The role of amyloid β peptide in synaptic alterations and spine loss

‘Amyloid β induced disturbance of synaptic signaling’

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Introduction

The human brain is the center of the nervous system and is a highly intricate structure. It enables us to use our senses and provide us the opportunity to move. Other important functions of the brain are the formation of thoughts, emotions and memories. For a proper performance of all its function, the brain needs a very good functioning signaling network.

An average adult human brain contains more than 10^{11} neurons. Communication between different neurons is from huge importance for a proper functioning of the brain and occurs at specialized junctions, the synapses. There are approximately 10^{16} synapses, concluding that one neuron is connected with multiple other neurons via the synapses. Neurons process and transmit information in the form of an electrical signal. This signal is transported to the axonal terminal, where it has to be converted into a neurotransmitter signal. This is because of the fact that neurons do not directly contact to each other, there is a small cleft between the axon of one neuron and the dendrite of the second neuron, the synaptic cleft. The development of the synapses and their connectivity are critical for a proper functioning of the brain. It is thought that the structure and chemistry of a synapse are very important for its functioning. Information is stored in form of an altered structure and chemistry of synapses and/ or by the formation of new synapses and the exclusion of the old synapses. This mechanism is named the plasticity of synapses and it seems to be the basis of learning and memory. [1]

Alterations in (the functioning of) the brain can lead to neurologic and psychiatric disorders, such as schizophrenia, Parkinson's disease and Alzheimer's disease (AD). Alzheimer's disease is the most prevalent neurologic disorder, it affects approximately 10% of the population over the age of 65. Within the population over the age of 85 even more than 50% has AD. The majority of cases (90-95%) is sporadic (SAD), the remainder is familial (FAD). FAD results from an inherited autosomal dominant gene mutation and causes AD at an age of 40-60 years. The most common mutations seen in FAD, are mutations in the genes encoding amyloid precursor protein (APP) and presenilin1 and presenilin2 (PS1 and PS2) . [2] All types of AD are characterized by a progressive dementia, which seems to be caused by an accumulation of intracellular neurofibrillary tangles (NFTs) and extracellular neuritic plaques. The affected parts of the brain are the hippocampus and the cortex. The intracellular NFTs are formed by hyperphosphorylated twisted filaments of the protein tau. Whereas the extracellular neuritic plaques are deposits of amyloid beta (Aβ) molecules, which are formed out of the amyloid precursor protein (APP). So it seems that there are three major players within the development of AD: tau protein, amyloid precursor protein and amyloid beta.

Tau protein is a microtubule-associated protein (MAP), which plays a role in the establishing and maintaining of the neuronal morphology. It regulates the microtubule polymer state, as well as it interacts with other cytoskeletal and subcellular components. Its presence is limited to the axonal parts of the neurons. The human gene encoding tau protein is located on chromosome 17q21 and contains 16 exons. Due to alternative splicing six main isoforms can be made, built of 352-441 amino acid residues. [3] The tau protein contains a repeat domain that is formed by three or four pseudo-repeats, which plays an important role in the binding to microtubules. The function of tau is regulated via its phosphorylation sites. Within the longest isoform of tau approximately 80 Ser or Thr residues are present, so about 20% of this isoform can be phosphorylated. In 1984 it was already shown that tau was the most efficient in promoting the microtubule assembly in a

dephosphorylated state.[4] Abnormally phosphorylated forms of tau protein resulted in paired helical filaments (PHFs), which subsequently formed the neurofibrillary tangles that were found in Alzheimer's disease brains. Mutations in the tau gene are known and cause rare autosomal dominant neurodegenerative diseases. This diseases are known as frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), whereby tha tau protein was abnormally hyperphosphoylated. This resulted in less interaction with the microtubules and the aggregation of tau proteins into the insoluble form, the NFTs. [5] Despite the enormous role of tau within the development of AD, the idea that tau protein was the main cause of neurodegenerative diseases was abandoned when it was discovered that there were mutations in other genes that seem to affect the production of amyloid beta, causing familial Alzheimer's disease. [6,7]

Amyloid beta is a cleavage product of the amyloid precursor protein (APP). APP is a member of a family of related proteins, which includes the amyloid precursor-like proteins (APLP-1 and APLP-2) in mammals and the amyloid precursor-protein-like (APPL) in Drosophila. All of the proteins out of this family are single-pass membrane structures with large extracellular domains. [8] In humans the gene encoding APP is located on chromosome 21, with eight isoforms arising from alternative splicing. Three isoforms are the most common: APP_{695}, APP_{751} and APP_{770}. Whereby the number refers to the amount of amino acid residues.[9] Both APP_{751} and APP_{770} are expressed in most tissues. APP_{695} however, is predominantly expressed in the central nervous system. The precise role of APP is still unclear, but it is suggested that it has a function in neurite outgrow, synaptogenesis, the trafficking of neuronal proteins along the axons and cell adhesion. Some studies showed the positive effect of APP on cell growth and cell health, for example the study of Oh *et al. (2009).* They showed that transgenic mice, which overexpressed APP, had enlarged neurons. The effect of APP was also studied in transiently transfected cell lines, whereby was shown that APP controls the cell growth, motility of the cell, neurite outgrow and even cell survival. [2]

Crucial steps within the processing of APP occur at the cell surface and in the Trans Golgi Network (TGN). Via the TGN, APP can be transported to the cell surface or an endosomal compartment. The APP on the cell surface can be cleaved in two different pathways, the non-amyloidogenic and the amyloidogenic pathway (See Fig.1.). Within the non-amyloidogenic pathway the APP is cleaved in the Amyloid-β domain by α-secretase, forming a soluble APP ectodomain (sAPPα) and a membrane-anchored C-terminal fragment, C83. The cleavage by a second secretase (γ-secretase) forms two different structures: the APP intracellular domain (AICD) and a smaller structure, the P3. Within the amyloidogenic pathway the APP is cleaved by β-secretase, forming a soluble structure, sAPPβ and a C-terminal structure, C99. Subsequently the C99 structure is cleaved by γ-secretase, thereby forming AICD and amyloid- β. This amyloid- β is the toxic peptide, that seems to be the major player in AD.

