Disruption of hippocampal long-term potentiation by amyloid β

A possible mechanism for memory impairment in Alzheimer's disease

Kirsten Bohmbach

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Image on front page:

The image shows a *hippocampus kuda* which is also known as the common seahorse. Arantius compared the human hippocampus to a seahorse in 1578 from which its name derives (Duvernoy, 2005).

Image Reference: *http://www.pragenturhamburg.de/opz/attachments/178_Kuda-Seepferdchen%20Hippocampus%20Kuda%20-%20hoch.jpg*

Bachelor Thesis

Disruption of hippocampal long-term potentiation by amyloid β

A possible mechanism for memory impairment in Alzheimer's disease

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- To my father, who hopefully will be able to see more of my scientific work -

Content

Abstract

The ability to obtain new memory is essential for our life quality and normal functioning. However, this process is disrupted in Alzheimer's disease (AD). The consolidation of explicit memory, i.e. learning, is associated with plastic changes. The most important form of synaptic plasticity is long-term potentiation (LTP). In AD this process is disrupted by Amyloid β, a peptide that is also found in plaques. By the use of electrophysiological measurements the effect of Amyloid β derived diffusible ligands (ADDL) on basal synaptic transmission and LTP is studied in acute hippocampal slices of 4 month old male C57BL/6 mice. The Input-Output curve shows a significant decrease in fEPSP slope by high stimulus intensities in the ADDL treated group. Furthermore a significant lower fEPSP slope in percent of baseline is found after LTP induction in the ADDL treated slices. These results show that besides the disruption of LTP, there is also an impairment of basal synaptic transmission caused by ADDL.

Abbreviations

5-HT	Serotonin receptor
AC	Adenyly cyclase
ACSF	Artificial cerebrospinal fluid
AD	Alzheimer's Disease
ADDL	Amyloid beta derived diffusible ligands
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAR	AMPA receptor
ApoE	Apolipoprotein E
APP	Amyloid Precursor Protein
AT	Angiotensin
Aβ	Amyloid beta
BDNF	Brain-derived neurotrophic factor
Ca^{2+}	Calcium ion
CaM	calmodulin
CaMKII	Ca^{2+}- and calmodulin-dependent protein kinase II
cAMP	cyclic adenosine monophosphate
CREB	Cyclic AMP-responsice element-binding protein
D1R	Dopamine 1 receptor
DMSO	Dimethyl sulfoxide
E-LTP	Early phase of LTP
EPSP	Excitatory postsynaptic potential
fEPSP	Field excitatory postsynaptic potential
GPCRs	G-protein coupled receptors
GSK3β	Glycogen synthase kinase 3β
GTP	Guanosine triphosphate
L-LTP	Late phase of LTP
LTD	Long-term depression
LTP	Long-term potentiation
mAChRs	Muscarinic acetylcholine receptors
MAPK	Mitogen-activated protein kinase
mGluR	Metabotropic glutamate receptor
mRNA	Messenger ribonucleic acid
mTOR	Mammalian target of rapamycin
MVB	Multivesicular bodies
nAChR	Nicotinic acetylchonine receptor
NFT	Neurofibrilar tangles
NFTs	Neurofibtillary tangles
NMDA	N-methyl-D-aspartic acid
NMDAR	NMDA receptor
NO	Nitric oxide
NOS	Nitric oxide synthase
NSF	N-ethylmleimide-sensitive factor
NT-4	Neurotrophine 4
PI3K	Phosphoinositide 3-kinase
PICK1	Protein interacting with C kinase 1
PIN1	Protein interacting with NIMA1
PKA	protein kinase A
PKC	Protein kinsase C
PKMζ	Protein kinase Mζ (Zeta)
PP1	Protein Phosphatase 1
PRPs	Plasticity-related proteins
PSD	Postsynaptic density
RAGE	Receptor for advaned glycation end products
ROS	Reactive oxigen species
SC	Schaffer collateral
STP	Short-term potentiation
trkB	Tyrosine kinase receptor B

1. Introduction

Every day we obtain new memory. This ability is essential for our normal functioning and life quality. However, in Alzheimer's disease (AD) this process is disrupted. Due to the neurological disorder patients experience impairment in the ability of gaining new memory. In a later stage also loss of existing memory takes place. In the whole world more than 24 million people suffer from dementia (Ferri *et al.*, 2005). Every seven second a new patient is sickened. It is estimated that in 2040 more than 81 million people will suffer from dementia.

This raises the questions how memory is obtained on a cellular level and how AD does influence these pathways. These questions have to been answered to establish a working medicine or a cure.

1.1. Memory in general

In general memory can be categorized into implicit and explicit (Kandel *et al.*, 2000). Implicit or non-declarative memory is often also called unconscious. It refers to motor and perceptual skills that are trained. Priming and conditioning are examples of memory that is formed unconsciously. The storage of these memories takes place in various brain areas depending on their function. For example, memory on priming stored in the neocortex and on habituation and sensitization in reflex pathways. Facts or events and their meaning for the organism are called explicit or declarative memory. This form of memory is also called conscious because it can be expressed by language. At first the information is processed in polymodal association cortices and sent towards the parahippocampal and pararhinal cortices (Figure 1). Next it is convoyed to the entorhinal cortex, dentate gyrus, hippocampus and subiculum. Finally its send back to the entorhinal cortex and to the other areas mentioned. The hippocampus plays a major role in the formation of memory because it is the major in- but also output area of the entorhinal cortex. As shown by Scoville *et al.* in 1957, a bilateral hippocampus lesion leads towards memory loss and the inability to acquire new memory (Scoville and Milner, 1957). However damage to the cortex area results in greater deficit in memory storage (Kandel *et al.*, 2000). From this point this thesis will on be focusing on the explicit memory, because it is primly disrupted in AD.

