(2), 363-369.

Caporaso G.L., Gandy S.E., Buxbaum J.D., Ramabhadran T.V., Greengard P., 1992. Protein phosphorylation regulates secretion of Alzheimer β/A4 amyloid precursor protein. Proc Natl Acad Sci U S A. 89(7), 3055-3059.

Catalano S.M., Dodson E.C., Henze D.A., Joyce J.G., Krafft G.A., Kinney G.G., 2006. The role of Aβ derived diffusible ligands (ADDLs) in Alzheimer's disease. Curr Top Med Chem. 6(6), 597-608.

Cedazo-Mínguez A., 2007. Apolipoprotein E and Alzheimer's disease: molecular mechanisms and therapeutic opportunities. J Cell Mol Med. 11(6), 1227-1238.

Chan C.S., Guzman J.N., Ilijic E., Mercer J.N., Rick C., Tkatch T., Meredith G.E., Surmeier D.J., 2007. 'Rejuvenation' protects neurons in mouse models of Parkinson's disease. Nature. 447(7148), 1081-1086.

Chapman P.F., White G.L., Jones M.W., Cooper-Blacketer D., Marshall V.J., Irizarry M., Younkin L., Good M.A., Bliss T.V., Hyman B.T., Younkin S.G., Hsiao K.K., 1999. Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. Nat Neurosci. 2(3), 271-276.

Chen G., Chen K.S., Knox J., Inglis J., Bernard A., Martin S.J., Justice A., McConlogue L., Games D., Freedman S.B., Morris R.G., 2000. A learning deficit related to age and β-amyloid plaques in a mouse model of Alzheimer's disease. Nature. 2000 408(6815), 975-979.

Chen T.Y., Liu P.H., Ruan C.T., Chiu L., Kung F.L., 2006. The intracellular domain of amyloid precursor protein interacts with flotillin-1, a lipid raft protein. Biochem Biophys Res Commun. 342(1), 266-272.

Chen Y. and Dong C., 2008. Aβ40 promotes neuronal cell fate in neural progenitor cells. Cell Death Differ. Advance online publication, 20 June 2008; doi:10.1038/cdd.2008.94.

Cheng G., Yu Z., Zhou D., Mattson M.P., 2002. Phosphatidylinositol-3-kinase-Akt kinase and p42/p44 mitogen-activated protein kinases mediate neurotrophic and excitoprotective actions of a secreted form of amyloid precursor protein. Exp Neurol. 175(2), 407-414.

Chishti M.A., Yang D.S., Janus C., Phinney A.L., Horne P., Pearson J., Strome R., Zuker N., Loukides J., French J., Turner S., Lozza G., Grilli M., Kunicki S., Morissette C., Paquette J., Gervais F., Bergeron C., Fraser P.E., Carlson G.A., George-Hyslop P.S., Westaway D., 2001. Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. J Biol Chem. 276(24), 21562-21570.

Chow N., Korenberg J.R., Chen X.N., Neve R.L., 1996. APP-BP1, a novel protein that binds to the carboxyl-terminal region of the amyloid precursor protein. J Biol Chem. 271(19), 11339-11346.

Ciallella J.R., Ikonomovic M.D., Paljug W.R., Wilbur Y.I., Dixon C.E., Kochanek P.M., Marion D.W., DeKosky S.T., 2002. Changes in expression of amyloid precursor protein and interleukin-1β after experimental traumatic brain injury in rats. J Neurotrauma. 19(12), 1555-1567.

Cirrito J.R., Yamada K.A., Finn M.B., Sloviter R.S., Bales K.R., May P.C., Schoepp D.D., Paul S.M., Mennerick S., Holtzman D.M., 2005. Synaptic activity regulates interstitial fluid Aβ levels in vivo. Neuron. 48(6), 913-922.

Citron M., Oltersdorf T., Haass C., McConlogue L., Hung A.Y., Seubert P., Vigo-Pelfrey C., Lieberburg I., Selkoe D.J., 1992. Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. Nature 360, 672-674.

Citron M., Vigo-Pelfrey C., Teplow D.B., Miller C., Schenk D., Johnston J., Winblad B., Venizelos N., Lannfelt L., Selkoe D.J., 1994. Excessive production of amyloid β-protein by peripheral cells of symptomatic and presymptomatic patients carrying the Swedish familial Alzheimer disease mutation. Proc Natl Acad Sci U S A. 91(25), 11993-11997.

Corder E.H., Saunders A.M., Strittmatter W.J., Schmechel D.E., Gaskell P.C., Small G.W., Roses A.D., Haines J.L., 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.

Corder E.H., Saunders A.M., Risch N.J., Strittmatter W.J., Schmechel D.E., Gaskell P.C. Jr, Rimmler J.B., Locke P.A., Conneally P.M., Schmader K.E., Small G. W., Roses A. D., Haines J. L., Pericak-Vance M. A. 1994. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet. 7(2), 180-184.

da Cruz e Silva E.F., da Cruz e Silva O.A., Zaia C.T., Greengard P., 1995. Inhibition of protein phosphatase 1 stimulates secretion of Alzheimer amyloid precursor protein. Mol Med. 1(5), 535-541.

Das A., Smalheiser N.R., Markaryan A., Kaplan A., 2002. Evidence for binding of the ectodomain of amyloid precursor protein 695 and activated high molecular weight kininogen. Biochim Biophys Acta. 1571(3), 225-238.

Davies P., 2006. A long trek down the pathways of cell death in Alzheimer's disease. J Alzheimers Dis. 9(3 Suppl), 265-269.

Davies P., Maloney A.J., 1976. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet. 2(8000), 1403.

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

De Felice F.G., Velasco P.T., Lambert M.P., Viola K., Fernandez S.J., Ferreira S.T., Klein W.L., 2007. Aβ oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. J Biol Chem. 282(15), 11590-11601.

Delacourte A., 2006. The natural and molecular history of Alzheimer's disease. J Alzheimers Dis. 9(3 Suppl), 187-194.

den Dunnen W.F., Brouwer W.H., Bijlard E., Kamphuis J., van Linschoten K., Eggens-Meijer E., Holstege G., 2008. No disease in the brain of a 115-year-old woman. Neurobiol Aging. 29(8), 1127-1132.

Dolmetsch RE, Xu K, Lewis RS., 1998. Calcium oscillations increase the efficiency and specificity of gene expression. Nature. 392(6679), 933-936.

Dosunmu R., Wu J., Basha M.R., Zawia N.H., 2007. Environmental and dietary risk factors in Alzheimer's disease. Expert Rev Neurother. 7, 887-900.

Dravid S.M., Murray T.F., 2004. Spontaneous synchronized calcium oscillations in neocortical neurons in the presence of physiological [$Mg^{(2+)}$]: involvement of AMPA/kainate and metabotropic glutamate receptors. Brain Res. 1006(1), 8-17.

Dreses-Werringloer U, Lambert JC, Vingtdeux V, Zhao H, Vais H, Siebert A, Jain A, Koppel J, Rovelet-Lecrux A, Hannequin D, Pasquier F, Galimberti D, Scarpini E, Mann D, Lendon C, Campion D, Amouyel P, Davies P, Foskett JK, Campagne F, Marambaud P., 2008.
A polymorphism in CALHM1 influences Ca^{2+} homeostasis, Aβ levels, and Alzheimer's disease risk. Cell. 2008 133(7), 1149-1161.

Drzezga A., Grimmer T., Peller M., Wermke M., Siebner H., Rauschecker J.P., Schwaiger M., Kurz A., 2005. Impaired cross-modal inhibition in Alzheimer disease. PLoS Med. 2(10):e288.

Dudal S., Krzywkowski P., Paquette J., Morissette C., Lacombe D., Tremblay P., Gervais F., 2004. Inflammation occurs early during the Aβ deposition process in TgCRND8 mice. Neurobiol Aging. 25(7), 861-871.

Echeverria, V., Ducatenzeiler, A., Alhonen, L., Janne, J., Grant, S.M., Wandosell, F., Muro, A., Baralle, F., Li, H., Duff, K., Szyf, M., Cuello, A.C., 2004a. Rat transgenic models with a phenotype of intracellular Aβ accumulation in hippocampus and cortex. J Alzheimers Dis. 6, 209-219.

Echeverria V., Ducatenzeiler A., Dowd E., Jänne J., Grant S.M., Szyf M., Wandosell F., Avila J., Grimm H., Dunnett S.B., Hartmann T., Alhonen L., Cuello A.C., 2004b. Altered mitogen-activated protein kinase signaling, tau hyperphosphorylation and mild spatial learning dysfunction in transgenic rats expressing the β-amyloid peptide intracellularly in hippocampal and cortical neurons. Neuroscience. 129(3), 583-592.

Echeverria V., Ducatenzeiler A., Chen C.H., Cuello A.C., 2005. Endogenous β-amyloid peptide synthesis modulates cAMP response element-regulated gene expression in PC12 cells. Neuroscience. 135(4), 1193-1202.

Erol A., 2008. An integrated and unifying hypothesis for the metabolic basis of sporadic Alzheimer's disease. J Alzheimers Dis. 13(3), 241-253.

Esch F.S., Keim P.S., Beattie E.C., Blacher R.W., Culwell A.R., Oltersdorf T., McClure D., Ward P.J., 1990. Cleavage of amyloid β peptide during constitutive processing of its precursor. Science. 248(4959), 1122-1124.

Essalmani R., Macq A.F., Mercken L., Octave J.N., 1996. Missense mutations associated with familial Alzheimer's disease in Sweden lead to the production of the amyloid peptide without internalization of its precursor. Biochem Biophys Res Commun. 218(1), 89-96.

Farzan M, Schnitzler CE, Vasilieva N, Leung D, Choe H., 2000. BACE2, a β-secretase homolog, cleaves at the β site and within the Aβ region of the amyloid-β precursor protein. Proc Natl Acad Sci U S A.97(17), 9712-9717.

Fassa A., Mehta P., Efthimiopoulos S., 2005. Notch 1 interacts with the amyloid precursor protein in a Numb-independent manner. J Neurosci Res. 82(2), 214-224.