The effect of Aβ on neurons is intensively investigated. Research with tissue cultures showed that the presence of Aβ molecules was acutely toxic to neurons, resulting in death of all the neurons within 24h of exposure. [11] It seemed that the neurons were affected by the toxic effects of Aβ, causing oxidative effects, which led to cell apoptosis. Mouse models showed that overexpression of mutant human APP resulted in Aβ deposition and neuronal injury. Also loss of

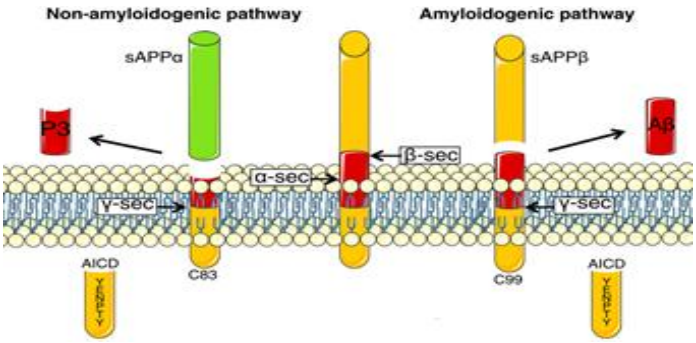

Fig. 1. Schematic diagram of APP processing. In the non-amyloidogenic pathway APP is cleaved in the Aβ-domain by α-secretase. Forming the soluble APP ectodomain (sAPPα) and the membrane-anchored C-terminal fragment, C83. Further cleavage by γ-secretase releases two peptides: P3 and the APP intracellular domain (AICD). In the amyloidogenic pathway APP is cleaved by β-secretase, thereby releasing soluble sAPPβ and the C-terminal fragment, C99. Which is subsequently cleaved into AID and Aβ. [Claeysen et al(2012)]

synaptic terminals and synaptic dysfunctioning were shown in these models. Despite this, there was proven that there was no correlation between the amount of Aβ plaques in the AD brain and the severity synapse loss. Further research, however, showed that there was a correlation between the amount of Aβ fibrils and synapse loss. Therefor my hypothesis is that amyloid β induces disturbance of synaptic signaling, resulting in synaptic alterations and spine loss.

The morphology and function of synapses

As described in the introduction the transmission of neuronal signals is of huge important for a proper functioning of the brain. Along the neurons the signaling occurs via electrical signals. Transmission of electrical signals between the different neurons is not possible, because there is a small cleft between the neurons, the synaptic cleft. Therefor the electrical signals are converted into neurotransmitter signals. In the presynaptic neuron the electrical signal is converted into the neurotransmitter signal within the presynaptic terminal. This neurotransmitter is released into the synaptic cleft, where it can bind to its receptor on the postsynaptic membrane. Thereby activating postsynaptic signaling cascades.

The presynaptic terminal:

The presynaptic terminal is loaded with a few hundred small, clear synaptic vesicles (SVs), although this number might varied among different synapses. [12] Not only the number of SVs is variable, also the size is variable. It depends largely on the type of neurotransmitter within the vesicles. SVs containing the fast-acting neurotransmitters, such as glutamate, are approximately 17-22 nm in diameter. However, SVs containing slower-acting transmitters or even proteins can have a diameter up to several hundred nanometers. Via active transport the neurotransmitters are taken up into the SVs, where they are stored. This active transport is driven by a vacuolar proton pump, which activity leads to an electrochemical gradient across the vesicle membrane. The uptake of neurotransmitters into the vesicles is mediated by seven different transporters. Glutamate uptake is mediated by three different transporters, whereas the uptake of monoamines is regulated by two transporters. For

both GABA and glycine a single transporter was found. Also the uptake of acetylcholine is mediated by only one transporter. The mechanism and precise regulation of the proton pump and the transporters is still unknown. [13]

When the SVs are filled with their cargo, they move closely to the presynaptic membrane, where they form clusters (See Fig.2.). This region of the presynaptic membrane is named the active zone.

In the active zone not only newly formed SVs can be found, also preexisting SVs are present. [14] To prepare for neurotransmitter release, the SVs dock at the presynaptic terminal, where they are primed to become sensitive to Ca^{2+}. This priming mechanism is mediated by SNARE proteins, complexins and synaptotagmins 1 and 2, which are anchored in the vesicle membranes. The involved SNARE proteins are synaptobrevin, which can be found on the SVs and syntaxin1 and SNAP-25, which both can be found on the presynaptic membrane. [15] In docked vesicles the SNARES and synaptotagmins do not interact. During priming the SNAREs of the vesicles interact with the SNAREs of the presynaptic membrane, forming SNARE complexes. Also the synaptotagmins interacts with the formed SNARE-complexes. Due to this interactions the vesicles are transported closely to the presynaptic membrane, which results in an unstable intermediate.