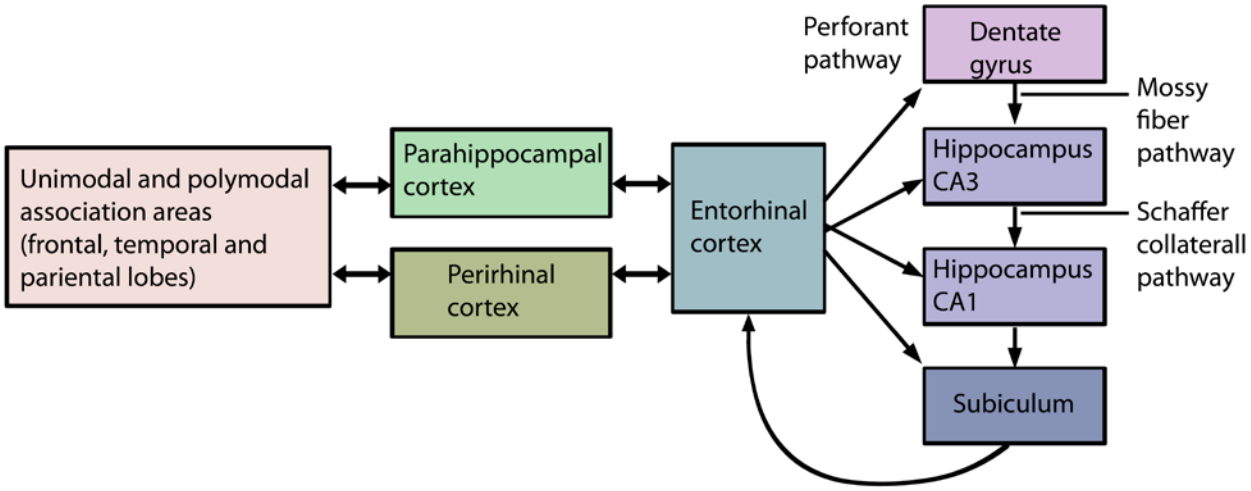

Figure 1: The organization of the hippocampal formation. The storage of explicit memory involves various areas of the medial temporal lobe. Information is processed by association areas and then convoyed via parahippocampal and perirhinal cortex towards the entorhinal cortex, dentate gyrus, hippocampus subiculum and then back. (Kandel *et al.*, 2000)

Memory can also be categorized by its temporality (Purves *et al.*, 2004). The first memory that is formed is referred to as immediate memory. This is only stored for a fraction of a second. Working memory is the category of memory that stays for seconds to minutes. A good example of working memory is the kind of memory you build up during searching your room for an object. During this search you are able to remember the places you just looked at. The third form is long-term memory which stays for hours, days or even lifespan. To establish long-term memory four types of processing are necessary (Kandel *et al.*, 2000): encoding, consolidation, storage and retrieval.

New information must first be encoded, which refers to the process when it is first encountered (Kandel *et al.*, 2000). For the formation of memory, it is important that information is deeply encoded. This is followed by consolidation, the first step of creating long-term memory. The information is made more stable by several molecular processes, which will be explained in the following section. The sites and mechanisms by which the memory is retained, is called storage. This information can be recalled by a process called retrieval. It also combines information's which are stored in different places in the brain.

1.2. Cellular mechanism of learning and memory formation

But how is it possible to consolidate memory? In 1973 Bliss and Lømo showed that synapses in the hippocampus have the capability of plastic changes (Bliss and Lomo, 1973), an ability, which is considered to be the cellular equivalent of learning. The most important form of synaptic plasticity is long-term potentiation (LTP). *In vivo* it is induced by high frequent stimulation (tetanus). It results is an enhanced synaptic transmission evidenced by a sustained increased of the excitatory post-synaptic potential (EPSP). The fact that in mice learning is associated with the induction of LTP supports it as a model for learning (Garcia *et al.*, 1993; Green *et al.*, 1990; Sharp *et al.*, 1985; Skelton *et al.*, 1987). Furthermore LTP is separated in to three phases: short-term potentiation (STP), early phase (E-LTP) and late phase (L-LTP). Due to the temporal changes observed in LTP, it is comparable to short- and long-term memory, and therefore a valuable model for learning and memory consolidation (Dash *et al.*, 2004; Reymann and Frey, 2007).

As stated before, the hippocampus plays a major role in the storage of explicit memory. Here we can differ between three pathways (Kandel *et al.*, 2000): (1) Perforant pathway; (2) Mossy fiber pathway; (3) Schaffer collateral (SC) pathway. The cellular mechanism of learning is different in each of those pathways. Because damage to the CA1 area of the SC pathway results in the inability to form new declarative memory ((Auer *et al.*, 1989; Zola-Morgan *et al.*, 1986)), it is linked to AD. Therefore the focus of the thesis will be on the SC pathway, which connects the CA3 area with the CA1 area.

1.2.1. Long-term potentiation in general

LTP in the SC pathway has several important characteristics. Firstly LTP is specific to the pathway were it is induced (Andersen *et al.*, 1980). The second characteristic is that for LTP induction, several axons must be activated together (Lee, 1983; McNaughton *et al.*, 1978). This feature is called cooperative. The third important feature is that LTP in the SC pathway is associative, because both, the pre- and postsynaptic cell are involved in the process (Levy and Steward, 1979; White *et al.*, 1988). The last two characteristics derived among others from the properties of the NMDA receptor (NMDAR), one of three ionotropic glutamergic receptors.

Weak synaptic activation and the induction of short-term potentiation (STP) solely require activation of the AMPA receptors (AMPAR) (Collingridge *et al.*, 1983) (Figure 2). This results in an influx of mainly Na^+ ions. Due to a voltage-dependent Mg^{2+} block of the NMDAR, there is no inward Ca^{2+} current (Nowak

et al., 1984). However, if the postsynaptic cell is already depolarized by a cooperative neuron, LTP can be induced by presynaptic glutamate release (Collingridge *et al.*, 1983; Nowak *et al.*, 1984). The influx of Ca^{2+} into the postsynaptic cell through the NMDAR is essential for the induction of LTP (Lynch *et al.*, 1983; Malenka *et al.*, 1988). In the cytoplasm Ca^{2+} binds to calmodulin (CaM). Together they activate Ca^{2+}- and calmodulin-dependent protein kinase II (CaMKII) (Malenka *et al.*, 1989; Malinow *et al.*, 1989). This kinase is essential for several processes involved in LTP induction (Otmakhov *et al.*, 1997) but must first be autophosphorylated at the position of Thr286 (Giese *et al.*, 1998).

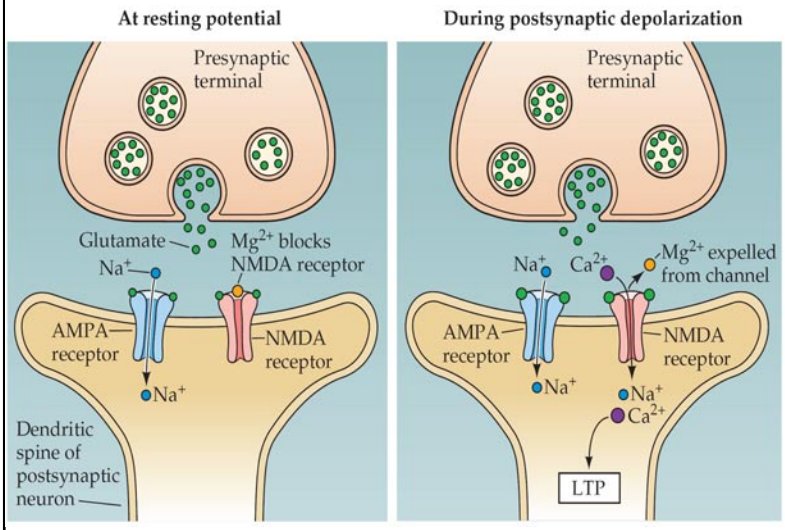

Figure 2: Short-term vs. long-term potentiation. If the post-synaptic cell is at resting potential, solely a short-term potentiation is induced. Due to the magnesium ion block of the NMDA receptor only AMPA receptors are activated. However, if the post-synaptic cell is depolarized, both AMPA and NMDA receptor are activated and there is calcium ion influx into the cell. This leads to the induction of long-term potentiation. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, N-methyl-D-aspartic acid; LTP, long-term potentiation. (Purves *et al.*, 2004)