Fath T., Eidenmüller J., Brandt R., 2002. Tau-mediated cytotoxicity in a pseudohyperphosphorylation model of Alzheimer's disease. J Neurosci. 22(22), 9733-9741.

Fiore F., Zambrano N., Minopoli G., Donini V., Duilio A., Russo T., 1995. The regions of Fe65 protein homologous to the phosphotyrosine interaction/phosphotyrosine binding domain of the Shc

bind the intracellular domain of the Alzheimer's amyloid precursor protein. J Biol Chem 270, 30853–30856.

Flood D.G., Lin Y.G., Lang D.M., Trusko S.P., Hirsch J.D., Savage M.J., Scott R.W., Howland D.S., 2007. A transgenic rat model of Alzheimer's disease with extracellular Aβ deposition. Neurobiol Aging. Advanced online publication: 2007 Nov 27.

Fluhrer R., Capell A., Westmeyer G., Willem M., Hartung B., Condron M.M., Teplow D.B., Haass C., Walter J., 2002. A non-amyloidogenic function of BACE-2 in the secretory pathway. J Neurochem. 81(5), 1011-1020.

Folkesson R., Malkiewicz K., Kloskowska E., Nilsson T., Popova E., Bogdanovic N., Ganten U., Ganten D., Bader M., Winblad B., Benedikz E., 2007. A transgenic rat expressing human APP with the Swedish Alzheimer's disease mutation. Biochem Biophys Res Commun. 358(3), 777-782.

Forsberg A., Engler H., Almkvist O., Blomquist G., Hagman G., Wall A., Ringheim A., Långström B., Nordberg A., 2008. PET imaging of amyloid deposition in patients with mild cognitive impairment. Neurobiol Aging. Neurobiol Aging. 29(10), 1456-1465.

Fratiglioni L., Rocca W.A., 2001. Epidemiology of dementia. In: Boller F, Cappa SF, eds. Handbook of neuropsychology. 2nd ed. Amsterdam: Elsevier, 193–215.

Fratiglioni L., Winblad B., von Strauss E., 2007. Prevention of Alzheimer's disease and dementia. Major findings from the Kungsholmen Project. Physiol Behav. 92(1-2), 98-104.

Furukawa K., Barger S.W., Blalock E.M., Mattson M.P., 1996a. Activation of K^+ channels and suppression of neuronal activity by secreted β-amyloid-precursor protein. Nature. 379(6560), 74-78.

Furukawa K., Sopher B.L., Rydel R.E., Begley J.G., Pham D.G., Martin G.M., Fox M., Mattson M.P., 1996b. Increased activity-regulating and neuroprotective efficacy of α-secretase-derived secreted amyloid precursor protein conferred by a C-terminal heparin-binding domain. J Neurochem. 67(5), 1882-1896.

Games D., Adams D., Alessandrini R., Barbour R., Berthelette P., Blackwell C., Carr T., Clemens J., Donaldson T., Gillespie F., Guido T., Hagopian S., Johnson-Wood K., Khan K., Lee M., Leibowitz P., Lieberburg I., Little S., Masliah E., McConlogue L., Montoya-Zavala M., Mucke L., Paganini L., Penniman E., Power M., Schenk D., Seubert P., Snyder B., Soriano F., Tan H., Vitale J., Wadsworth S., Wolozin B., Zhao J., 1995. Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. Nature. 373(6514), 523-527.

Garaschuk O., Linn J., Eilers J., Konnerth A., 2000. Large-scale oscillatory calcium waves in the immature cortex. Nat Neurosci. 3(5), 452-459.

García-Sierra F., Mondragón-Rodríguez S., Basurto-Islas G., 2008. Truncation of tau protein and its pathological significance in Alzheimer's disease. J Alzheimers Dis. 14(4), 401-409.

Gatz M., Fratiglioni L., Johansson B., Berg S., Mortimer J.A., Reynolds C.A., Fiske A., Pedersen N.L., 2005. Complete ascertainment of dementia in the Swedish Twin Registry: the HARMONY study. Neurobiol Aging.26(4), 439-447.

Geddes-Klein D.M., Serbest G., Mesfin M.N., Cohen A.S., Meaney D.F., 2006. Pharmacologically induced calcium oscillations protect neurons from increases in cytosolic calcium after trauma. J Neurochem. 97(2), 462-474.

German D.C., Nelson O., Liang F., Liang C.L., Games D., 2005. The PDAPP mouse model of Alzheimer's disease: locus coeruleus neuronal shrinkage. J Comp Neurol. 492(4), 469-476.

Gibson G.E., Vestling M., Zhang H., Szolosi S., Alkon D., Lannfelt L., Gandy S., Cowburn R.F., 1997. Abnormalities in Alzheimer's disease fibroblasts bearing the APP670/671 mutation. Neurobiol Aging. 18(6), 573-580.

Giedraitis V., Sundelöf J., Irizarry M.C., Gårevik N., Hyman B.T., Wahlund L.O., Ingelsson M., Lannfelt L., 2007. The normal equilibrium between CSF and plasma Aβ levels is disrupted in Alzheimer's disease. Neurosci Lett. 427(3), 127-131.

Gilman S., Koller M., Black R.S., Jenkins L., Griffith S.G., Fox N.C., Eisner L., Kirby L., Rovira M.B., Forette F., Orgogozo J.M., 2005. Clinical effects of Aβ immunization (AN1792) in patients with AD in an interrupted trial. Neurology. 64(9), 1553-1562.

Golan M.P., Styczyńska M., Jóźwiak K., Walecki J., Maruszak A., Pniewski J., Lugiewicz R., Filipek S., Zekanowski C., Barcikowska M., 2007. Early-onset Alzheimer's disease with a de novo mutation in the presenilin 1 gene. Exp Neurol.208(2), 264-268.

Gong Y., Meyer E.M., Meyers C.A., Klein R.L., King M.A., Hughes J.A., 2006. Memory-related deficits following selective hippocampal expression of Swedish mutation amyloid precursor protein in the rat. Exp Neurol. 200(2), 371-377.

Goodman Y., Mattson M.P., 1994. Secreted forms of β-amyloid precursor protein protect hippocampal neurons against amyloid β-peptide-induced oxidative injury. Exp Neurol. 128(1), 1-12.

Gorman P.M., Kim S., Guo M., Melnyk R.A., McLaurin J., Fraser P.E., Bowie J.U., Chakrabartty A., 2008. Dimerization of the transmembrane domain of amyloid precursor proteins and familial Alzheimer's disease mutants. BMC Neurosci. 9:17.

Green KN, Demuro A, Akbari Y, Hitt BD, Smith IF, Parker I, LaFerla FM., 2008. SERCA pump activity is physiologically regulated by presenilin and regulates Aβ production. J Cell Biol. 181(7), 1107-1116.

Götz J., Chen F., van Dorpe J., Nitsch R.M., 2001. Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Aβ 42 fibrils. Science. 293(5534), 1491-1495.

Guo Q., Robinson N., Mattson M.P., 1998. Secreted β-amyloid precursor protein counteracts the proapoptotic action of mutant presenilin-1 by activation of NF-κB and stabilization of calcium homeostasis. J Biol Chem. 273(20), 12341-12351.

Haass C., Lemere C.A., Capell A., Citron M., Seubert P., Schenk D., Lannfelt L., Selkoe D. J., 1995. The Swedish Mutation Causes Early-Onset Alzheimers-Disease by β-Secretase Cleavage Within the Secretory Pathway. Nature Medicine. 1, 1291-1296.

Haan, M.N., 2006. Therapy Insight: type 2 diabetes mellitus and the risk of late-onset Alzheimer's disease. Nat. Clin. Pract. Neurol. 2, 159–166.

Hamid R., Kilger E., Willem M., Vassallo N., Kostka M., Bornhövd C., Reichert A.S., Kretzschmar H.A., Haass C., Herms J., 2007. Amyloid precursor protein intracellular domain modulates cellular calcium homeostasis and ATP content. J Neurochem. 102(4), 1264-1275.

Hansson C.A., Frykman S., Farmery M.R., Tjernberg L.O., Nilsberth C., Pursglove S.E., Ito A., Winblad B., Cowburn R.F., Thyberg J., Ankarcrona M. (2004). Nicastrin, presenilin, APH-1, and PEN-2 form active γ-secretase complexes in mitochondria. J Biol Chem. 279(49), 51654-51660.

Hardy J., Allsop D., 1991. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci. 12(10), 383-388.

Hardy J.A, Higgins G.A., 1992. Alzheimer's disease: the amyloid cascade hypothesis. Science. 256(5054), 184-185.

Hardy J., 2006. Alzheimer's disease: the amyloid cascade hypothesis: an update and reappraisal. J Alzheimers Dis. 9(3 Suppl), 151-153.

Hass S. Fresser F., Köchl S., Beyreuther K., Utermann G., Baier G., 1998. Physical interaction of ApoE with amyloid precursor protein independent of the amyloid Aβ region in vitro. J Biol Chem. 273(22), 13892-13897.

Heber S., Herms J., Gajic V., Hainfellner J., Aguzzi A., Rülicke T., von Kretzschmar H., von Koch C., Sisodia S., Tremml P., Lipp H.P., Wolfer D.P., Müller U., 2000. Mice with combined gene knock-outs reveal essential and partially redundant functions of amyloid precursor protein family members. J Neurosci. 20(21), 7951-7963.

Hebert L.E., Scherr P.A., McCann J.J., Beckett L.A., Evans D.A., 2001. Is the risk of developing Alzheimer's disease greater for women than for men? Am J Epidemiol. 153(2), 132-136.

Hebert S.S., Serneels L., Tolia A., Craessaerts K., Derks C., Filippov M.A., Müller U., de Strooper B., 2006. Regulated intramembrane proteolysis of amyloid precursor protein and regulation of expression of putative target genes. EMBO Rep 7, 739–745.

Helton T.D., Xu W., Lipscombe D., 2005. Neuronal L-type calcium channels open quickly and are inhibited slowly. J Neurosci. 25(44), 10247-10251.