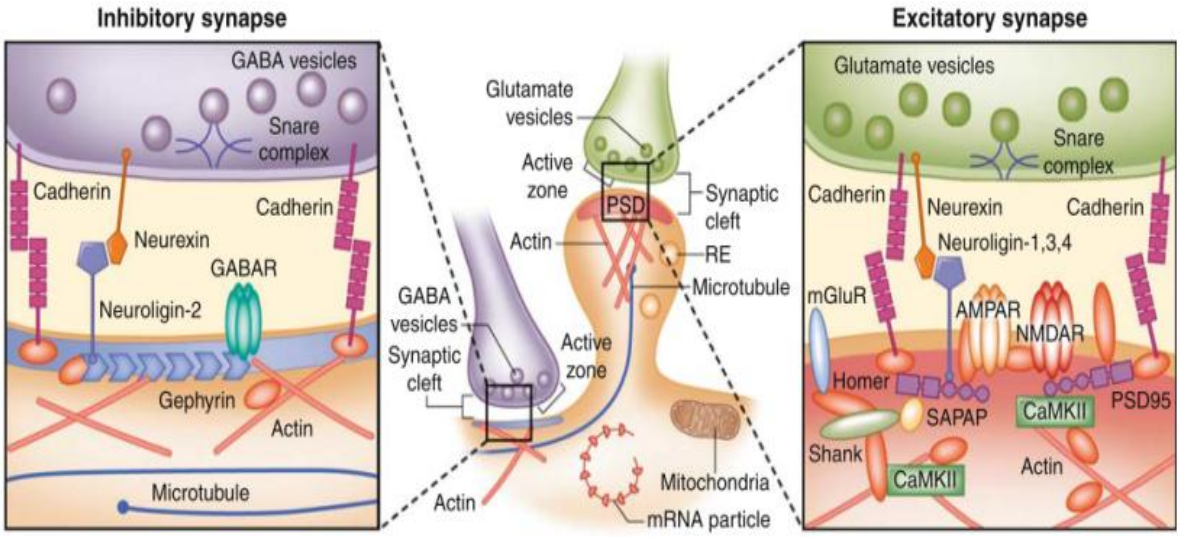

Fig. 2. The molecular architecture of inhibitory and excitatory synapses. The excitatory synapses target on mushroom-shaped spines, which contain the postsynaptic density (PSD). The inhibitory synapses are present along the dendritic shaft and lack such a region. Within the synapses different organelles can be found. The mitochondria provide energy, whereas the ribosomes and RNA particles are needed for local protein synthesis. The recycling endosomes play a role in the transport of internalized synaptic receptors back to the plasma membrane. The cytoskeleton regulates the spine dynamics. The actin cytoskeleton is connected to the PSD and is the primary determinant of spine shape and motility. Transient invasion of dynamic microtubule into dendritic spines can regulate formation of spine head protrusions and rapid spine growth. Both synapse types consist of a unique set of channels, scaffolding proteins and other postsynaptic molecules. The molecular architecture of the inhibitory and exhibitory synapses and their organization of proteins and protein-protein interactions are depicted in the left and right panels respectively. In the inhibitory synapse GABA is released and interacts with its receptor, GABAR. In the excitatory synapse glutamate is released after an action potential and interacts with one of its receptors, AMPAR, NMDAR or mGluR. The pre- and postsynaptic membranes of the synapse are held together by the cell-adhesion molecules cadherin, neurexin and neuroligin. Also some scaffolding proteins are depicted, such as gephyrin and PSD-95. Homer, Shank and CamKII play an important role in the signaling within the post-synaptic membrane. [Van Spronsen (2010)]

If an action potential reaches the presynaptic terminal, the voltage-gated Ca^{2+}-channels open. In most synapses the Ca^{2+}-influx goes via P/Q- ($Ca_v2.1$) or N-type ($Ca_v2.2$) Ca^{2+}-channels. Other Ca^{2+}channels, such as R- (Cav2.3) and the l-type Ca^{2+}-channel are only rarely involved. [16] Due to the Ca^{2+}-influx, the intracellular Ca^{2+}-concentration raises, leading to further destabilization of the intermediate, which causes the forming of a fusion pore. [17] Via the fusion pore, the contents of the SVs are released into the synaptic cleft. Not every action potential however leads to the release of neurotransmitters, only 10-20% triggers the release of neurotransmitters.

After fusion with the presynaptic membrane and the release of the SVs contents, the lipids and proteins of the vesicles are endocytosed for reuse. For this reuse, three alternative pathways are known. The first one is the kiss-and-stay pathway, whereby the SVs are reacidified and refilled with neurotransmitters without undocking. Which enables the SVs to be released immediately. Another pathway is the kiss-and-run pathway, in this pathway the SVs undock, reacidify and refill with neurotransmitters locally. In the third pathway the SVs are endocytosed via clathrin-coated pits. The reacidification and refill of the SVs can occur directly of after passing through an endosomal intermediate. [17]

The synaptic cleft:

The active zone of the presynaptic membrane and the postsynaptic membrane are separated from each other by the synaptic cleft, which is approximately 20-25 nm wide. The synaptic cleft is filled with extracellular matrix, that contains different extracellular proteins. These extracellular proteins mediate the synapse formation and stabilization. Examples of such proteins are cell adhesion molecules, such as N-cadherin, neuroligin (NLGN) and neurexin (NRXN). N-cadherin is the most widely distributed neuronal cadherin. Together with its cytosolic partner, the catenin, it promotes adhesive functions. N-cadherin and catenin are present on both the presynaptic and the postsynaptic membrane. [18]

NLGN and NRXN both interact with cytoplasmic scaffolding proteins, which might influence their role in the synaptic functions. NLGN binds to PSD-95, an important player in the postsynaptic terminal. It induces the clustering of NLGNs in the postsynaptic membrane, which in turn might initiate the clustering of NRXNs in the presynaptic membrane.

The postsynaptic membrane:

The neurotransmitters that are released from the presynaptic membrane interacts with their receptors on the postsynaptic membrane. Only two types of postsynaptic receptors are able to recognize neurotransmitters: the ligated-gated ion channels and G-protein-coupled receptors. The ligand-gated ion channels are amino-3-hydroxy-5-methyl-4-isoazaolepropionate (AMPA) or N-methyl-D-aspartate (NMDA) and the G-protein-coupled receptor is the metabotropic glutamate receptor, or mGluR. When glutamate interacts with one of its receptor, it causes an excitatory synaptic signal. The binding of GABA with its receptor, $GABA_A$, however, causes an inhibitory synaptic signal. Next to the neurotransmitter receptors, also scaffolding proteins such as PSD-95, signaling proteins such as Ca^{2+}/calmodulin-dependent kinase II (CamKII) , NLGN, Shank family proteins, synapse-associated protein (SAPAP) and actin can be found within the postsynaptic membrane. [13)] All these molecules are assembled into an organized structure, named the postsynaptic density (PSD). This is an electron dense thickening, which is situated opposite to the

active zone of the presynaptic membrane. The PSD is not static, but highly dynamic during development and synaptic activity. It is about 200-800 nm wide and 30-50 nm thick. [19] The PSD usually lies at the distal tip of a protrusion of the dendrite, the dendritic spine, and it is specialized in excitatory post-synaptic signaling. Region with inhibitory synapses lack a PSD.