1.2.2. AMPA receptor trafficking

One important process for signal enhancement in LTP is the trafficking of AMPARs. Already in 1989 an enhanced sensitivity of AMPARs was shown (Davies *et al.*, 1989). A possible mechanism could be the phosphorylation of AMAPRs catalyzed by CaMKII (Barria *et al.*, 1997). This phosphorylation may be important for the increased channel conductance of AMPARs after LTP induction (Benke *et al.*, 1998) and thereby enhance the sensitivity and synaptic transmission. Another mechanism for the enhanced AMPA component (Shi *et al.*, 1999) could be the exocytosis of AMPARs (Carroll *et al.*, 1999). In this process the accumulation of CaMKII and therefore the thickening of the postsynaptic density (PSD) (Dosemeci *et al.*, 2001) could play a major role. Here CaMKII is colocalized with (Gardoni *et al.*, 1998) and binds to (Strack *et al.*, 2000) the NR2B subunit of the NMDAR. This interaction is involved in the facilitation of the response of CaMKII to Ca^{2+} (Bayer *et al.*, 2001); the suppression of inhibitory second

autophosphorylation of CaMKII (Bayer *et al.*, 2001) by protein phosphatase 1 (PP1) (Shen *et al.*, 2000) and the reduction of the down regulation of NMDAR activity (Bayer *et al.*, 2001). The colocalization with the NMDAR may also be involved in the delivery of further AMPARs out of the ready-releasable pool towards the postsynaptic membrane. Thus, Lisman *et al.* proposed that an anchoring link for AMPARs is provided by an interaction between CaMKII, NMDA and actin (Lisman and Zhabotinsky, 2001). Another model was proposed in 2002 after showing the involvement of Ras-Guanosin triphosphate (GTP) in the AMAPR trafficking (Zhu *et al.*, 2002). After LTP induction Ras-GTP is formed which results in an activation of p42/44 mitogen-associated protein kinase (MAPK). This cascade results in a delivery of AMPAR to the postsynaptic membrane and thereby enhances the synaptic transmission in STP. Another important protein in this process is PSD-95, the clustering of which is enhanced after LTP induction (El-Husseini *et al.*, 2000). It may function as stabilization for AMPARs (El-Husseini *et al.*, 2002; Schnell *et al.*, 2002). Taken together, insertion of AMPARs, containing the GluR1 subunit, into the post-synaptic membrane is activated by CaMKII (Malinow and Malenka, 2002).

1.2.3. Induction of the late phase of long-term potentiation

In contrast to the early phase, the induction of L-LTP requires gene transcription and the synthesis of new proteins ((Frey *et al.*, 1988; Frey *et al.*, 1996). For the induction of L-LTP two conserved pathways are necessary: cAMP/PKA pathway and MAPK/ERK pathway.

1.2.3.1. cAMP/PKA pathway

The first step in the cyclic adenosine monophosphate (cAMP)/Protein kinase A (PKA) pathway is the activation of Adenylyl cyclase (AC) by either Ca^{2+} and CaM (Eliot *et al.*, 1989) or the α subunits of G-protein-coupled receptors (Tang and Gilman, 1991) (Figure 3). Examples for the latter are the metabotropic glutamate receptors (mGluR) (Winder and Conn, 1993), Dopamine 1 receptor (D1R) (Monsma *et al.*, 1990) and adrenergic receptors (Segal, 1981). AC forms cAMP, which for the activation of PKA, must bind to two binding sites of each of the two regulatory domains of the protein (Taylor *et al.*, 1990). That way a dissociation of the two catalytic domains is possible. To establish this, sufficient cAMP is necessary. Both, AC1 and AC8, provide it (Wong *et al.*, 1999; Wu *et al.*, 1995). However, solely inhibition of both enzymes results in LTP deficits, indicating that one can produce sufficient cAMP for L-LTP induction. Once PKA is active, several pathways are activated by phosphorylation, including disinhibition of E-LTP by PP-1 suppression (Blitzer *et al.*, 1995). Also the phosphorylation of AMPAR, as mentioned above, is mediated by PKA (Song and Huganir, 2002).

However, the involvement of the cAMP/PKA pathway in the induction of the L-LTP is even more important. This role was studied in transgenic mice who expressed an inhibitory form of the regulatory subunit of PKA (Abel *et al.*, 1997). As a result the activity of PKA was reduced which lead to a significant impairment of L-LTP. E-LTP was unaffected. In a healthy brain PKA phosphorylates several transcription factors. One of those is the cAMP response element binding protein (CREB) (Gonzalez and Montminy, 1989).

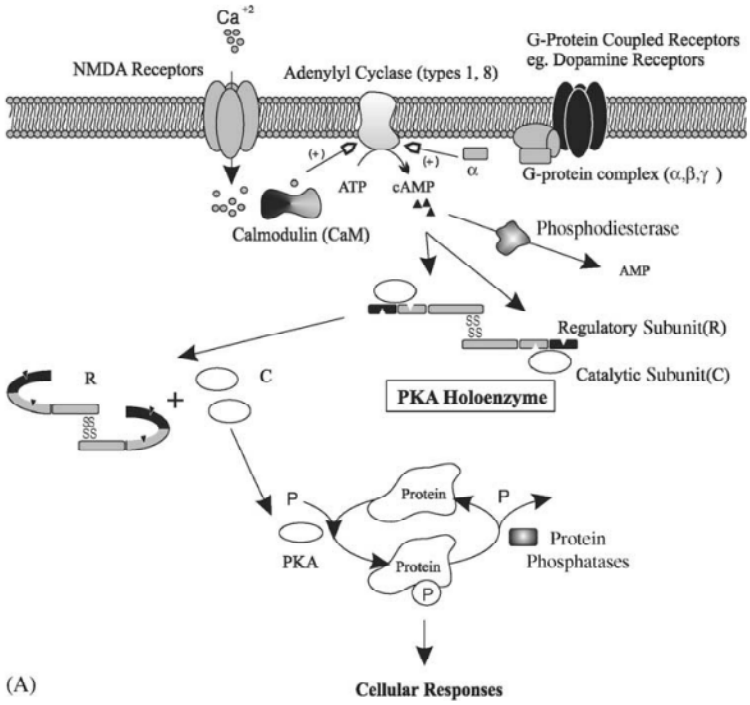

Figure 3: The cAMP/PKA pathway. To activate the cAMP/PKA pathway are two mechanisms possible: (1) via calcium ions and calmodulin due to long-term potentiation or (2) due to activation of the α subunit of a G-protein coupled receptors. This leads to production of cyclic adenosine monophosphate (cAMP) which activates protein kinase A (PKA) by binding to its regulatory subunit. The catalytic subunit dissociate and activates a relular response by phosphorylation of proteins and transcription factors like cAMP response element binding protein (CREB). (Nguyen and Woo, 2003)

1.2.3.2. MAPK/ERK pathway

The transcription of CREB can also be activated by MAPK, formely called extracellular signal-related protein kinase (ERK) (English and Sweatt, 1997). MAPK is activated by high levels of intracellular cAMP (Impey *et al.*, 1998; Vossler *et al.*, 1997) and then also phosphorylates the transcription factor CREB.