Henneberry R.C., Novelli A., Vigano M.A., Reilly J.A., Cox J.A., Lysko P.G., 1989. Energy-related neurotoxicity at the NMDA receptor: a possible role in Alzheimer's disease and related disorders. Prog Clin Biol Res. 317, 143-156.

Hernández F., Borrell J., Guaza C., Avila J., Lucas J.J., 2002. Spatial learning deficit in transgenic mice that conditionally over-express GSK-3β in the brain but do not form tau filaments. J Neurochem. 83(6), 1529-1533.

Hernández-SanMiguel E., Vay L., Santo-Domingo J., Lobatón C.D., Moreno A., Montero M., Alvarez J., 2006. The mitochondrial Na^{+}/Ca^{2+} exchanger plays a key role in the control of cytosolic Ca^{2+} oscillations. Cell Calcium. 40(1), 53-61.

Ho A., Südhof T.C., 2004. Binding of F-spondin to amyloid-β precursor protein: a candidate amyloid-β precursor protein ligand that modulates amyloid-β precursor protein cleavage. Proc Natl Acad Sci U S A. 101(8), 2548-2553.

Hoe H.S., Tran T.S., Matsuoka Y., Howell B., Rebeck G.W., 2006. DAB1 and Reelin effects on amyloid precursor protein and ApoE receptor 2 trafficking and processing. J Biol. Chem. 281, 35176-35185.

Hoe H.S., Rebeck W.G., 2008. Functional interactions of APP with the apoE receptor family. J Neurochem. Advanced online publication: 2008 Jul 28.

Hoffmann J, Pietrzik CU, Kummer MP, Twiesselmann C, Bauer C, Herzog V., 1999. Binding and selective detection of the secretory N-terminal domain of the alzheimer amyloid precursor protein on cell surfaces. J Histochem Cytochem. 47(3), 373-382.

Holcomb L., Gordon M.N., McGowan E., Yu X., Benkovic S., Jantzen P., Wright K., Saad I., Mueller R., Morgan D., Sanders S., Zehr C., O'Campo K., Hardy J., Prada C.M., Eckman C., Younkin S., Hsiao K., Duff K., 1998. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. Nat Med. 4(1), 97-100.

Holcomb L.A., Gordon M.N., Jantzen P., Hsiao K., Duff K., Morgan D., 1999. Behavioral changes in transgenic mice expressing both amyloid precursor protein and presenilin-1 mutations: lack of association with amyloid deposits. Behav Genet. 29(3), 177-185.

Holmes C., Boche D., Wilkinson D., Yadegarfar G., Hopkins V., Bayer A., Jones R.W., Bullock R., Love S., Neal J.W., Zotova E., Nicoll J.A.R., 2008. Long-term eff ects of Aβ42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. Lancet 372, 216–223.

Holtzman D.M., Bales K.R., Tenkova T., Fagan A.M., Parsadanian M., Sartorius L.J., Mackey B., Olney J., McKeel D., Wozniak D., 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.

Honig L.S., Tang M.X., Albert S., Costa R., Luchsinger J., Manly J., Stern Y., Mayeux R., 2003. Stroke and the risk of Alzheimer disease. Arch Neurol. 60(12), 1707-1712.

Hooper C., Killick R., Lovestone S., 2008. The GSK3 hypothesis of Alzheimer's disease. J Neurochem. 104(6), 1433-1439.

Horie M., Suzuki M., Takahashi E., Tanigami, 1999. Cloning, expression, and chromosomal mapping of the human 14-3-3 γ gene (YWHAG) to 7q11.23. Genomics 60, 241–243.

Hornsten A., Lieberthal J., Fadia S., Malins R., Ha L., Xu X., Daigle I., Markowitz M., O'Connor G., Plasterk R., Li C., 2007. APL-1, a Caenorhabditis elegans protein related to the human β-amyloid precursor protein, is essential for viability. Proc Natl Acad Sci U S A. 104(6), 1971-1976.

Howell B.W., Lanier L.M., Frank R., Gertler F.B., Cooper J.A., 1999. The Disabled 1 phosphotyrosine-binding domain binds to the internalization signals of transmembrane glycoproteins and to phospholipids. Mol Cell Biol 19, 5179–5188.

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

Hung A.Y., Koo E.H., Haass C., Selkoe D.J., 1992. Increased expression of β-amyloid precursor protein during neuronal differentiation is not accompanied by secretory cleavage. Proc Natl Acad Sci U S A. 89(20), 9439-9443.

Ikezu T., Trapp B.D., Song K.S., Schlegel A., Lisanti M.P., Okamoto T., 1998. Caveolae, plasma membrane microdomains for α-secretase-mediated processing of the amyloid precursor protein. J Biol Chem. 273(17), 10485-10495.

Irizarry M.C., McNamara M., Fedorchak K., Hsiao K., Hyman B.T., 1997a. APPSw transgenic mice develop age-related Aβ deposits and neuropil abnormalities, but no neuronal loss in CA1. J Neuropathol Exp Neurol. 56(9), 965-973.

Irizarry M.C., Soriano F., McNamara M., Page K.J., Schenk D., Games D., Hyman B.T., 1997b. Aβ deposition is associated with neuropil changes, but not with overt neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse. J Neurosci. 17(18), 7053-7059.

Irizarry M.C., Rebeck G.W., Cheung B., Bales K., Paul S.M., Holzman D., Hyman B.T., 2000. Modulation of Aβ deposition in APP transgenic mice by an apolipoprotein E null background. Ann N Y Acad Sci. 920, 171-178.

Irizarry M.C., Locascio J.J., Hyman B.T., 2001. β-site APP cleaving enzyme mRNA expression in APP transgenic mice: anatomical overlap with transgene expression and static levels with aging. Am J Pathol.158(1), 173-177.

Isaacs A.M., Senn D.B., Yuan M., Shine J.P., Yankner B.A., 2006. Acceleration of amyloid β-peptide aggregation by physiological concentrations of calcium. J Biol Chem. 281(38), 27916-27923.

Ishii K., Hirose K., Iino M., 2006. Ca^{2+} shuttling between endoplasmic reticulum and mitochondria underlying Ca^{2+} oscillations. EMBO Rep. 7(4), 390-396.

Itzhaki R.F., Wozniak M.A., 2008. Herpes simplex virus type 1 in Alzheimer's disease: the enemy within. J Alzheimers Dis. 13(4), 393-405.

Jankowsky J.L., Younkin L.H., Gonzales V., Fadale D.J., Slunt H.H., Lester H.A., Younkin S.G., Borchelt D.R., 2007. Rodent Aβ modulates the solubility and distribution of amyloid deposits in transgenic mice. J Biol Chem. 282(31), 22707-22720.

Janus C., Pearson J., McLaurin J., Mathews P.M., Jiang Y., Schmidt S.D., Chishti M.A., Horne P., Heslin D., French J., Mount H.T., Nixon R.A., Mercken M., Bergeron C., Fraser P.E., St George-Hyslop P., Westaway D., 2000. Aβ peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. Nature. 2000 408(6815), 979-982.

Jaworski J., Sheng M., 2006. The growing role of mTOR in neuronal development and plasticity. Mol Neurobiol. 34(3), 205-219.

Jin L.W., Ninomiya H., Roch J.M., Schubert D., Masliah E., Otero D.A., Saitoh T., 1994. Peptides containing the RERMS sequence of amyloid β/A4 protein precursor bind cell surface and promote neurite extension. J Neurosci. 14(9), 5461-5470.

Johansen T.E., Schøller M.S., Tolstoy S., Schwartz T.W., 1990. Biosynthesis of peptide precursors and protease inhibitors using new constitutive and inducible eukaryotic expression vectors. FEBS Lett. 267(2), 289-294.

Johnstone E.M., Chaney M.O., Norris F.H., Pascual R., Little S.P., 2003. Conservation of the sequence of the Alzheimer's disease amyloid peptide in dog, polar bear and five other mammals by cross-species polymerase chain reaction analysis. Brain Res Mol Brain Res. 10(4), 299-305.

Jorgensen et al., 2007. Cloned Pigs Help Scientists Towards A Breakthrough In Alzheimer's. (University of Copenhagen (2007, June 29). ScienceDaily. Retrieved from: http://www.sciencedaily.com /releases/2007/06/070629083416.htm).

Kaether C, Haass C., 2004. A lipid boundary separates APP and secretases and limits amyloid beta-peptide generation. J Cell Biol. 167(5), 809-812.

Kagan B.L., Hirakura Y., Azimov R., Azimova R., Lin M.C., 2002. The channel hypothesis of Alzheimer's disease: current status. Peptides. 23(7), 1311-1315.

Kallai J., Makany T., Csatho A., Karadi K., Horvath D., Kovacs-Labadi B., Jarai R., Nadel L., Jacobs J.W., 2007. Cognitive and affective aspects of thigmotaxis strategy in humans. Behav Neurosci. 121(1), 21-30.

Kamal A., Stokin G.B., Yang Z., Xia C.H., Goldstein L.S. 2000. Axonal transport of amyloid precursor protein is mediated by direct binding to the kinesin light chain subunit of kinesin-I. Neuron 28, 449–459.

Kamal A., Almenar-Queralt A., LeBlanc J.F., Roberts E.A., Goldstein L.S., 2001. Kinesin-mediated axonal transport of a membrane compartment containing β-secretase and presenilin-1 requires APP. Nature. 2001 414(6864), 643-648.

Kamenetz F., Tomita T., Hsieh H., Seabrook G., Borchelt D., Iwatsubo T., Sisodia S., Malinow R., 2003. APP processing and synaptic function. Neuron. 37(6), 925-937.

Kang J., Lemaire H.G., Unterbeck A., Salbaum J.M., Masters C.L., Grzeschik K.H., Multhaup G., Beyreuther K., Müller-Hill B., 1987. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 325(6106), 733-736.