The dendritic spine:

Dendritic spines are small membranous compartments, which protrude from the dendrites and receive input from glutamate-releasing axons. They are approximately 0,5-2 µm in length and occur at a density of 1-10 spines per µm. Spines contain all the necessary postsynaptic elements, including the NMDA- and AMPA-receptor, the PSD, an actin cytoskeleton and a variety of organelles. A typical spine has a bulbous head, which is connected to the dendrite via a spine neck. This neck is very thin, which hinders the transport of molecules in and out of the spine. Therefor a spine can be considered as a microcompartement, in which the environment can differ from the environment within the dendrite itself.

Spines can varied greatly in both size and shape. Based on their shape, they are categorized as mushroom, stubby, thin or cup shaped. Most of the spines have constricted necks and are mushroom shaped or thin shaped, with smaller heads. The mushroom shaped spines contain a larger and much more complex PSD, with a higher density of glutamate receptors. Thereby making them more sensitive for glutamate. [20] It is believed that larger spines contains SER, polyribosomes and endosomal compartments. This suggests that larger spines are able to response stronger to glutamate and have more endosomal recycling, protein translation and degradation. Smaller spines however, might be more flexible and capable of rapidly enlarging or shrinking. [13]

The formation and morphology of the spines is regulated by actin filaments. [21] The filamentous actin, or F-actin, forms organized bundles in the neck of the spines. The shapes of the spine heads can be altered via polymerization and depolymerization of the actin filaments. A mechanism that is regulated via synaptic transmission. Under normal circumstances, glutamate is release out of the SVs at the presynaptic terminal and act on its postsynaptic receptors. Thereby activating and opening the AMPA- receptor, causing a flow of Na^+ into the postsynaptic terminal, subsequently leading to a depolarization of it. Due to this depolarization also the NMDA receptor becomes active, it removes its Mg^{2+} blockade, thereby allowing Na^+ and Ca^{2+} to flow into the dendritic spine. Resulting in an increase of intracellular Na^+ and Ca^{2+} levels. The Ca^{2+} binds to calmodulin, thereby activating CaMKII. CaMKII undergoes autophosphorylation, thereby maintaining its activity after Ca^{2+} concentrations returns to its basal levels. CaMKII phosphorylates the AMPARs on the post-synaptic membrane, increasing their single-channel conductance. Next to this CaMKII seems to play a role in the delivering of more AMPARs to the post-synaptic membrane, thereby making it more sensitive for neurotransmitter release.

Due to long-lasting enhancement in signal transmission the postsynaptic NMDA receptor is activated continuously, causing a continuously polymerization of the actin filaments, leading to the forming of a bigger and more mushroom shaped spine head. This mechanism is called long term potentiation (LTP) (See Fig.3.). Long term depression (LTD) on the other hand, causes depolymerization of the actin and shrinkage of the spine heads. [13] For the regulation of the actin filaments, actin-binding proteins are needed. Profilin promotes the actin polymerization, which could facilitate an LTP-

induced actin assembly and enlargement of the spines. Cofilin promotes the depolymerization of actin. During LTP cofilin is inhibited, thereby inhibiting the depolymerization of the actin filaments. Under influence of an induction of LTP the actin filaments depolymerize, thereby diminishing the spine head. [1]

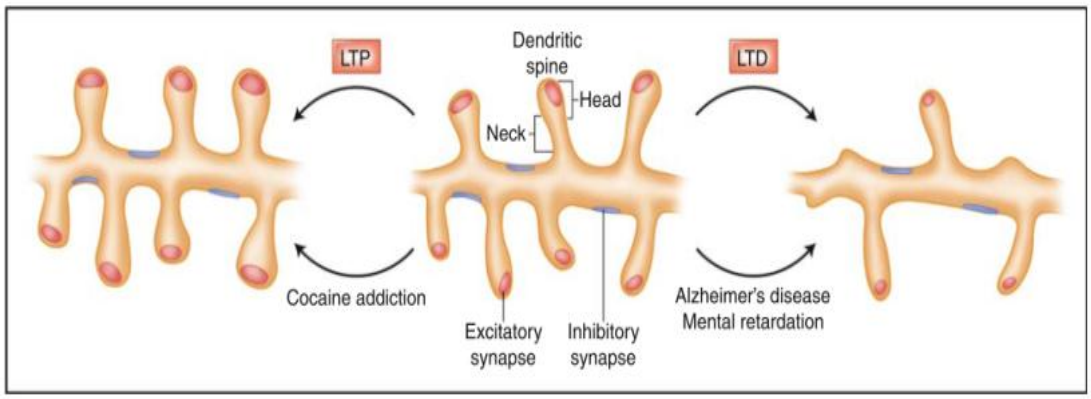

Fig. 3. The major morphologic events occurring in dendritic spines upon LTP and LDP. Under influence of LTP or cocaine addiction the shape of the spines change, they become bigger and more mushroom-shaped mature spines. In contrast, LTD or mental retardation and Alzheimer's disease, causes thinner and smaller spines. [van Spronsen 2010]

Alzheimer's disease:

As previous mentioned in the introduction, it is thought that the presence of amyloid beta (Aβ) in the hippocampus and cerebral cortex is the main cause of the cognitive deficits of Alzheimer's disease. Aβ is generated out of its precursor protein APP, within the amyloidogenic pathway by various cleavage steps. Within the non-amyloidogenic pathway APP is cleaved into P3 and sAPPα, a molecule that seems to have a protective role in neuronal plasticity. Due to missense mutations the formation of Aβ might be increased. Also age related accumulation of Aβ is seen. Both accumulations lead to the deposits of dense amyloid plaques, which are formed by Aβ fibrils.