1.2.3.3. Involvement of BDNF in L-LTP

The phosphorylation of CREB results in the transcription of CRE-associated genes (Yamamoto *et al.*, 1988). One of those genes is the brain-derived neurotrophic factor (BDNF) (Shieh *et al.*, 1998; Tao *et al.*, 1998). Increased expression of BDNF has several consequences. Firstly a significant increase in synaptic branches is seen (Danzer *et al.*, 2002). Secondly the number of basal dendrites is increased. Thirdly the transmitter release is enhanced by increased levels of synaptophysin, synpatobrevin and synaptotagmin (Tartaglia *et al.*, 2001; Tyler and Pozzo-Miller, 2001).

1.2.4. Maintenance of the late phase of long-term potentiation

To maintain LTP an ongoing transcription of genes is necessary. This raises the question, how the effect is maintained for hours or even days. As solution a constantly active protein is necessary. In contrast to most second messengers that are only active for a few minutes an isoform of protein kinase C (PKC), protein kinase Mζ (PKMζ), is constantly active after formation (Hernandez *et al.*, 2003). Due to the lack of the regulatory domain autoinhibition is not possible (Figure 4a). Under normal circumstances, the translation of PKMζ mRNA is blocked by protein interacting with NIMA1 (PIN1) (Westmark *et al.*, 2010). However, after induction of LTP several proteins and processes are activated, as for example CAMKII, Phophoinositide 3-kinase (PI3K), MAPK, PKA, mammalian target of rapamycin (mTOR) and actin filament formation (Kelly *et al.*, 2007a; Kelly *et al.*, 2007b). Together they result in a release of the block by PIN1 and that way enable translation of PKMζ in the dendrite (Westmark *et al.*, 2010). To obtain it full potential, PKMζ has to bind to phosphoinositde-dependent kinase 1 (PDK1), so that the conformation of the former is changed to the high constitutive stage (Kelly *et al.*, 2007a). Now PKMζ can initiate a positive feedback loop. Due to inhibition of PIN1, an increased translation is established (Sacktor, 2010; Westmark *et al.*, 2010).

To maintain memory, PKMζ enhances the amount of AMPARs in the postsynaptic membrane (Figure 4b) (Ling *et al.*, 2002; Ling *et al.*, 2006; Yao *et al.*, 2008). AMPAR can bind to two types of proteins that influence the trafficking of the receptor. Binding to protein interacting with C kinase 1 (PICK1) dimers (Hanley *et al.*, 2002), brefeldin resistant Arf-GEF2 protein (BRAG2), Arf6 and adaptor protein 2 results in trafficking away from the membrane (Lee *et al.*, 2002). However, interaction with either N-ethylmaleimide-sensitive factor (NSF) (Hanley *et al.*, 2002) or PKMζ increases the insertion of AMPARs (Yao *et al.*, 2008). During the induction of LTP PKMζ binds PICK1 dimers. Therefore the endocytosis of AMPARs is decreased. Furthermore the action of NSF is increased. Both these processes enhance the

synaptic transmission. This is maintained during LTP so that the number of GluR2 containing AMPARs is increased and stabilized. The importance of this process is shown by inhibition of PKMζ (Ling *et al.*, 2002; Serrano *et al.*, 2005). Even when the inhibitor is applied days after LTP induction, the maintenance is reversed (Pastalkova *et al.*, 2006).

Another question is how it is possible that newly synthesized proteins act specifically on tetanized synapses. For the latter are two theories proposed: "synaptic tag" and "local protein synthesis".

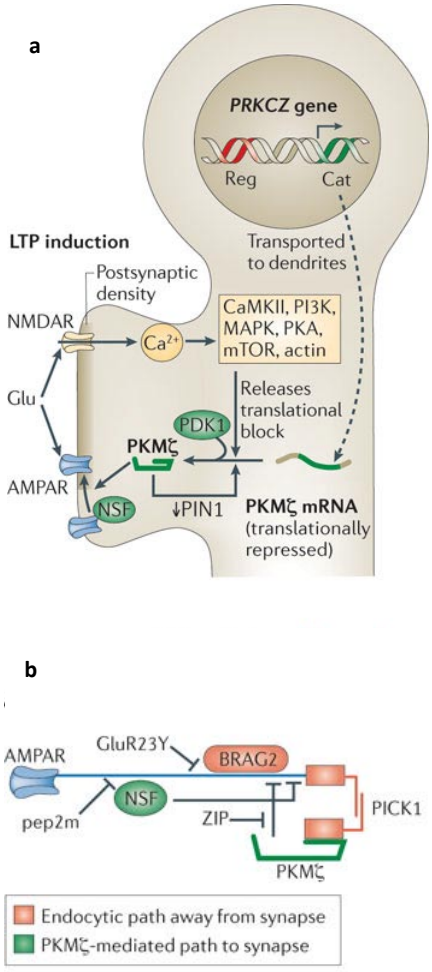

Figure 4: The role of PKMζ during long-term potentiation.

(a) Formation of PKMζ. Due to a internal promoter, the gene of the protein kinase C, zeta (PRKCZ) is transcribed without the regulatory domain (red). The mRNA of the catalytic domain (green) is transported to the dendrite. However, the translation is suppressed by PIN1 (protein interacting with NIMA1). Solely during long-term potentiation (LTP) the block is released due to activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), phosphatidylinositol 3-kinase (PIK3), mitogen-activated protein kinase (MAPK), protein kinase A (PKA), mammalian target of rapamycin (mTOR) and the formation of actin filaments. To gain its full activity, the newly synthesized protein kinase Mζ (PKMζ) is phosphorylated by phosphoinositide-dependent protein kinase 1 (PDK1). Now a positive feedback loop, to inhibit PIN1, is initiated. Furthermore, the amount of AMPA (α-amino-2-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors is increased by PKMζ via N-ethylmaleimide-sensitive factor (NSF).

(b) Influence on AMPA receptors. Several proteins can bind to the carboxyl terminal of the AMPA receptor and so influence the trafficking. Stimulation of endocytosis (red) is induced by brefeldin resistant Arf-GEF 2 protein (BRAG2) and protein interacting with C kinase 1 (PICK1). Exocytosis is enhanced by N-ethylmaleimide-sensitive factor (NSF) and PKMζ. Zeta inhibitory peptide (ZIP) and pep2m can disrupt AMPA trafficking towards the post-synaptic membrane by inhibition of PKMζ or NSF, respectively. The effect of ZIP can be blocked by GluR23Y, which binds to BRAG2. (Sacktor, 2011)

1.2.4.1. Theory of synaptic tagging

A "synaptic tag" is thought to be responsible for the fact that plasticity-related proteins (PRPs) are solely transported to the synapse where a tag is formed (Frey and Morris, 1997). This means that a synapse (S1) where E-LTP is induced by weak tetanization, although no protein synthesis is induced, can experience L-LTP owning to another synapse (S2) where L-LTP and hence protein synthesis is evoked.