Khachaturian, Z. S., 1989. Calcium, membranes, aging, and Alzheimer's disease. Introduction and overview. Ann. NY Acad. Sci. 568, 1–4.

Kim H.S., Park C.H., Cha S.H., Lee J.H., Lee S., Kim Y., Rah J.C., Jeong S.J., Suh Y.H., 2000. Carboxyl-terminal fragment of Alzheimer's APP destabilizes calcium homeostasis and renders neuronal cells vulnerable to excitotoxicity. FASEB J. 14(11), 1508-1517.

Kim J.H., Anwyl R., Suh Y.H., Djamgoz M.B., Rowan M.J., 2001. Use-dependent effects of amyloidogenic fragments of β-amyloid precursor protein on synaptic plasticity in rat hippocampus in vivo. J Neurosci. 21(4), 1327-1333.

Kimberly W.T., Zheng J.B., Guenette S.Y., Selkoe D.J., 2001. The intracellular domain of the β-amyloid precursor protein is stabilized by Fe65 and translocates to the nucleus in a Notch-like manner. J Biol Chem 276, 40288–40292.

Kinoshita A., Whelan C.M., Smith C.J., Berezovska O., Hyman B.T., 2002. Direct visualization of the γ secretase-generated carboxyl-terminal domain of the amyloid precursor protein: association with Fe65 and translocation to the nucleus. J Neurochem 82, 839–847.

Kloskowska E, Pham T.M., Nilsson T., Zhu S., Öberg J., Pedersen L.Ø., Pedersen J.T., Malkiewicz K., Winblad B., Folkesson R., Benedikz E., 2008. Memory impairment in the Tg6590 transgenic rat model of Alzheimer's disease. Submitted, paper II of this thesis.

Klunk W.E., Price J.C., Lopresti B.J., Huang G., Debnath M.L., Shao L., Tsopelas N.D., DeKosky S.T., Mathis C.A.. Can *in vivo* amyloid imaging with Pittsburgh compound-B (PIB) tell us anything about the time course of amyloid deposition in Alzheimer's disease? Program No. 587.5. *2005 Abstract Viewer/Itinerary Planner.* Washington, DC: Society for Neuroscience, 2005. Online.

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

Koo E.H., Squazzo S.L., 1994. Evidence that production and release of amyloid β-protein involves the endocytic pathway. J Biol Chem. 269(26), 17386-17389.

Koson P., Zilka N., Kovac A., Kovacech B., Korenova M., Filipcik P., Novak M., 2008. Truncated tau expression levels determine life span of a rat model of tauopathy without causing neuronal loss or correlating with terminal neurofibrillary tangle load. Eur J Neurosci. 28(2), 239-246.

Kourie J.I., 2001. Mechanisms of amyloid β protein-induced modification in ion transport systems: implications for neurodegenerative diseases. Cell Mol Neurobiol. 21(3), 173-213.

Lafay-Chebassier C., Paccalin M., Page G., Barc-Pain S., Perault-Pochat M.C., Gil R., Pradier L., Hugon J., 2005. mTOR/p70S6k signalling alteration by Aβ exposure as well as in APP-PS1 transgenic models and in patients with Alzheimer's disease. J Neurochem. 94(1), 215-225.

LaFerla F.M., 2002. Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. Nat Rev Neurosci.3(11), 862-872.

Lanz T.A., Carter D.B., Merchant K.M., 2003. Dendritic spine loss in the hippocampus of young PDAPP and Tg2576 mice and its prevention by the ApoE2 genotype. Neurobiol Dis. 13(3), 246-253.

Lawlor P.A., Bland R.J., Das P., Price R.W., Holloway V., Smithson L., Dicker B.L., During M.J., Young D., Golde T.E., 2007. Novel rat Alzheimer's disease models based on AAV-mediated gene transfer to selectively increase hippocampal Aβ levels. Mol Neurodegener. 2:11.

Lee V.M., Trojanowski J.Q., 1992. The disordered neuronal cytoskeleton in Alzheimer's disease. Curr Opin Neurobiol. 2(5), 653-656.

Leissring M.A., Murphy M.P., Mead T.R., Akbari Y., Sugarman M.C., Jannatipour M., Anliker B., Müller U., Saftig P., De Strooper B., Wolfe M.S., Golde T.E., LaFerla F.M., 2002. A physiologic signaling role for the γ-secretase-derived intracellular fragment of APP. Proc Natl Acad Sci U S A. 99(7), 4697-4702.

Lesné S., Koh M.T., Kotilinek L., Kayed R., Glabe C.G., Yang A., Gallagher M., Ashe K.H.., 2006. A specific amyloid-β protein assembly in the brain impairs memory. Nature. 440(7082), 352-357.

Levinoff E.J., Saumier D., Chertkow H., 2005. Focused attention deficits in patients with Alzheimer's disease and mild cognitive impairment. Brain Cogn. 57(2), 127-130.

Levy E., Prelli F., Frangione B., 2006. Studies on the first described Alzheimer's disease amyloid β mutant, the Dutch variant. J Alzheimers Dis. 9 (3 Suppl), 329-339.

Lewis J., Dickson D.W., Lin W.L., Chisholm L., Corral A., Jones G., Yen S.H., Sahara N., Skipper L., Yager D., Eckman C., Hardy J., Hutton M., McGowan E., 2001. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. Science. 293(5534), 1487-1491.

Li W., Llopis J., Whitney M., Zlokarnik G., Tsien R.Y., 1998. Cell-permeant caged InsP3 ester shows that Ca^{2+} spike frequency can optimize gene expression. Nature 392(6679), 936-941.

Li R.X., Yu X.Z., Fu M., Zong M., Liu R.L., Xie Z.P., 2008. Frequency modulation of synchronized $Ca(^{2+})$ spikes in cultured hippocampal networks through mTOR. Neurosci Lett. 441(1), 50-55.

Li Z.W., Stark G., Götz J., Rülicke T., Gschwind M., Huber G., Müller U., Weissmann C., 1996. Generation of mice with a 200-kb amyloid precursor protein gene deletion by Cre recombinase-mediated site-specific recombination in embryonic stem cells. Proc Natl Acad Sci U S A. 93(12), 6158-6162.

Lin P., Li F., Zhang Y.W., Huang H., Tong G., Farquhar M.G., Xu H., 2007. Calnuc binds to Alzheimer's β-amyloid precursor protein and affects its biogenesis. J Neurochem. 100(6), 1505-1514.

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., Rozmyslowicz T., Stwora-Wojczyk M., Wojczyk B., 1999. Posttranslational Modifications of the Amyloid Precursor Protein. Springer Protocols: Alzheimer's Disease: Methods and Protocols, 32, 169-190.

Liu F., Su Y., Li B., Ni B., 2003. Regulation of amyloid precursor protein expression and secretion via activation of ERK1/2 by hepatocyte growth factor in HEK293 cells transfected with APP751. Exp Cell Res. 287(2), 387-396.

Liu F.L., Liu P.H., Shao H.W., Kung F.L., 2006. The intracellular domain of amyloid precursor protein interacts with FKBP12. Biochem Biophys Res Commun. 350(2), 472-477.

Liu L., Orozco I.J., Planel E., Wen Y., Bretteville A., Krishnamurthy P., Wang L., Herman M., Figueroa H., Yu W.H., Arancio O., Duff K., 2008. A transgenic rat that develops Alzheimer's disease-like amyloid pathology, deficits in synaptic plasticity and cognitive impairment. Neurobiol Dis. 31(1), 46-57.

Lopez J.R., Lyckman A., Oddo S., Laferla F.M., Querfurth H.W., Shtifman A., 2008. Increased intraneuronal resting [Ca^{2+}] in adult Alzheimer's disease mice. J Neurochem. 105(1), 262-271.

Lorent K., Overbergh L., Moechars D., De Strooper B., Van Leuven F., Van den Berghe H., 1995. Expression in mouse embryos and in adult mouse brain of three members of the amyloid precursor protein family, of the α-2-macroglobulin receptor/low density lipoprotein receptor-related protein and of its ligands apolipoprotein E, lipoprotein lipase, α-2-macroglobulin and the 40,000 molecular weight receptor-associated protein. Neuroscience. 65, 1009-1025.

Lorenzo A., Yuan M., Zhang Z., Paganetti P.A., Sturchler-Pierrat C., Staufenbiel M., Mautino J., Vigo F.S., Sommer B., Yankner B.A., 2000. Aβ interacts with the amyloid precursor protein: a potential toxic mechanism in Alzheimer's disease. Neurosci. 3(5), 460-464.

Lozsadi D.A., Larner A.J., 2006. Prevalence and causes of seizures at the time of diagnosis of probable Alzheimer's disease. Dement Geriatr Cogn Disord. 22(2), 121-124.

Luo L., Tully T., White K., 1992. Human amyloid precursor protein ameliorates behavioral deficit of flies deleted for Appl gene. Neuron. 9(4), 595-605.

Ma Q.H., Futagawa T., Yang W.L., Jiang X.D., Zeng L., Takeda Y., Xu R.X., Bagnard D., Schachner M., Furley A.J., Karagogeos D., Watanabe K., Dawe G.S., Xiao Z.C., 2008. A TAG1-APP signalling pathway through Fe65 negatively modulates neurogenesis. Nat Cell Biol. 10(3), 283-294.

Margallo-Lana M.L., Moore P.B., Kay D.W., Perry R.H., Reid B.E., Berney T.P., Tyrer S.P., 2007. Fifteen-year follow-up of 92 hospitalized adults with Down's syndrome: incidence of cognitive decline, its relationship to age and neuropathology. J Intellect Disabil Res. 51, 463-477.

Markesbery W.R., Schmitt F.A., Kryscio R.J., Davis D.G., Smith C.D., Wekstein D.R.., 2006. Neuropathologic substrate of mild cognitive impairment. Arch Neurol. 63(1), 38-46.

Marquez-Sterling N.R., Lo A.C.Y., Sisodia S.S., Koo E.H., 1997. Trafficking of cell-surface b-amyloid precursor protein: evidence that a sorting intermediate participates in synaptic vesicle recycling. J Neurosci 17, 140–151.