The non-amyloidogenic pathway:

Within the non-amyloidogenic pathway APP is first cleaved by α-secretase, thereby forming sAPPα and the membrane-anchored C-terminal fragment, C83. This cleavage is followed by a cleavage mediated by γ-secretase, thereby forming extracellular P3 and the APP intracellular domain (AICD). The α-secretase cleaves APP at the Lys16-Leu17 bond, preventing the formation of an Aβ-molecule, because the cleavage occurs within the Aβ domain. The first α-secretase studies suggested that it was a membrane-bound endoprotease, that cleaved the APP primarily at the plasma membrane. [22] Further research showed that only three specific metalloproteinase molecules possessed the α-secretase-like activity. These three molecules were member of the A Disintegrin And Metalloproteinase (ADAM) family. The members of the ADAM-family that have been suggested as the α-secretase were ADAM9, ADAM10 and ADAM17. All of these molecules are, like APP, a type-I transmembrane protein. The functioning of α-secretase can be constitutive, but it can also be regulated by several agents, like drugs. Both ADAM9 and ADAM17 are mostly regulated, while ADAM10 can be both constitutive and regulated. The concentrations of the three ADAMs are not equal within the brain, the concentration of ADAM10 is highly expressed in the brain, while ADAM9

and ADAM17 are found in low concentrations. Which might suggest that ADAM10 plays the major role as α-secretase within the human brain. [2]

ADAM10 is encoded by a gene on chromosome 15 and it is built of 750 amino acids. It is a zymogen, which is synthesized in the ER. It needs the removal of its prodomain, before it can be activated. The prodomain is bound to the catalytic region of ADAM10 via a consensus sequence (RKKR), which is recognized by PC7, a proprotein convertase and furin. Cleavage by PC7 and furin results in the unmasking of the active site of ADAM10. It was shown that mutations in the region that is recognized by PC7, resulted in the inhibition of ADAM10 activation. [23] Other studied showed that the prodomain has a fundamental role in preserving ADAM10 inactive and thereby protecting from degradation within the secretory pathway. [24] The effect of ADAM10 in APP processing and in the development of Alzheimer's disease has been studies frequently. Overexpression of ADAM10 can cause an increase of the α-cleavage, whereas downregulation of it results in a decreased α-cleavage. [25] ADAM17 or tumor necrosis factor-α converting enzyme (TACE) contains an extracellular domain, which can be proteolytically cleaved into a soluble TGF-α. [26] It seems to play an important role within the APP processing. When ADAM17 is inhibited, the regulated α-secretase activity in human neurons is diminished. [27] Multiple other studies confirmed that ADAM17 likely affects the regulated α-cleavage of APP. Studies with ADAM9 showed that RNAi of ADAM9 did not affect the generation of sAPPα. A treatment with phorbol ester, however, showed a upregulation of sAPPα, concluding that ADAM9 only is involved in regulated α-cleavage. [25, 28]

The sAPPα, that was formed by the α-cleavage, seems to play an huge role in both neuronal plasticity as survival. Next to this it protects against excitotoxicity and regulates neural stem cell proliferation. The C83 fragment, that was formed by the α-secretase activity is further cleaved by γ-secretase into a P3 fragment and an APP intracellular domain (AICD), is rapidly degraded and seems to possess no important role. [2]

The amyloidogenic pathway:

Within the amyloidogenic pathway APP is first cleaved by β-secretase at the N-terminal site of Asp1, thereby forming sAPPβ and a C-terminal fragment (C99). sAPPβ differs from sAPPα by lacking the Aβ1-16 region. The cleavage by β-secretase is followed by a cleavage of the C-terminal fragment by γ-secretase, which forms the Aβ molecule and the AICD. Several studies focused on the identification of the β-secretase. Eventually BACE1, also called Asp2 or memapsin2, was identified as the major β-secretase. It is a membrane-bound aspartyl protease with near the C-terminus a characteristic type I transmembrane domain. Alterations in the expression of BACE1 affects the cleavage of APP: overexpression of BACE1 induces the cleavage, whereas downregulation results in the inhibition of APP cleavage. *In vitro* studies showed that synthetic APP peptides indeed were cleaved by BACE1, providing convincing evidence for BACE1 to be the β-secretase involved in the APP processing. [29]. BACE1 is encoded by a gene on chromosome 11 and contains 501amino acid residues. The larger precursor, named pro-BACE1, is reformed by different modification steps such as glycosylation and phosphorylation, after which it is cleaved by a furin-like endoprotease, thereby forming the mature BACE1 molecule. For an optimal functioning of BACE1 it is important to have an environment of pH 5. [30]

Because of its function BACE1 is an interesting therapeutic target and therefor it is investigated in multiple studies. Luo *et al.* (2001) showed that BACE1 knockout mice had no detectable levels of Aβ and that they had no severe phenotypic abnormalities.[31] More recent studies however, showed that there were several phenotypic abnormalities. In one study a significant amount of BACE1 null mice died within the first weeks after birth. The mice that did survive, showed multiple problems. They were smaller than their littermates and were hyperactive, which might indicate that BACE1 is important for a proper development. [32]

Not only BACE1 shows β-secretase activity, also its homolog, BACE2, seems to function as a β-secretase. The gene encoding BACE2 is mapped on chromosome 21q22.3. This region is critical for Down's syndrome (DS). Within DS patients Aβ accumulation is often seen, suggesting that there is a link between BACE2 activity and APP processing. *In vitro* studies showed that BACE2 cleaves APP in a similar way as BACE1 does. The expression of BACE2 in neurons however, is significantly lower compared to BACE1. [2]