Due to the "synaptic tag", established by E-LTP, PRPs can be captured by S1. This process maintains the input specificity of LTP. One of those PRP is PKMζ (Sajikumar *et al.*, 2005). It was shown that PKMζ is active in both synpases, S1 and S2. There are also indications that BDNF functions as a PRP (Barco *et al.*, 2005).

1.2.4.2. Theory of local protein synthesis

This theory implicate that the synthesis of proteins during L-LTP takes place in the dendrite instead of in the soma. Evidence is found in the fact that polyribosomes are preferably found in dendritic spines (Steward and Levy, 1982) and that for example CaMKII and PKMζ are synthesized in the dendrite (Ouyang *et al.*, 1999; Westmark *et al.*, 2010). Furthermore it was shown that inhibition of dendritic protein synthesis impairs L-LTP (Bradshaw *et al.*, 2003).

1.2.5. Retrograde messaging during LTP

Additionally to all these post-synaptic mechanisms, retrograde messengers and therefore pre-synaptic mechanisms are also necessary for the induction of LTP (Bolshakov and Siegelbaum, 1994; Emptage *et al.*, 2003). A possible retrograde messenger can be nitric oxide (NO) (Böhme *et al.*, 1991; O'Dell *et al.*, 1991; Schuman and Madison, 1991). It is synthesized after Ca^{2+}/CaM dependent activation of nitric oxide synthase (NOS) (Christopherson *et al.*, 1999). In the pre-synaptic cell, NO enhances the potential by activating guanylyl cylcase and therefore the production of cGMP (Son *et al.*, 1998). This results in an enhanced release of neurotransmitter.

1.2.6. Long-term depression

Besides enhancing the synaptic transmission, it is also possible to weaken it (Purves *et al.*, 2004). This process is called long-term depression (LTD). The decision whether LTP or LTD is induced depends on the amount of Ca^{2+} entering the post-synaptic cell. Low concentrations of Ca^{2+} result in enhanced endocytosis of AMPARs. Protein phosphatases play a major role in this process. Without phosphorylation it is not possible to induce most processes of LTP. By LTD PKMζ is not translated. Due to the lack of this enzyme, PICK1 (Hanley *et al.*, 2002) and BRAG2 (Lee *et al.*, 2002) are not inhibited. This directly enhances the endocytosis of AMPARs.

1.2.7. Importance of the interplay between long-term potentiation and long-term depression

The interplay of LTP and LTD regulates the synaptic transmission (Purves *et al.*, 2004). This process is not only important in the formation of memory but also in the maturation of silent synapses. These synapses do not contain AMPARs. However, if another synapse is activated, also the post-synaptic membrane of the silent synapse is depolarized. By simultaneous stimulation of the silent synapse with glutamate, induction of LTP is possible because a "synaptic tag" is established. Owing to this process the synapses matures. Furthermore, growth of new dendritic spines is induced by LTP.

Taken together the activity-dependent plasticity, mediated by LTP and LTD, leads to functional changes of synapses in neuronal circuit. A process, that is among others, important for the formation of memory.

1.3. Alzheimer's disease

AD was first described by Alois Alzheimer in 1907 (Alzheimer, 1907). A 51-year-old woman had great memory impairment along with cognitive deficits. After her death, 4 years later, a histological analysis of her brain was performed. Alzheimer found neurofibrillary tangles (NFTs) and inclusions that became later been known as amyloid plaques.

Nowadays AD is the most common form of dementia in patients over 65 years of age (Ferri *et al.*, 2005). However, early-onset AD also affects much younger patients. The key symptom is the impairment of memory formation (Stahl, 2008). The language, motor function, recognition and working memory can also be impaired. Furthermore, changes in personality are seen. These impairments are caused by synaptic loss that also correlates best with the cognitive decline. But how do the two hallmark features, Amyloid β-containing plaques and NFTs fit into the pathology?

1.3.1. Amyloid β in Alzheimer's disease

Amyloid β (Aβ) derives from the amyloid precursor protein (APP), which can be cleaved by different enzymes (Figure 5) (Stahl, 2008). One mechanism involves α-secretase. The product, αAPPs, is further cleaved by γ-secretase, resulting in P3. However, if APP is cleaved by β- and γ-secretase, it will result in a toxic peptide, Aβ, which is likely to accumulate. Commonly Aβ consists of 39 or 43 amino acids. Several genetic mutations and risk factors have been implicated to be involved in the production of Aβ. So is

early-onset AD caused by mutations in the genes of APP, presenilin 1 or presenilin 2. Presenilin 1 and 2 are components of γ-secretase .Moreover the expression of apolipoprotein (apo)-E4 enhances the risk of developing AD at old age. Normally ApoE is involved in the removal of Aβ, except for the E4 allel of this gene.

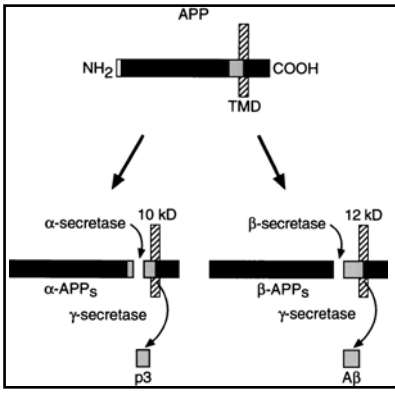

Figure 5: Amyloid precursor protein cleavage. On the left side the process without toxic product is shown. At first Amyloid precursor protein (APP) is cleaved by α-secretase, resulting in α-APPs. The second step is splitting by γ-secretase. P3 is produced by this step. However when APP is cleaved by β- and γ-secretase instead, a toxic protein, Aβ, is produced. TMD, transmembrane domain; αAPP_S and βAPP_S, secreted fragments generated by α- and β-secretase cleavage, respectively. (Ross *et al.*, 1998)

Aβ is a hydrophobic peptide and therefore aggregates into monomers, dimers, oligomers, protofibrils and mature fibrils, which are seen as plaques. Hardy and Higgins proposed in 1992 the "amyloid cascade hypothesis" (Hardy and Higgins, 1992). At first it was thought that the plaques are responsible for the damage. However, the appearance of plaques does not correlate with the disease progression. For this reason the "amyloid cascade hypothesis" is review in 2002 (Hardy and Selkoe, 2002). It is shown that Aβ derived diffusible ligands (ADDL) are the more potent in causing the damage, observed in AD patients (El-Agnaf *et al.*, 2000; Roher *et al.*, 1996; Wang *et al.*, 1999).

1.3.1.1. Generation of increased levels of Amyloid β by AD

Several studies have shown the involvement of G-protein coupled receptors (GPCRs) in the regulation of secretases and hence Aβ production. The effects will be discussed by subtype.