Martins I.C., Kuperstein I., Wilkinson H., Maes E., Vanbrabant M., Jonckheere W., Van Gelder P., Hartmann D., D'Hooge R., De Strooper B., Schymkowitz J., Rousseau F., 2008. Lipids revert inert Aβ amyloid fibrils to neurotoxic protofibrils that affect learning in mice. EMBO J. 27(1), 224-233.

Masliah E., Sisk A., Mallory M., Mucke L., Schenk D., Games D., 1996. Comparison of neurodegenerative pathology in transgenic mice overexpressing V717F β-amyloid precursor protein and Alzheimer's disease. J Neurosci. 16(18), 5795-5811.

Mathews P.M., Garcia-Osta A., Mazzella M.J., Alberini C.M., Endogenous Aβ enhances memory retention in the rat. Oral presentation at the 2008 ICAD conference in Chicago, abstract no: O2-02-01.

Matsuda S., Yasukawa T., Homma Y., Ito Y., Niikura T., Hiraki T., Hirai S., Ohno S., Kita Y., Kawasumi M., Kouyama K., Yamamoto T., Kyriakis J.M., Nishimoto I., 2001. c-Jun N-terminal kinase (JNK)-interacting protein-1b/Islet-brain-1 scaffolds Alzheimer's amyloid precursor protein with JNK. J Neurosci 21, 6597–6607.

Mattson M.P., Cheng B., Davis D., Bryant K., Lieberburg I., Rydel R.E., 1992. β-Amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. J Neurosci. 12(2), 376-389.

Mattson M.P., Cheng B., Culwell A.R., Esch F.S., Lieberburg I., Rydel R.E., 1993. Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the β-amyloid precursor protein. Neuron. 10(2), 243-254.

McGowan E., Eriksen J., Hutton M., 2006. A decade of modeling Alzheimer's disease in transgenic mice. Trends Genet. 22(5), 281-289.

McPhie D.L., Coopersmith R., Hines-Peralta A., Chen Y., Ivins K.J., Manly S.P., Kozlowski M.R., Neve K.A., Neve R.L., 2003. DNA synthesis and neuronal apoptosis caused by familial Alzheimer disease mutants of the amyloid precursor protein are mediated by the p21 activated kinase PAK3. J Neurosci. 23(17), 6914-6927.

Mega M.S., 2000. The cholinergic deficit in Alzheimer's disease: impact on cognition, behaviour and function. Int J Neuropsychopharmacol. 3(7), 3-12.

Moehlmann T., Winkler E., Xia X., Edbauer D., Murrell J., Capell A., Kaether C., Zheng H., Ghetti B., Haass C., Steiner H., 2002. Presenilin-1 mutations of leucine 166 equally affect the generation of the Notch and APP intracellular domains independent of their effect on Aβ 42 production. Proc Natl Acad Sci U S A. 99(12), 8025-8030.

Molnár Z., Soós K., Lengyel I., Penke B., Szegedi V., Budai D., 2004. Enhancement of NMDA responses by β-amyloid peptides in the hippocampus in vivo. Neuroreport. 15(10), 1649-1652.

Mondadori C.R., Buchmann A., Mustovic H., Schmidt C.F., Boesiger P., Nitsch R.M., Hock C., Streffer J., Henke K., 2006. Enhanced brain activity may precede the diagnosis of Alzheimer's disease by 30 years. Brain. 129(Pt 11), 2908-2922.

Morgan D., Diamond D.M., Gottschall P.E., Ugen K.E., Dickey C., Hardy J., Duff K., Jantzen P., DiCarlo G., Wilcock D., Connor K., Hatcher J., Hope C., Gordon M., Arendash G.W., 2000. Aβ peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature. 408(6815), 982-985.

Morley J., Farr S., Banks W., Johnson N.S., Yamada A.K, and Xu L., 2008. A Physiological Role for Amyloid β Protein: Enhancement of Learning and Memory. Available from Nature Precedings (http://hdl.handle.net/10101/npre.2008.2119.1).

Morris R., 1984. Developments of a water-maze procedure for studying spatial learning in the rat. J. Neurosci. Methods 11, 47-60.

Moussa C.E., Fu Q., Kumar P., Shtifman A., Lopez J.R., Allen P.D., LaFerla F., Weinberg D., Magrane J., Aprahamian T., Walsh K., Rosen K.M., Querfurth H.W., 2006. Transgenic expression of β-APP in fast-twitch skeletal muscle leads to calcium dyshomeostasis and IBM-like pathology. FASEB J. 20(12), 2165-2167.

Moya K.L., Benowitz L.I., Schneider G.E., Allinquant B., 1994. The amyloid precursor protein is developmentally regulated and correlated with synaptogenesis. Dev Biol. 161(2), 597-603.

Mudher A., Lovestone S., 2002. Alzheimer's disease-do tauists and baptists finally shake hands? Trends Neurosci. 25(1), 22-26.

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 β-amyloid. Nat Genet.1(5), 345-347.

Murphy T.H., Blatter L.A., Wier W.G., Baraban J.M., 1992. Spontaneous synchronous synaptic calcium transients in cultured cortical neurons. J Neurosci. 12(12), 4834-4845.

Murray J.N., Igwe O.J., 2003. Regulation of β-amyloid precursor protein and inositol 1,4,5-trisphosphate receptor gene expression during differentiation of a human neuronal cell line. Prog Neuropsychopharmacol Biol Psychiatry. 27(3), 351-563.

Muyllaert D, Terwel D, Borghgraef P, Devijver H, Dewachter I, Van Leuven F., 2006. Transgenic mouse models for Alzheimer's disease: the role of GSK-3B in combined amyloid and tau-pathology. Rev Neurol (Paris). 162(10), 903-907.

Nadkarni S., Jung P., 2003. Spontaneous oscillations of dressed neurons: a new mechanism for epilepsy? Phys Rev Lett. 91(26 Pt 1), 268101.

Nilsberth C., Westlind-Danielsson A., Eckman C.B., Condron M.M., Axelman K., Forsell C., Stenh C., Luthman J., Teplow D.B., Younkin S.G., Näslund J., Lannfelt L., 2001. The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Aβ protofibril formation. Nat Neurosci. 4(9), 887-893.

Ninomiya H, Roch JM, Sundsmo MP, Otero DA, Saitoh T., 1993. Amino acid sequence RERMS represents the active domain of amyloid β/A4 protein precursor that promotes fibroblast growth. J Cell Biol. 121(4), 879-886.

Nishimoto I., Okamoto T., Matsuura Y., Takahashi S., Okamoto T., Murayama Y., Ogata E., 1993. Alzheimer amyloid protein precursor complexes with brain GTP-binding protein G(o). Nature. 362(6415), 75-79.

Nixon R.A., Cataldo A.M., 2006. Lysosomal system pathways: genes to neurodegeneration in Alzheimer's disease. J Alzheimers Dis. 9(3 Suppl), 277-289.

Nizzari M., Venezia V., Repetto E., Caorsi V., Magrassi R., Gagliani M.C., Carlo P., Florio T., Schettini G., Tacchetti C., Russo T., Diaspro A., Russo C., 2007. Amyloid precursor protein and Presenilin1 interact with the adaptor GRB2 and modulate ERK 1,2 signaling. J Biol Chem. 282(18), 13833-13844.

Oddo S., Caccamo A., Kitazawa M., Tseng B.P., LaFerla F.M., 2003a. Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. Neurobiol Aging. 24(8), 1063-1070.

Oddo S., Caccamo A., Shepherd J.D., Murphy M.P., Golde T.E., Kayed R., Metherate R., Mattson M.P., Akbari Y., LaFerla F.M., 2003b. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Aβ and synaptic dysfunction. Neuron. 39(3), 409-421.

Oh Y.S., Turner R.J., 2006. Effect of γ -secretase inhibitors on muscarinic receptor-mediated calcium signaling in human salivary epithelial cells. Am J Physiol Cell Physiol. 291(1), C76-82.

Ohm T.G., Hamker U., Cedazo-Minguez A., Röckl W., Scharnagl H., März W., Cowburn R., Müller W., Meske V., 2001. Apolipoprotein E and β A4-amyloid: signals and effects. Biochem Soc Symp. 67, 121-129.

Ohsawa I., Takamura C., Kohsaka S., 2001. Fibulin-1 binds the amino-terminal head of β-amyloid precursor protein and modulates its physiological function. J Neurochem. 76(5), 1411-1420.

Ohkubo N., Mitsuda N., Tamatani M., Yamaguchi A., Lee Y.D., Ogihara T., Vitek M.P., Tohyama M., 2001. Apolipoprotein E4 stimulates cAMP response element-binding protein transcriptional activity through the extracellular signal-regulated kinase pathway. J Biol Chem. 276(5), 3046-3053.

Olsson A., Csajbok L., Ost M., Höglund K., Nylén K., Rosengren L., Nellgård B., Blennow K., 2004. Marked increase of β-amyloid(1-42) and amyloid precursor protein in ventricular cerebrospinal fluid after severe traumatic brain injury. J Neurol. 251(7), 870-876.

Osterfield M., Egelund R., Young L.M., Flanagan J.G., 2008. Interaction of amyloid precursor protein with contactins and NgCAM in the retinotectal system. Development. 135(6), 1189-1199.

Palop J.J., Chin J., Mucke L., 2006. A network dysfunction perspective on neurodegenerative diseases. Nature. 443(7113), 768-773.

Palop J.J., Chin J., Roberson E.D., Wang J., Thwin M.T., Bien-Ly N., Yoo J., Ho K.O., Yu G.Q., Kreitzer A., Finkbeiner S., Noebels J.L., 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.

Pardossi-Piquard R., Petit A., Kawarai T., Sunyach C., da Costa C.A., Vincent B., Ring S., D'Adamio L., Shen J., Müller U., Hyslop P.S.G., Checler F., 2005. Presenilin-dependent transcriptional control of the Aβ-degrading enzyme neprilysin by intracellular domains of βAPP and APLP. Neuron 46: 541–554.