After cleavage by β-secretase the C-terminal fragment or C99 remains membrane associated and will be cleaved by γ-secretase. This secretase is a complex that contains at least four different components: presenilin (PS), nicastrin (Nct), anterior pharynx-defective-1 (APH-1) and presenilin enhancer-2 (PEN-2). The different components of γ-secretase accomplish different tasks: presenilin contains the active site, whereas nicastrin plays a role in the anchoring of a C99 molecule. PEN-2 stabilizes presenilin in the complex and the function of APH-1 is still unclear. [33] PS is a multi-transmembrane protein, with 7-9 transmembrane domains. It contains two highly conserved aspartate residues, which are crucial for γ-secretase activity. In mammals two different homologs of presenilin are known, PS1 and PS2. Mutations affecting the genes of presenilin, mainly PS1, are causative in the majority of FAD. Nct is a type I transmembrane glycoprotein, which is thought to function as the scaffolding protein of the γ-secretase complex. The ectodomain of Nct interacts with both APP and Notch and is capable of recruiting them into the γ-secretase complex. This suggests that Nct might act as the γ-secretase receptor.

γ-secretase cleaves C99 within the transmembrane domain, thereby forming an Aβ-molecule and the AICD. The precise site of cleaves might varied, resulting in different Aβ-molecules and AICDs. The Aβ-molecules that are associated with AD are Aβ40 and Aβ42. 10% of the produced Aβ-molecules are Aβ42. Despite its low concentration, Aβ42 plays an important role within the development of AD, it might initiate the formation of fibrils. [34 Studies with FAD mutations showed an increased ratio of Aβ42/Aβ40, indicating that any disturbance of the ratio is critical for AD pathogenesis. Aβ might function as the core for Aβ assembly into oligomers, fibrils and even amyloidogenic plaques. [35]

The processing of APP occurs mostly on the plasma membrane, thereby secreting the formed Aβ outside the neurons, however, a small amount of it is formed intracellular. This occurs when APP, bound the ER, Golgi/TGN or endosome/lysosome, is cleaved. It can also happen that extracellular Aβ is internalized by the neuron for degradation. This intracellular Aβ might accumulate in the neurons and contribute to dysfunctioning of the neurons. In DS patients it is often seen that the accumulation of intracellular Aβ precedes extracellular plaque formation. Later on in the development of AD it is shown that the level of intracellular Aβ declines, whereas the extracellular Aβ plaques accumulate. Transgenic mouse models showed similarities with these observations. In

the early stage of AD high concentrations of intracellular Aβ accumulation was detected, which decreased at the moment of the formation of more extracellular plaques. [2]

Pathogenesis:

The AD brain is characterized by atrophy of the hippocampus and the cerebral cortex. Believing the amyloid hypothesis, this atrophy is caused by a disturbances of production and aggregation of the Aβ peptide causing neuron degeneration. Accumulation of aggregated amyloid fibrils, which are believed to be toxic, are responsible for disrupting the neurons calcium ion homeostasis, inducing apoptosis.

There are different factors that might cause an increased production of Aβ. There are dominantly inherited forms of AD or nondominant forms. The dominantly inherited forms are caused by mutations. There are more than 30 autosomal-dominant APP mutations known, that are linked to AD. Via different mechanisms these mutations seems to increase aggregation of Aβ. A mutation that facilitates APP cleavage near the β-secretase site, called the Swedish mutation, causes an increase of the formation of Aβ. Not only the level of Aβ42 is increased, all the types of Aβ seems to be upregulation due to this mutation. [36)] The Arctic mutation, which is located in codon 693 of Aβ, replaces a glutamic acid for glycine (E693G). Carriers of this mutation showed decreased levels of Aβ 40 and 42 in plasma. Even *in vitro* studies showed low levels of Aβ42 in APP_{E693G} transfected cells. Also fibrillization studies showed no difference in fibrillization rate, however it was shown that the Aβ formed protofibrils at a much higher rate and in larger quantities compared to wild-type Aβ. [37] Multiple mutations near the γ-secretase site are known, causing an increase in the formation of Aβ42.

Also other molecules involved in the APP processing can affect the production of Aβ. A missense mutation in the presenilin genes causes an increased formation of Aβ42, while the total amount of produced Aβ remains the same. Concluding that the Aβ42:Aβ40 ratio is disturbed. Also studies with transgenic mouse models that over-expressed mutant PS-1 showed an increase of Aβ-42 in the brain, which suggest presenilin-1 plays an important role in beta-amyloid regulation and can be highly related to Alzheimer's disease.

Synaptic alterations within Alzheimer's disease :

Despite the important role of Aβ in Alzheimer's disease and its deposition as β-amyloid plaques in the brain, there was no direct link between the amount of β-amyloid plaques and behavioral symptoms, in both human and transgenic mouse models. Suggesting that not the Aβ plaques, but smaller Aβ complexes, the Aβ oligomers or protofibrils, were the main cause of the behavioral symptoms. It was thought that changes in the levels of Aβ in the brain initiate the so called amyloid cascade. Changes in the Aβ metabolism can be caused by an increased Aβ production, an increased Aβ42/Aβ40 ratio or a decreased degradation/clearance of Aβ. This altered metabolism results in oligomerization of Aβ and initiates the formation of (diffuse) Aβ42 deposits. As a reaction on the formed Aβ42 oligomers there are subtle changes in the synaptic functioning. Eventually the presence of the oligomers causes severe and permanent changes in the synaptic functioning. In time the Aβ oligomers forms Aβ plaques, which are microscopically visible. As these plaques begin to acquire Aβfibrils, local inflammatory responses can be observed. Leading to progressive synaptic and

neuronal injury, which subsequently causes an altered neuronal ionic homeostasis and oxidative stress. Which leads to additional biochemical changes. After that NFTs are induced by altered kinase and phosphatase activities and contribute to additional defects. The cascade culminates in widespread synaptic/neuronal dysfunctioning and cell death, leading to progressive dementia with plaque and tangle pathology. [38]