The activation of α-secretase precludes the generation of toxic Aβ. Hence could this be an interesting drug target and source of the elevated Aβ levels found in AD brains. Muscarinic acetylcholine receptors (mAChRs) as well as mGluRs and serotonin receptors (5-HT) are involved in the regulation of α-secretase. Thus, a stimulation of M1 and M3 mAChRs results in an enhanced production of αAPPs (Buxbaum *et al.*, 1992; Nitsch *et al.*, 1992). This cascade includes the activation of PKC, MAPK and glycogen synthase kinase 3β (GSK3β), which also reduce tau phosphorylation (Caccamo *et al.*, 2006). However, mGluRs are negatively coupled to α-secretase activity (Kim *et al.*, 2010). There is evidence that mGluR2 is overexpressed in the hippocampus of AD patients (Lee *et al.*, 2004). This subtype and other group II

mGluRs stimulate tau phophorytion and reduce cAMP production (Kim *et al.*, 2010; Lee *et al.*, 2009). Another receptor involved in the pathogenesis of AD is 5-HT_6. A polymorphism in this gene is associated with a higher risk for AD (Tsai *et al.*, 1999) and the expression is decreased in the prefrontal cortex (Lorke *et al.*, 2006). An antagonist for the 5-HT_6 receptor significantly improves memory formation and cognition (Foley *et al.*, 2003) and successfully completed the phase II clinical trial (Maher-Edwards *et al.*, 2010).

The opponent of α-secretase is β-secretase, which produces βAPP_s, the precursor for Aβ. The activity is regulated by opioid receptors and angiotensin (AT) type 2A receptor. Recent research showed that the δ-opiod receptor can modulate BACE1 (β-secretase) and Aβ regulation {{653 Teng,Lin 2010}}, hence be a therapeutic target. The angiotensin receptor, $AT_{2A}R$, is abundantly expressed in the hippocampus of AD patients (Angulo *et al.*, 2003). Moreover the high Aβ levels cause dimerization/oligomerization of the receptor and therefore decrease the activation of mAChR M1 via Gα (AbdAlla *et al.*, 2009). Due to this oligomerization enhanced tau phosphorylation, further production of Aβ and impairment of memory are observed. However, an antagonist for $AT_{2A}R$, caffeine, has neuroprotective function and could improve cognition (Dall'lgna *et al.*, 2003).

1.3.2. Impairment of synaptic transmission by amyloid β

As mentioned before synaptic loss correlates best with the observed cognitive decline (Bancher *et al.*, 1996; DeKosky and Scheff, 1990). High levels of Aβ as shown by *in vivo* and *in vitro* studies may account for it (Cirrito *et al.*, 2005; Kamenetz *et al.*, 2003). The production and secretion of Aβ is regulated by the neuronal activity (Kamenetz *et al.*, 2003). In hyperactive neurons, e.g. during epileptiform activity, the release of Aβ is enhanced (Cirrito *et al.*, 2005). Hence Kamenetz *et al.* proposed that Aβ may act as a negative feedback loop to control hyperactivity (Kamenetz *et al.*, 2003). This theory is further supported by a study conducted by Abramov *et al.* (Abramov *et al.*, 2009). They showed that Aβ regulates presynaptic release of glutamate. By the use of small concentrations of Aβ, the synaptic transmission can be enhanced via a α7-nicotinic acetylcholine receptor (nACHR)-dependent pathway (Puzzo *et al.*, 31 December 2008). This lead to the proposition made by Palop & Mucke, that Aβ regulates synaptic transmission in a bell-shaped manner (Figure 6) (Palop and Mucke, 2010). Low as well as high concentrations of Aβ result in depression of pre- or postsynaptic activity, respectively. However, intermediate concentrations enhance presynaptic glutamate release.

Of these three effects, the postsynaptic one is the most intensively studied. Normally the activity of excitatory synapses is regulated by the interplay between LTP and LTP. Aβ causes postsynaptic depression by tempering with this process and therefore impairs LTP (Chapman *et al.*, 1999; Chen *et al.*, 2000; Cleary *et al.*, 2005; Cullen *et al.*, 1997) and enhances LTD (Kim *et al.*, 2001; Li *et al.*, 2009). Several molecular processes are involved in this.

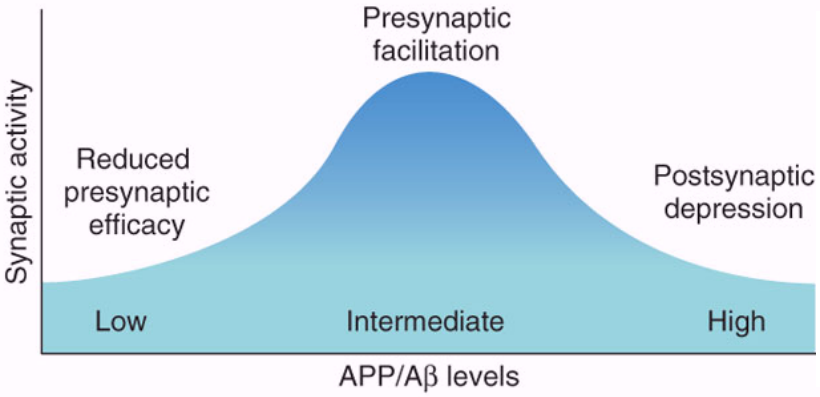

Figure 6: Hypothetical interaction between synaptic activity and Amyloid β. Intermediate concentrations of Amyloid β (Aβ) result in an increase in synaptic activity, whereas low or high levels cause a decrease. (Palop and Mucke, 2010)

1.3.2.1. Amyloid β and disruption of the NMDA Receptor-Dependent Signaling pathway of LTP

The impairment of LTP by Aβ is studied in several ways. As well *in vivo* (Cullen *et al.*, 1997; Stéphan *et al.*, 2001) as *in vitro* (Chen *et al.*, 2000) the NMDAR dependent decrease in LTP was studied. Moreover it was shown that in transgenic mice, having the Swedish mutation of the APP gen, LTP in the CA1 and dentate gyrus is impaired (Chapman *et al.*, 1999). However, STP was normal. Furthermore, the effect seems to correlate with the age of the mice. Where does Aβ disrupt the process? The chronic administration of Aβ results in decreased NMDAR (Chen *et al.*, 2002) and AMPAR (Parameshwaran *et al.*, 2007) currents. But it is thought that the interaction is not directly with the receptors, hence there must be downstream targets of Aβ (Raymond *et al.*, 2003).

Normally the current of AMPARs is enhanced during LTP by phosphorylation (Barria *et al.*, 1997). Due to Aβ, there is disruption of CaMKII autophosphorylation and therefore also AMPAR phosphorylation (Zhao *et al.*, 2004). This process seems to be dependent on tyrosine kinase receptor B (trkB) (Zeng *et al.*, 2010). By co-treatment with neurotrophin 4 (NT-4) or BDNF, both ligands for trkB, an enhanced autophosphorylation of CaMKII and phosphorylation of AMPAR is established. Furthermore Aβ induces AMPAR endocytosis via a mechanism similar to LTD (Hsieh *et al.*, 2006).