Park J.H., Gimbel D.A., GrandPre T., Lee J.K., Kim J.E., Li W., Lee D.H., Strittmatter S.M., 2006. Alzheimer precursor protein interaction with the Nogo-66 receptor reduces amyloid-β plaque deposition. J Neurosci. 26(5), 1386-1395.

Parisiadou L., Bethani I., Michaki V., Krousti K., Rapti G., Efthimiopoulos S., 2008. Homer2 and Homer3 interact with amyloid precursor protein and inhibit Aβ production. Neurobiol Dis. 30(3), 353-364.

Pastor P., Roe C.M., Villegas A., Bedoya G., Chakraverty S., García G., Tirado V., Norton J., Ríos S., Martínez M., Kosik K.S., Lopera F., Goate A.M., 2003. Apolipoprotein E ε4 modifies Alzheimer's disease onset in an E280A PS1 kindred. Ann Neurol. 54(2), 163-169.

Pastorino L., Sun A., Lu P.J., Zhou X.Z., Balastik M., Finn G., Wulf G., Lim J., Li S.H., Li X., Xia W., Nicholson L.K., Lu K.P., 2006. The prolyl isomerase Pin1 regulates amyloid precursor protein processing and amyloid-β production. Nature. 440(7083), 528-534.

Pawlik M., Otero D.A., Park M., Fischer W.H., Levy E., Saitoh T., 2007. Proteins that bind to the RERMS region of β amyloid precursor protein. Biochem Biophys Res Commun. 355(4), 907-912.

Perry E.K., Tomlinson B.E., Blessed G., Bergmann K., Gibson P.H., Perry R.H., 1978. Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. Br Med J. 2(6150), 1457-1459.

Petersen R.C., 2007. Mild cognitive impairment: current research and clinical implications.. Semin Neurol. 27(1), 22-31.

Pfeifer M., Boncristiano S., Bondolfi L., Stalder A., Deller T., Staufenbiel M., Mathews P.M., Jucker M., 2002. Cerebral hemorrhage after passive anti-Aβ immunotherapy. Science. 298(5597), 1379.

Piccininni M., Di Carlo A., Baldereschi M., Zaccara G., Inzitari D., 2005. Behavioral and psychological symptoms in Alzheimer's disease: frequency and relationship with duration and severity of the disease. Dement Geriatr Cogn Disord. 19(5-6), 276-281.

Pierrot N., Ghisdal P., Caumont A.S., Octave J.N., 2004. Intraneuronal amyloid-β 1-42 production triggered by sustained increase of cytosolic calcium concentration induces neuronal death. J Neurochem. 88(5), 1140-1150.

Pierrot N., Santos S.F., Feyt C., Morel M., Brion J.P., Octave J.N., 2006. Calcium-mediated transient phosphorylation of tau and amyloid precursor protein followed by intraneuronal amyloid-β accumulation. J Biol Chem. 281(52), 39907-39914.

Pietrzik CU, Hoffmann J, Stöber K, Chen CY, Bauer C, Otero DA, Roch JM, Herzog V., 1998. From differentiation to proliferation: the secretory amyloid precursor protein as a local mediator of growth in thyroid epithelial cells. Proc Natl Acad Sci U S A. 95(4), 1770-1775.

Pike C.J., Walencewicz A.J., Glabe C.G., Cotman C.W., 1991. In vitro aging of β-amyloid protein causes peptide aggregation and neurotoxicity. Brain Res. 563(1-2), 311-314.

Pinnix I., Musunuru U., Tun H., Sridharan A., Golde T., Eckman C., Ziani-Cherif C., Onstead L., Sambamurti K., 2001. A novel γ-secretase assay based on detection of the putative C-terminal fragment- γ of amyloid β protein precursor. J Biol Chem. 276(1), 481-487.

Popp GM, Graebert KS, Pietrzik CU, Rosentreter SM, Lemansky P, Herzog V., 1996. Growth regulation of rat thyrocytes (FRTL-5 cells) by the secreted ectodomain of β-amyloid precursor-like proteins. Endocrinology. 137(5), 1975-1983.

Querfurth H.W., Selkoe D.J., 1994. Calcium ionophore increases amyloid β peptide production by cultured cells. Biochemistry. 33(15), 4550-4561.

Radde R., Bolmont T., Kaeser S.A., Coomaraswamy J., Lindau D., Stoltze L., Calhoun M.E., Jäggi F., Wolburg H., Gengler S., Haass C., Ghetti B., Czech C., Hölscher C., Mathews P.M., Jucker M., 2006. Aβ42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. EMBO Rep. 7(9), 940-946.

Reynish W., Andrieu S., Nourhashemi F., Vellas B., 2001. Nutritional factors and Alzheimer's disease. J Gerontol A Biol Sci Med Sci. 56(11), M675-680.

Ring S., Weyer S.W., Kilian S.B., Waldron E., Pietrzik C.U., Filippov M.A., Herms J., Buchholz C., Eckman C.B., Korte M., Wolfer D.P., Müller U.C., 2007. The secreted beta-amyloid precursor protein ectodomain APPs alpha is sufficient to rescue the anatomical, behavioral, and electrophysiological abnormalities of APP-deficient mice. J Neurosci. 27(29), 7817-7826.

Robinson D.M., Keating G.M., 2006. Memantine: a review of its use in Alzheimer's disease. Drugs. 66(11), 1515-1534.

Roch J.M., Masliah E., Roch-Levecq A.C., Sundsmo M.P., Otero D.A., Veinbergs I., Saitoh T., 1994. Increase of synaptic density and memory retention by a peptide representing the trophic domain of the amyloid β/A4 protein precursor. Proc Natl Acad Sci U S A. 91(16), 7450-7454.

Rogaeva E., Meng Y., Lee J.H., Gu Y., Kawarai T., Zou F., Katayama T., Baldwin C.T., Cheng R., Hasegawa H., Chen F., Shibata N., Lunetta K.L., Pardossi-Piquard R., Bohm C., Wakutani Y., Cupples L.A., Cuenco K.T., Green R.C., Pinessi L., Rainero I., Sorbi S., Bruni A., Duara R., Friedland R.P., Inzelberg R., Hampe W., Bujo H., Song Y.Q., Andersen O.M., Willnow T.E., Graff-Radford N., Petersen R.C., Dickson D., Der S.D., Fraser P.E., Schmitt-Ulms G., Younkin S., Mayeux R., Farrer L.A., St George-Hyslop P., 2007. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. Nat Genet. 39(2), 168-177.

Rojas G., Cárdenas A.M., Fernández-Olivares P., Shimahara T., Segura-Aguilar J., Caviedes R., Caviedes P., 2008. Effect of the knockdown of amyloid precursor protein on intracellular calcium increases in a neuronal cell line derived from the cerebral cortex of a trisomy 16 mouse. Exp Neurol. 209(1), 234-242.

Roncarati R., Sestan N., Scheinfeld M.H., Berechid B.E., Lopez P.A., Meucci O., McGlade J.C., Rakic P., D'Adamio L., 2002. The g-secretase-generated intracellular domain of β-amyloid precursor protein binds Numb and inhibits Notch signaling. Proc Natl Acad Sci U S A 99, 7102–7107.

Rossner S., Fuchsbrunner K., Lange-Dohna C., Hartlage-Rübsamen M., Bigl V., Betz A., Reim K., Brose N., 2004. Munc13-1-mediated vesicle priming contributes to secretory amyloid precursor protein processing. J Biol Chem. 279(27), 27841-27844.

Rowan M.J., Klyubin I., Wang Q., Hu N.W., Anwyl R., 2007. Synaptic memory mechanisms: Alzheimer's disease amyloid β-peptide-induced dysfunction. Biochem Soc Trans. 35(Pt 5), 1219-1223.

Ruiz-Opazo N., Kosik K.S., Lopez L.V., Bagamasbad P., Ponce L.R., Herrera V.L., 2004. Attenuated hippocampus-dependent learning and memory decline in transgenic Tg6590 Fischer-344 rats. Mol. Med. 10, 36-44.

Ruscheweyh R., Sandkühler J., 2005. Long-range oscillatory Ca^{2+} waves in rat spinal dorsal horn. Eur J Neurosci. 22(8), 1967-1976.

Ryan K.A., Pimplikar S.W., (2005) Activation of GSK-3 and phosphorylation of CRMP2 in transgenic mice expressing APP intracellular domain. J Cell Biol 171, 327–335.

Sadile A.G., 1996. Long-term habituation of theta-related activity components of albino rats in the làt-maze, in: Sanberg, P.R., Ossenkopp, K.P., Kovaliers, M. (Eds), Motor Activity and Movement Disorders. Humana Press, Totowa., pp. 3-55.

Schäfer S., Wirths O., Multhaup G., Bayer T.A., 2007. Gender dependent APP processing in a transgenic mouse model of Alzheimer's disease. J Neural Transm. 114(3), 387-394.

Scheff S.W., Price D.A., Schmitt F.A., DeKosky S.T., Mufson E.J., 2007. Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. Neurology. 68(18), 1501-1508.

Scheinfeld M.H., Roncarati R., Vito P., Lopez P.A., Abdallah M, D'Adamio L., 2002. Jun NH2-terminal kinase (JNK) interacting protein 1 (JIP1) binds the cytoplasmic domain of the Alzheimer's β-amyloid precursor protein (APP). J Biol Chem 277, 3767–3775.

Schenk D., Barbour R., Dunn W., Gordon G., Grajeda H., Guido T., Hu K., Huang J., Johnson-Wood K., Khan K., Kholodenko D., Lee M., Liao Z., Lieberburg I., Motter R., Mutter L., Soriano F., Shopp G., Vasquez N., Vandevert C., Walker S., Wogulis M., Yednock T., Games D., Seubert P., 1999. Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature. 400(6740), 173-177.

Scheuermann S., Hambsch B., Hesse L., Stumm J., Schmidt C., Beher D., Bayer T.A., Beyreuther K., Multhaup G., 2001. Homodimerization of amyloid precursor protein and its implication in the amyloidogenic pathway of Alzheimer's disease. J Biol Chem. 276(36), 33923-33929.