The presence of Aβ plaques and NFTs are the main criteria for a postmortem diagnosis of AD. With use of staining techniques it was possible to study the amount of plaques and link this to the severity of premortem cognitive deficits. Most of the studies that investigated the correlation between the amount of plaques and cognitive deficits showed no correlation. Biochemical analyses showed that both presynaptic and postsynaptic proteins are downregulated in the brain of AD patients. Suggesting that there are substantial synaptic alterations. [39]

Within AD patients synaptic loss plays an important role. To gain more insight in this role, several transgenic mouse models of AD have been analyzed for dendritic spine anomalies and synaptic loss. The studies varied from determination of the level of pre- and postsynaptic markers, such as PSD-95 to the counting of dendritic spines or the analysis of spine morphology and dynamics. The results of the studies showed consistent evidence that synaptic loss occurs consistently in an age-dependent manner in all of the models. However, the question of such loss occurs before Aβ plaque deposition or not was still unanswered. Further research in the role of Aβ oligomers showed incompatible results.

Biochemical analyzes showed that several pre- an postsynaptic proteins are downregulated in an AD brain. One important protein of the pre-synaptic membrane that is downregulated is synaptophysin. Multiple studies showed that there was a correlation between the degree of cognitive decline in AD patients and the decrease in synaptophysin expression. Masliah *et al* (2001) analyzed the presence of synaptophysin in the cortex of patients with minimal cognitive impairment (MCI) and compared it with the presence in age-matched subjects, with a normal memory functioning. Immunoreactivity assays showed an decrease of approximately 25% of synaptophysin in the cortex of patients with MCI.[40] Experiments with mouse models by Hsia *et al* (1999) showed that the amount of synaptophysin-positive presynaptic membranes were decrease with more than 30%, compared to age-matched mice. The mouse models used were 2 till 3 months old, indicating that it was Aβ plaque independent. Concluding that the presynaptic terminals were already affected in the young mice as their soluble levels of aβ rise, but independent of the Aβ plaques. [41]

Larson *et al.* (1999) studied the synaptic transmission and plasticity in both young and aged transgenic mice. The transgenic mouse model had a mutation at residue 717 of the APP molecule, the valine was changed into a phenylalanine. Earlier studies showed accumulation of Aβ oligomers and the formation of Aβ plaques in an aged-dependent manner within this mouse model. The young transgenic mice showed an enhanced paired-pulse facilitation and a rapid decay of LTP, compared to the age-matched non-transgenic mice. The older mice showed a diminished synaptic response. Concluding that there was an altered synaptic communication.[42]

Amyloid β-induced disturbance of synaptic signaling:

The studies done in AD models showed morphological and biochemical alterations, changes that highly affect the functioning of the brain. Almost all of the studies suggest that there is a direct

negative link of Aβ oligomers on synaptic signaling, whereby different mechanisms might cause the effect.

There is growing evidence that direct interaction of Aβ with postsynaptic receptors (NMDA and AMPA) results in a disturbance of the synaptic signaling. Lacor *et al*. used small neurotoxins, which contains soluble Aβ-oligomers, to show the effect of Aβ. The Aβ-derived diffusible ligands (ADDLs) specifically interact with NMDA receptors within the excitatory synapses. Continuous exposure of ADDLs lead to an altered spine morphology and it decreases the spine density. Due to the interaction of ADDLs with the postsynaptic membrane, the number of NMDA receptors diminished. [43] Another research showed that Aβ decreases the expression of NMDA receptors on the surface. This was caused by upregulation of endocytosis of the NMDA receptors. Not affecting other ionotropic receptors. This effect was partially blocked by a nACh receptor antagonist. [44] More evidence for the involvement of NMDA receptor in the disturbance of synaptic signaling was provided in three additional studies.

Shankar *et al* (2007) showed the effect of Aβ on the hippocampal synapses of rats. The rats were treated with naturally secreted Aβ dimers and trimers, thereby inducing progressive loss of hippocampal synapses. Both the density of the dendritic spines and the amount of electrophysiologically active synapses were decreased. Treatment with Aβ-specific antibodies resulted in a neutralization of the Aβ effect on the spines, suggesting that the spine loss was reversible. To unravel the role of the NMDA receptor in this mechanism, NMDAR antagonists were used. These antagonists mimicked or blocked the effects of Aβ, resulting in a proper functioning of the spines. Concluding that NMDAR activity is necessary for Aβ-mediated spine loss. Due to the inhibition of the NMDAR by Aβ oligomers the influx of calcium is reduced, causing synapse weakening, elimination and loss. [45]

Calabrese *et al* (2007) studied the effect of Aβ in the pre- and postsynaptic morphology and connectivity in cultured hippocampal neurons. They found that an one hour incubation with Aβ resulted in a diminished size and amount of synaptophysin clusters. Not all the synapses were affected by incubation, only the glutamergic synapses. There was a 40% decrease in number of clusters, while the size of the remaining clusters was diminished with almost 10%. They also showed that the one hour incubation already had effects on the dendritic spine number and its morphology. The heads of the spines decreased in width for 22-28%, whereas the average length of the protrusions increased with 35%. Also the effect of Aβ on the NMDAR was investigated. Hippocampal neurons were treated with noncompetitive NMDAR antagonists, incubated with Aβ for two hours and compared to hippocampal neurons that also were incubated with Aβ. It was shown that the neurons treated with NMDAR antagonists were less affected by Aβ. [46]

Dewachter *et al* (2009) studied the effects of Aβ on the NMDA receptor in both *in vitro* and *in vivo* models. The effect of Aβ oligomers on the NMDARs was tested in cultured hippocampal and cortical neurons. It was shown that the Aβ oligomers bind in close proximity of the NR2B domain of the NMDARs, a domain that plays a crucial role in the binding of glutamate. Further research showed that the influx of calcium was inhibited due to the interaction of Aβ oligomers with the NMDARs, thereby influencing the down-stream pathways. They also proved that there was a decrease in the expression of NR2B-contianing NMDARs after long-term incubation with Aβ. *In vivo* studies were done in APP[V717I] transgenic mice, which overexpressed human mutant APP[V717I]. PSD fractions

of the mouse brain were purified, analyzed and compared to PSD fractions of non-transgenic mice. The concentrations of important postsynaptic proteins, such as NR2B, PSD-95 and phosphorylated α-CaMKII, was decreased. [47]