The reduced NMDAR dependent LTP can be rescued by administration of a calcineurin inhibitor (Chen *et al.*, 2002; Shankar *et al.*, 2007). The role of calcineurin is activation of PP1, which results in dephosphorylation and hence deactivation of CREB (Bito *et al.*, 1996). Therefore inhibition of PP1 should also result in the rescuing of LTP from impairment as demonstrated by Knobloch *et al.* in 2007 (Knobloch *et al.*, 2007). Moreover it was shown that this impairment is sufficient to induce synaptic loss (Shankar *et al.*, 2007).

Another mechanism that reduces the current of NMDARs is found in transgenic ApoE4 mice (Chen *et al.*, 2010). The expression of the ApoE4 allel results in a reduced expression of Apoer2. This protein acts as a functional receptor for Reelin, NMDAR and AMAPR. Due to this reduced expression the ability of Reelin to enhance synaptic activity via phosphorylation is impaired.

1.3.2.2. Amyloid β and Calcium Homeostasis

The regulation of calcium is essential for the survival of a cell because elevated intracellular Ca^{2+} concentrations cause cell death (Berridge, 1998). Therefore the impairment of the calcium homeostasis by Aβ in AD plays a major role in the pathology (Demuro *et al.*, 2005). Aβ increased Ca^{2+} by an enhanced influx.

In contrast to the decrease in NMDAR current by chronic Aβ administration (Shankar *et al.*, 2008; Snyder *et al.*, 2005), acute treatment with Aβ results in an enhanced Ca^{2+} influx (Kelly and Ferreira, 2006). Due to the enhanced Ca^{2+} concentration, degeneration of dynamin 1 takes placed. This process is mediated by calpain activation. To disrupt this process, the NMDAR is studied as a drug target. Memantin, an NMDAR antagonist, is found to be effective and used as a treatment for moderate to severe dementia (Reisberg *et al.*, 2003). Moreover cytosolic Ca^{2+} levels can also be enhanced by increased influx through voltage-gated Ca^{2+} channels (MacManus *et al.*, 2000).

Another target of Aβ is the cell membrane itself. It was shown that Aβ not only decreases the membrane fluidity (Müller *et al.*, 1995) but also causes an enhanced permeability of the cell membrane (Wong *et al.*, 2009). This leads to a higher membrane conductance (Kayed *et al.*, 2004; Sokolov *et al.*, 2006). It was further shown that there is more interaction of Aβ with alkaline membrane as seen in endosomes. Therefore Aβ42 is colocolized with multivescular bodies (MVBs) (Takahashi *et al.*, 2002). This accumulation appears to be inhibiting the ubiquitin-proteasome system and hence influence the MVB sorting (Almeida *et al.*, 2006; Oh *et al.*, 2005). This impairment of the proteasome system not only enhances Aβ but also tau accumulation (Tseng *et al.*, 2008).

Large amounts of intracellular Ca^{2+} are stored in the endoplasmic reticulum and mitochondria (Berridge, 1998). Uptake and release is regulated by inositol trisphosphate receptors and ryanodine receptors. Due to Aβ, the release mediated by both receptors is increased (Schapansky *et al.*, 2007; Shtifman *et al.*, 2010). Furthermore, a direct interaction between Aβ and mitochondria is found (Manczak *et al.*, 2006) and lead to impairment of the respiratory chain (Caspersen *et al.*, 2005). A result is the production of reactive oxygen species (ROS), which furthermore increases the possibility of cell death.

1.3.3. The role of Neurofibrilar tangles in Alzheimer's disease

In addition to Aβ, there are also NFTs. How does the second hallmark feature fit into the amyloid cascade hypothesis? In transgenic mice it was shown that the formation of Aβ induces hyperphosphorylation of tau (Gotz *et al.*, 2004). Tau is normally involved in the stabilization of microtubules (Stahl, 2008). However, if it is hyperphosphorylated as in AD, it forms tangles. NFTs can be seen at the end stage of AD pathology (Bancher *et al.*, 1996), which would fit into the theory that Aβ induces NFTs (Ittner and Götz, 2011). Another possibility is that both lesions are causing the neurotoxicity of AD together. Evidence supporting this theory would be that both are induced due to defects in GPCRs regulation. Furthermore, both proteins affect for example the respiratory chain, but via different targets. Lastly there is the possibility that NFTs mediate the toxicity of Aβ. Support comes from $tau^{-/-}$ neurons, which are protected against Aβ toxicity.

1.4. Aim of the work

With this work I like to show the impairment of NMDAR-dependent LTP in the CA1 region of the hippocampus by ADDL 1-42 *in vitro* in acute hippocampal slices.

2. Material & Methods

2.1. Preparation of hippocampal slices

For the preparation of acute hippocampal slices, four month old male mice (*mus musculus*; C57BL/6; Institute breeding stock) were used. Animals were sacrificed by cervical dislocation and decapitation. Next the pelage of the head was cut off; the skull sliced up along the sutura sagittalis and the skullcap removed. Then the brain was transferred from the skull into artificial cerebrospinal fluid (ACSF; 124nM NaCl, 4.9mM KCl, 1.3mM $MgSO_4$, 2mM $CaCl_2$, 1.2mM KH_2PO_4, 25.6mM $NaHCO_3$, 10mM D-Glucose), which before was gased with carbogen (95% oxygen; 5% carbon dioxide) and cooled to a temperature of 0°C. It is essential that those first three steps are performed fast, because the brain is without oxygen. Then the corpus callosum as well as the hippocampal connections with the fornix and entorhinal cortex where intersected. Afterwards the isolated hippocampi were sectioned to 400µm thick slices at a cutting angle of 70°. This way the pathway of longitudinal signal processing is preserved. In the end the slices are put for two hours in the pre-incubation chamber filled with carbogen gased ACSF and the test substance: 4µL Dimethyl sulfoxide (DMSO; Control) or 500nM ADDL 1-42, solved in 4µL DMSO.

2.2. Preparation of ADDL

Monomerization of solid Aβ 1-42 peptides is performed according to the protocol of Klein *et al.* (Klein, 2002). At the day of use, the storred Aβ peptides are solved in 4µL DMSO. The sides of the tube are washed down by thoroughly pipetting. Afterwards the solution is vortexed for one minute and put twice for two minutes in an ultrasonic bath, followed by another minute of vortexing.

2.3. Basics of electrophysiological measurements

The general aim of electrophysiology is to generate an artificial activation of nerves. Therefore stimulation above the threshold potential is necessary. For that reason it is important to understand the electrical characteristics of the cell membrane. The lipid-bilayer is comparable to a capacitor (Ranck, 1979) and its ion channels to battery in series with a resistor (Hille, 2001). A resistor alone does not include the concentration gradient at the channel.