Schmidt C., Lepsverdize E., Chi S.L., Das A.M., Pizzo S.V., Dityatev A., Schachner M., 2007a. Amyloid precursor protein and amyloid β-peptide bind to ATP synthase and regulate its activity at the surface of neural cells. Mol Psychiatry. Advanced online publication: 2007 Aug 28.

Schmidt V., Sporbert A., Rohe M., Reimer T., Rehm A., Andersen O.M., Willnow T.E., 2007b. SorLA/LR11 regulates processing of amyloid precursor protein via interaction with adaptors GGA and PACS-1. J Biol Chem. 282(45), 32956-32964.

Schultzberg M., Lindberg C., Aronsson A.F., Hjorth E., Spulber S.D., Oprica M., 2007. Inflammation in the nervous system--physiological and pathophysiological aspects. Physiol Behav.92(1-2), 121-128.

Seabrook G.R., Smith D.W., Bowery B.J., Easter A. Reynolds T., Fitzjohn S.M., Morton R.A., Zheng H., Dawson G.R., Sirinathsinghji D.J., Davies C.H., Collingridge G.L., Hill R.G., 1999. Mechanisms contributing to the deficits in hippocampal synaptic plasticity in mice lacking amyloid precursor protein. Neuropharmacology. 38, 349-359.

Selivanova A., Winblad B., Dantuma N.P., Farmery M.R., 2007. Biogenesis and processing of the amyloid precursor protein in the early secretory pathway. Biochem Biophys Res Commun. 357(4), 1034-1039.

Senior K., 2002. Dosing in phase II trial of Alzheimer's vaccine suspended. Lancet Neurol. 1(1), 3.

Sennvik K., Fastbom J., Blomberg M., Wahlund L.O., Winblad B., Benedikz E., 2000. Levels of alpha- and beta-secretase cleaved amyloid precursor protein in the cerebrospinal fluid of Alzheimer's disease patients. Neurosci Lett. 278(3), 169-172.

Seubert P., Vigo-Pelfrey C., Esch F., Lee M., Dovey H., Davis D., Sinha S., Schlossmacher M., Whaley J., Swindlehurst C., McCormack R., Wolfert R., Selkoe D., Lieberburg I., Schenk D., 1992. Isolation and quantification of soluble Alzheimer's β-peptide from biological fluids. Nature. 359(6393), 325-327.

Shankar G.M., Bloodgood B.L., Townsend M., Walsh D.M., Selkoe D.J., Sabatini B.L., 2007. Natural oligomers of the Alzheimer amyloid-β protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. J Neurosci. 27(11), 2866-2875.

Sherrington R., Rogaev E.I., Liang Y., Rogaeva E.A., Levesque G., Ikeda M., Chi H., Lin C., Li G., Holman K., Tsuda T., Mar L., Foncin J.-F., Bruni A.C., Montesi M.P., Sorbi S., Rainero I., Pinessi L., Nee L., Chumakov I., Pollen D., Brookes A., Sanseau P., Polinsky R.J., Wasco W., Da Silva H.A.R., Haines J.L., Pericak-Vance M.A., Tanzi R.E., Roses A.D., Fraser P.E., Rommens J.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.

Singh A., Gebhart M., Fritsch R., Sinnegger-Brauns M.J., Poggiani C., Hoda J.C., Engel J., Romanin C., Striessnig J., Koschak A., 2008. Modulation of Voltage- and Ca^{2+}-dependent Gating of $Ca_V1.3$ L-type Calcium Channels by Alternative Splicing of a C-terminal Regulatory Domain. J Biol Chem. 283(30), 20733-20744.

Sisodia S.S., Koo E.H., Beyreuther K., Unterbeck A., Price D.L., 1990. Evidence that β-amyloid protein in Alzheimer's disease is not derived by normal processing. Science. 248(4954), 492-495.

Sisodia S.S., 1992. Secretion of the β-amyloid precursor protein. Ann N Y Acad Sci. 674, 53-57.

Sisodia S.S., Koo E.H., Hoffman P.N., Perry G., Price D.L., 1993. Identification and transport of full-length amyloid precursor proteins in rat peripheral nervous system. J Neurosci. 13(7), 3136-3142.

Sisodia S.S., Thinakaran G., Slunt H.H., Kitt C.A., Von Koch C.S., Reed R.R., Zheng H., Price D.L., 1996. Studies on the metabolism and biological function of APLP2. Ann N Y Acad Sci. 777, 77-81.

Sleegers K., Brouwers N., Gijselinck I., Theuns J., Goossens D., Wauters J., Del-Favero J., Cruts M., van Duijn C.M., Van Broeckhoven C., 2006. APP duplication is sufficient to cause early onset Alzheimer's dementia with cerebral amyloid angiopathy. Brain. 129, 2977-2983.

Small D.H., Nurcombe V., Reed G., Clarris H., Moir R., Beyreuther K., Masters C.L., 1994. A heparin-binding domain in the amyloid protein precursor of Alzheimer's disease is involved in the regulation of neurite outgrowth. J Neurosci. 14(4), 2117-2127.

Small D.H., Clarris H.L., Williamson T.G., Reed G., Key B., Mok S.S., Beyreuther K., Masters C.L., Nurcombe V., 1999. Neurite-outgrowth regulating functions of the amyloid protein precursor of Alzheimer's disease. J Alzheimers Dis. 1(4-5), 275-285.

Small S.A., Gandy S., 2006. Sorting through the cell biology of Alzheimer's disease: intracellular pathways to pathogenesis. Neuron. 52(1), 15-31.

Soba P., Eggert S., Wagner K., Zentgraf H., Siehl K., Kreger S., Löwer A., Langer A., Merdes G., Paro R., Masters C.L., Müller U., Kins S., Beyreuther K., 2005. Homo- and heterodimerization of APP family members promotes intercellular adhesion. EMBO J. 24(20), 3624-3634.

Sola Vigo F., Kedikian G., Heredia L., Heredia F., Añel A.D., Rosa A.L., Lorenzo A., 2008. Amyloid-β precursor protein mediates neuronal toxicity of amyloid β through Go protein activation. Neurobiol Aging. Advance online publication: 2008 Jan 8.

Sorbi S., Nacmias B., Forleo P., Piacentini S., Latorraca S., Amaducci L., 1995. Epistatic effect of APP717 mutation and apolipoprotein E genotype in familial Alzheimer's disease. Ann Neurol. 38(1), 124-127.

Spasic D., Annaert W., 2008. Building γ-secretase: the bits and pieces. J Cell Sci. 121(Pt 4):413-420.

Steen E., Terry B.M., Rivera E.J., Cannon J.L., Neely T.R., Tavares R., Xu X.J., Wands J.R., de la Monte S.M., 2005. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease--is this type 3 diabetes? J Alzheimers Dis. 7(1), 63-80.

Steinhilb M.L., Turner R.S., Gaut J.R., 2002. ELISA analysis of β-secretase cleavage of the Swedish amyloid precursor protein in the secretory and endocytic pathways. J Neurochem. 80(6), 1019-1028.

Sturchler-Pierrat C., Abramowski D., Duke M., Wiederhold K.H., Mistl C., Rothacher S., Ledermann B., Bürki K., Frey P., Paganetti P.A., Waridel C., Calhoun M.E., Jucker M., Probst A., Staufenbiel M., Sommer B., 1997. Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. Proc Natl Acad Sci U S A. 94(24), 13287-13292.

Sturchler-Pierrat, Staufenbiel, 2000. Pathogenic mechanisms of Alzheimer's disease analyzed in the APP23 transgenic mouse model. Ann N Y Acad Sci. 920:134-139.

Sun X, He G, Song W., 2006. BACE2, as a novel APP τ-secretase, is not responsible for the pathogenesis of Alzheimer's disease in Down syndrome. FASEB J. 20(9), 1369-1376.

Supnet C., Grant J., Kong H., Westaway D., Mayne M., 2006. Amyloid-β-(1-42) increases ryanodine receptor-3 expression and function in neurons of TgCRND8 mice. J Biol Chem. 281(50), 38440-38337.

Suzuki N., Cheung T.T., Cai X.D., Odaka A., Otvos L. Jr., Eckman C., Golde T.E., Younkin S.G., 1994. An increased percentage of long amyloid β protein secreted by familial amyloid β protein precursor (β APP717) mutants. Science. 264(5163), 1336-1340.

Suzuki T., Nakaya T., 2008. Regulation of APP by phosphorylation and protein interactions. J Biol Chem. Advance online publication: 2008 Jul 23.

Swanson T.L., Knittel L.M., Coate T.M., Farley S.M., Snyder M.A., Copenhaver P.F., 2005. The insect homologue of the amyloid precursor protein interacts with the heterotrimeric G protein Go α in an identified population of migratory neurons. Dev Biol. 288(1), 160-178.

Swerdlow R.H., Khan S.M., 2004. A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease. Med Hypotheses. 63(1), 8-20.

Takeuchi A., Irizarry M.C., Duff K., Saido T.C., Hsiao Ashe K., Hasegawa M., Mann D.M., Hyman B.T., Iwatsubo T., 2000. Age-related amyloid β deposition in transgenic mice overexpressing both Alzheimer mutant presenilin 1 and amyloid β precursor protein Swedish mutant is not associated with global neuronal loss. Am J Pathol. 157(1), 331-339.

Tang F., Dent E.W., Kalil K., 2003. Spontaneous calcium transients in developing cortical neurons regulate axon outgrowth. J Neurosci. 23(3), 927-936.

Tang B.L., Liou Y.C., 2007. Novel modulators of amyloid-β precursor protein processing. J Neurochem. 100(2), 314-323.

Tarr P.E., Roncarati R., Pelicci G., Pelicci P.G., D'Adamio L. 2002. Tyrosine phosphorylation of the β-amyloid precursor protein cytoplasmic tail promotes interaction with Shc. J Biol Chem 277, 16798–16804.