Not only the NMDA receptor seems to be affected due to Aβ, also the number and functioning of the AMPA receptor is affected by Aβ. Hsieh *et al*. (2006) showed that Aβ uses parts of the LTD pathway to affect the neurons. Incubation of pyramidal neurons with Aβ, resulted in an upregulation of AMPAR endocytosis, causing a decrease in both surface and synaptic AMPARs. Next to this, there was shown that this endocytosis resulted in dysfunctioning of the NMDAR, leading to spine loss.[48]

Alterations in downstream signaling:

The changes in AMDAR and AMPAR expression led to changed currents and a decreased calcium influx. These changes might influence the activity of various downstream signaling molecules. The first steps of the signaling cascade involves multiple phosphatases and kinases. The balance between these two is of huge importance for a proper signaling. In both human and rodents, changes in the phosphatase activity were detected and associated with AD. [39] Numerous downstream signaling molecules are calcium-dependent, such as CaMKII and calcineurin. Due to the altered Ca^{2+}-influx calcineurin moves the neuron into an LTD like state, it down regulates the NDMAR expression, increases the AMPAR endocytosis and initiates spine loss.

Also the protein kinases are affected by Aβ, mostly affecting the tau hyperphosphorylation. An important player in this field is GSK3, a tau kinase. In both *in vitro* and *in vivo* studies was shown that GSK3 promoted neurodegeneration and the formation of the Alzheimer's disease plaques and NFTs.

Via the downstream signaling pathways also gene expression is altered. An example of a signaling pathway that is affected by Aβ is the cAMP response element-binding protein (CREB) pathway. CREB functions as a transcription factor. In multiple studies, in either AD patients, transgenic mouse models, cultured neurons and hippocampal slices, was shown that Aβ decreased the phosphorylation of CREB. Subsequently there is an altered gene expression.

Conclusion

There is many evidence indicating that amyloid β induces disturbance of synaptic signaling, resulting in synaptic alterations and spine loss. Subsequently resulting in the onset and progression of AD. It was shown that the presynaptic terminals seemed to be affected by the exposure of Aβ. Multiple studies also showed that Aβ oligomers affected the synaptic activity by influencing both the functioning of the NMDA- and AMPA- receptor. Likewise, further downstream signaling pathways were affected, resulting in an altered Ca^{2+} influx and different gene expression.

Discussion

Although there is a lot of evidence for the hypothesis that amyloid β induces disturbance of synaptic signaling, also other mechanisms might be possible.

One of these mechanisms is the disruption of the cytoskeletal network under the influences of Aβ. Aβ oligomers seems to affect the activity of multiple actin-remodeling proteins, thereby affecting the formation of a proper cytoskeletal network. Cofilin seems to be such a protein. Under normal conditions active cofilin binds to actin filaments of the cytoskeletal network, removing actin monomers and enhancing the filament severing and depolymerization. In presence of Aβ oligomers cofilin seems to dephosphorylate (activate) and form rod-shaped actin bundles. A study with cultured rat hippocampal neurons showed rod formation in 18% of the neurons after Aβ exposure. Another research showed that overexpression of Aβ not only led to rod formation, but also to synapse loss and an impaired synaptic plasticity. [49]

Another molecule that might play an important role in the Aβ- induced disruption of the cytoskeletal network is drebrin. This is, just as cofilin, an actin-binding protein. It actually is closely linked to cofilin. Drebrin functions as a stabilizer of the cytoskeletal network by the inhibition of actin-myosin interactions and by competing in the filamentous actin binding with other actin-binding molecules, for example tropomyosin. The prominent isoform in the neurons is drebrin-A, which is abundantly found in the postsynaptic terminals of excitatory synapses. Overexpression of drebrin-A results in elongation of spines, whereas downregulation of the expression leads to a reduced spine density and the formation of thin spines. Concluding that drebrin-A is an important player in the regulation of spine morphology. Next to this drebrin-A also plays a huge role in the accumulation of PSD-95 and accumulation of NMDA receptors in the PSD. [49]

Early studies showed that drebrin disappeared from the hippocampus of AD patients [50)]. Further research by Hatanpaa *et al*. (1999) showed that this was also the case in the cerebral cortex. Analysis of neurological disorders that are accompanied by mild cognitive impairments showed that there was a decrease in drebrin concentrations. Concluding that pathological changes of the debrin concentration in neurological disorders is accompanied by cognitive defects. [51]

Another effect of a decrease in drebrin levels is a diminished exposure of NMDA receptors. Under normal circumstances an increase of AMPA receptor activity leads to an accumulation of drebrin in the spines. Thereby increasing the NMDA receptor transport to the postsynaptic membrane, that is mediated via actin cytoskeletal dynamics. A decrease in AMPA receptor expression diminishes the drebrin expression. Subsequently leading to less NMDA receptor transport to the postsynaptic membrane. [49]

The functioning of both drebrin and cofilin is regulated via PI3 kinase and P21-activated kinase (PAK). In normal brains PI3 kinase activates PAK, which subsequently stimulates drebrin and inhibits cofilin. This actions leads to normal cytoskeletal dynamics and a proper functioning synapse. Under the circumstances of Aβ exposure, PI3 kinase is inhibited, resulted in less PAK activation and subsequently less drebrin stimulation. Because there is less active PAK, cofilin is less inhibited, resulting in disrupted actin cytoskeletal dynamics and a dysfunctional synapse. [49]

Further unraveling the mechanisms of APP metabolism and their effect on the synapse will be important for identifying of new potential therapies to reduce Aβ oligomers accumulation and combat AD.

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