Taylor C.J., Ireland D.R., Ballagh I., Bourne K., Marechal N.M., Turner P.R., Bilkey D.K., Tate W.P., Abraham W.C., 2008. Endogenous secreted amyloid precursor protein-α regulates hippocampal NMDA receptor function, long-term potentiation and spatial memory. Neurobiol Dis. 31(2), 250-260.

Terwel D., Muyllaert D., Dewachter I., Borghgraef P., Croes S., Devijver H., Van Leuven F., 2008. Amyloid activates GSK-3β to aggravate neuronal tauopathy in bigenic mice. Am J Pathol. 172(3), 786-798.

Thal D.R., Griffin W.S., de Vos R.A., Ghebremedhin E., 2008. Cerebral amyloid angiopathy and its relationship to Alzheimer's disease. Acta Neuropathol. 115(6), 599-609.

Theuns J., Brouwers N., Engelborghs S., Sleegers K., Bogaerts V., Corsmit E., De Pooter T., van Duijn C.M., De Deyn P.P., Van Broeckhoven C., 2006a. Promoter mutations that increase amyloid precursor-protein expression are associated with Alzheimer disease. Am J Hum Genet. 78(6), 936-946.

Theuns J., Marjaux E., Vandenbulcke M., Van Laere K., Kumar-Singh S., Bormans G., Brouwers N., Van den Broeck M., Vennekens K., Corsmit E., Cruts M., De Strooper B., Van Broeckhoven C., 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 "β-secretase" site occurs in the golgi apparatus. J Biol Chem. 271(16), :9390-9397.

Thinakaran G., Koo E.H., 2008. APP trafficking, processing and function. J Biol Chem. Advanced online publication: 2008 Jul 23.

Trojanowski J.Q., Lee V.M., 2005. Rous-Whipple Award Lecture. The Alzheimer's brain: finding out what's broken tells us how to fix it. Am J Pathol. 167(5), 1183-1188.

Tu H., Nelson O., Bezprozvanny A., Wang Z., Lee S.F., Hao Y.H., Serneels L., De Strooper B., Yu G., Bezprozvanny I., 2006. Presenilins form ER Ca^{2+} leak channels, a function disrupted by familial Alzheimer's disease-linked mutations. Cell. 126(5), 981-993.

Turner P.R., O'Connor K., Tate W.P., Abraham W.C., 2003. Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. Prog Neurobiol. 70(1), 1-32.

Vaisid T., Kosower N.S., Katzav A., Chapman J., Barnoy S., 2007. Calpastatin levels affect calpain activation and calpain proteolytic activity in APP transgenic mouse model of Alzheimer's disease. Neurochem Int. 51(6-7), 391-397.

Van Dam D., D'Hooge R., Staufenbiel M., Van Ginneken C., Van Meir F., De Deyn P.P., 2003. Age-dependent cognitive decline in the APP23 model precedes amyloid deposition. Eur J Neurosci. 17(2), 388-396.

Vandenberghe R., 2006b. Alzheimer dementia caused by a novel mutation located in the APP C-terminal intracytosolic fragment. Hum Mutat. 27(9), 888-896.

Van Den Heuvel C., Thornton E., Vink R., 2007. Traumatic brain injury and Alzheimer's disease: a review. Prog Brain Res. 161, 303-316.

Van Nostrand W.E., Farrow J.S., Wagner S.L., Bhasin R., Goldgaber D., Cotman C.W., Cunningham D.D., 1991. The predominant form of the amyloid β-protein precursor in human brain is protease nexin 2. Proc Natl Acad Sci U S A. 88(22), 10302-10306.

Vassar R., Bennett B.D., Babu-Khan S., Kahn S., Mendiaz E.A., Denis P., Teplow D.B., Ross S., Amarante P., Loeloff R., Luo Y., Fisher S., Fuller J., Edenson S., Lile J., Jarosinski M.A., Biere A.L., Curran E., Burgess T., Louis J.C., Collins F., Treanor J., Rogers G., Citron M., 1999. β-Secretase Cleavage of Alzheimer's Amyloid Precursor Protein by the Transmembrane Aspartic Protease BACE. Science 286, 735.

Veinbergs I., Everson A., Sagara Y., Masliah E., 2002 Neurotoxic effects of apolipoprotein E4 are mediated via dysregulation of calcium homeostasis. J Neurosci Res. 67(3), 379-387.

von Rotz R.C., Kohli B.M., Bosset J., Meier M., Suzuki T., Nitsch R.M., Konietzko U., 2004. The APP intracellular domain forms nuclear multiprotein complexes and regulates the transcription of its own precursor. J Cell Sci 117: 4435–4448.

Walker E.S., Martinez M., Brunkan A.L., Goate A., 2005. Presenilin 2 familial Alzheimer's disease mutations result in partial loss of function and dramatic changes in Aβ42/40 ratios. J Neurochem. 92(2), 294-301.

Walter J., Haass C., 1999. Posttranslational Modifications of Amyloid Precursor Protein. Springer Protocols: Alzheimer's Disease: Methods and Protocols, 32: 149-168.

Wang H.Y., Lee D.H., D'Andrea M.R., Peterson P.A., Shank R.P., Reitz A.B., 2000. β-Amyloid(1-42) binds to α7 nicotinic acetylcholine receptor with high affinity. Implications for Alzheimer's disease pathology. J Biol Chem. 275(8), 5626-5632.

Westerman M.A., Cooper-Blacketer D., Mariash A., Kotilinek L., Kawarabayashi T., Younkin L.H., Carlson G.A., Younkin S.G., Ashe K.H., 2002. The relationship between Aβ and memory in the Tg2576 mouse model of Alzheimer's disease. J Neurosci. 22(5):1858-67.

Whitehouse P., Price D., Struble R., Clarke A., Coyle J., Delong M., 1982. Alzheimer's disease in senile dementia: loss of neurones in the basal forebrain. Science. 215, 1237-1239.

Wijsman E.M., Daw E.W., Yu X., Steinbart E.J., Nochlin D., Bird T.D., Schellenberg G.D., 2005. APOE and other loci affect age-at-onset in Alzheimer's disease families with PS2 mutation. Am J Med Genet B Neuropsychiatr Genet. 132B(1), 14-20.

Wilcock D.M., Jantzen P.T., Li Q., Morgan D., Gordon M.N., 2007. Amyloid-β vaccination, but not nitro-nonsteroidal anti-inflammatory drug treatment, increases vascular amyloid and microhemorrhage while both reduce parenchymal amyloid. Neuroscience. 144(3), 950-960.

Wimmer B., Friedburg H., Hennig J., Kauffmann G.W., 1986. Diagnostic imaging potentialities of nuclear resonance tomography. Changes in vertebrae, ligaments and intervertebral disks in comparison with computed tomography. Radiologe. 26, 137-143.

Wirths O., Bayer T.A., 2008. Motor impairment in Alzheimer's disease and transgenic Alzheimer's disease mouse models. Genes Brain Behav. 7 Suppl 1:1-5.

Wise L.E., Lichtman A.H., 2007. The uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist memantine prolongs spatial memory in a rat delayed radial-arm maze memory task. Eur J Pharmacol. 575(1-3), 98-102. Advance online publication: 2007 Aug 3.

Wu J, Anwyl R, Rowan MJ., 1995a. β-Amyloid-(1-40) increases long-term potentiation in rat hippocampus in vitro. Eur J Pharmacol. 284(3), R1-3.

Wu J, Anwyl R, Rowan MJ., 1995b. β-Amyloid selectively augments NMDA receptor-mediated synaptic transmission in rat hippocampus. Neuroreport. 6(17), 2409-2413.

Yamazaki Y, Jia Y, Niu R, Sumikawa K., 2006. Nicotine exposure in vivo induces long-lasting enhancement of NMDA receptor-mediated currents in the hippocampus. Eur J Neurosci. 23(7):1819-1828.

Young-Pearse T.L., Bai J., Chang R., Zheng J.B., LoTurco J.J., Selkoe D.J., 2007. A critical function for β-amyloid precursor protein in neuronal migration revealed by in utero RNA interference. J Neurosci. 27(52), 14459-14469.

Young-Pearse T.L., Chen A.C., Chang R., Marquez C., Selkoe D.J., 2008. Secreted APP regulates the function of full-length APP in neurite outgrowth through interaction with integrin β1. Neural Develop. 3:15.

Zambrano N., Bruni P., Minopoli G., Mosca R., Molino D., Russo C., 1998. The Fe65 adaptor protein interacts through its PID1 domain with the transcription factor CP2/LSF/LBP1. J Biol Chem 273, 20128–20133.

Zhao J., Paganini L., Mucke L., Gordon M., Refolo L., Carman M., Sinha S., Oltersdorf T., Lieberburg I., McConlogue L., 1996. β-secretase processing of the β-amyloid precursor protein in transgenic mice is efficient in neurons but inefficient in astrocytes. J Biol Chem. 271(49), 31407-31411.

Zheng H., Jiang M., Trumbauer M.E., Sirinathsinghji D.J., Hopkins R., Smith D.W., Heavens R.P., Dawson G.R., Boyce S., Conner M.W., Stevens K.A., Slunt H.H., Sisoda S.S., Chen H.Y., Van der Ploeg L.H., 1995. β-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. Cell. 81(4), 525-531.

Zheng P., Eastman J., Pol S.V., Pimplikar S.W., 1998. PAT1, a microtubule-interacting protein, recognizes the basolateral sorting signal of amyloid precursor protein. Proc Natl Acad Sci U S A 95, 14745–14750.

Zhu X, Lee HG, Perry G, Smith MA., 2007. Alzheimer disease, the two-hit hypothesis: an update. Biochim Biophys Acta. 1772(4), 494-502.

Zilka N., Filipcik P., Koson P., Fialova L., Skrabana R., Zilkova M., Rolkova G., Kontsekova E., Novak M., 2006. Truncated tau from sporadic Alzheimer's disease suffices to drive neurofibrillary degeneration in vivo. FEBS Lett. 580(15), 3582-3588.