In the unrealistic case, in which the membrane is exclusively permeable to potassium, a transmembrane potential can be generated by changing the extracellular potassium concentration to a lower level (Ranck, 1979). As a result a few potassium ions will diffuse out off the cell through its ion channels. This outwards ionic current charges the capacitor (inwards capacitavice current) with a potential. Therefore the net flow over the cell membrane is zero. This is comparable to adding a battery to a parallel circuit of a capacitor and resistor (Figure 7). The battery will charge the capacitor with a resistive current flowing out of the resistor. The current flow stops when the potential in the battery is the same as in the capacitor. This situation is comparable with an equilibrium of ion concentration or/and charge of a cell.

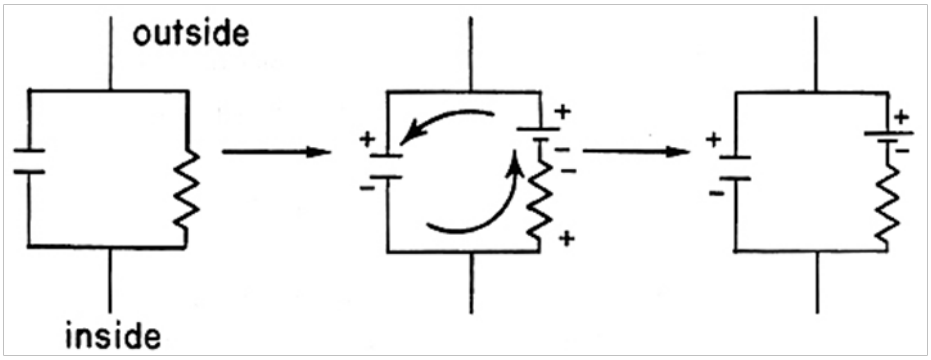

Figure 7: The cell membrane as a capacitor and resistor. The cell membrane with its ion channels can be compared to a capacitor in a parallel circuit with a resistor (left). By adding a battery to the circuit an outwards resistive current is generated which charges the capacitor (middle). This process is comparable to a cell, which is solely permeable for potassium ions, with a high intracellular and low extracellular concentration of potassium. After the battery and capacitor have the same potential, the current flow stops (right). This way an equilibrium is developed. (Ranck 1979)

To generate a depolarization in a fiber with an extracellular electrode, an outwards current is necessary (Ranck, 1975), but for this to happen there must be an inwards current elsewhere. When stimulating a neuron with a cathode, it is important to realize that although the current is outwards (depolarizing) near the electrode, the surroundings current will be inwards and therefore hyperpolarize the cell (Figure 8B). This effect is called anodal surrounding. However, the hyperpolarizing current is spread over a greater distance and therefore its extent is less. Thus it will still be possible to evoke an action potential that is able to propagate through the hyperpolarized part of the fiber. With an anode it is exactly the opposite (Figure 8A). To evoke EPSPs *in vitro* biphasic stimulation is used to prevent the production of hydroxide gas near the cathode. Furthermore, this has the advantage that not-depolarized, surrounding fibers are not affected at all.

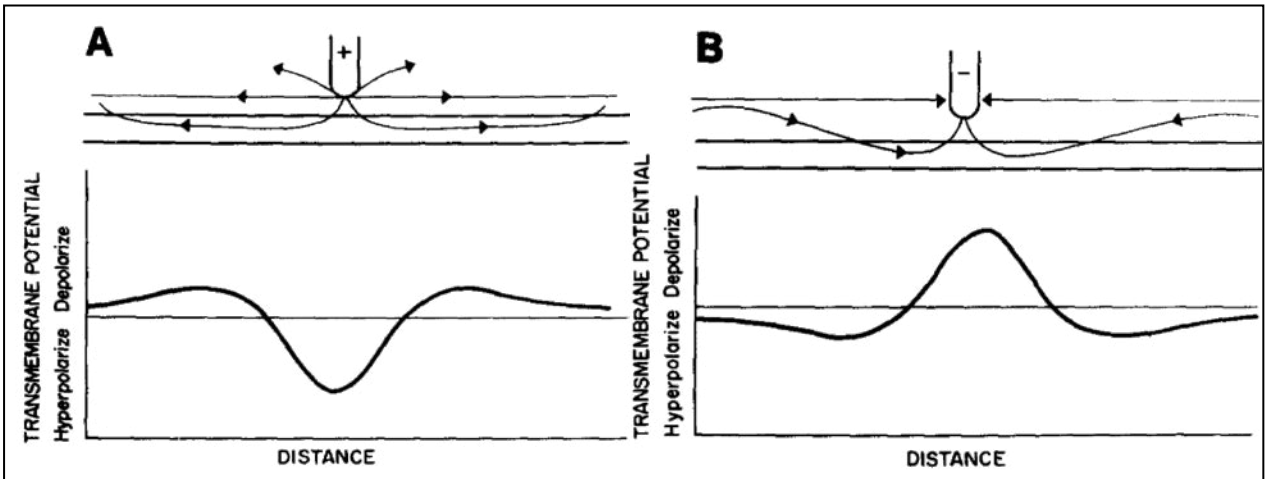

Figure 8: Effect of an monopolar electrode on the current flow near a fiber. A: Anode; B: Cathode. An outwards current across the cell membrane is necessary for depolarization of the fiber. The second electrode is not shown and placed in greater distance. (Ranck 1975)

In general a short negative current is used for stimulation. This results in a biochemical reaction by which electrons are freed. These electrons bind to positive ions in the ACSF. This results in a change in extracellular ion concentration and therefore changes the membrane potential. If the threshold for an action potential is reached, the activation of AMPARs at the synapses takes place. The extracellular potential resulting from this process can be measured by the slope of the fEPSP. This means that the EPSPs of several synapses are detected simultaneously, which makes it possible to measure a current in the range of mV. The changes in extracellular ion concentration due to the EPSP are measured with a microelectrode filled with ACSF. The concentration of ions in the microelectrode changes together with the concentration in the extracellular space. This change is detected by an Ag/AgCl pallet by a mechanism comparable to the stimulation.

By the use of high frequent stimulation it is possible to evoke a NMDAR-dependent LTP in the SC pathway of the hippocampus. Three repeats results in a persistent change of the synaptic plasticity, L-LTP (Frey *et al.*, 1993). This enhanced extracellular STP can be seen for eight hours after tetanization. However, four hours are sufficient because the enhancement of STP by E-LTP is only present for two to three hours. Afterwards the effect of L-LTP and therefore the involvement of protein synthesis can be studied.

2.4. Electrophysiological measurements

After the two hours incubation period the hippocampal slices are transferred to a submerged-type recording chamber (Figure 9a). Here continuous perfusion with carbogen purged ACSF at a rate of 1.5 ml/min at 32±1°C takes place. After a recovery period of 25 minutes the lacquer-coated stainless-steel-stimulating electrode is placed in the Schaffer collateral pathway – commissural fibers in the stratum radiatum of CA1 region. To measure fEPSP the glass microelectrode (filled with ACSF, 1 – 4 Ω) is set in the apical dendrite layer (Figure 9b). A chlorinated silver wire functions as a reference electrode.

By the use of an Input-Output graph the maximum EPSP is determined. The test stimuli are adjusted to evoke a fEPSP of about 30% of the maximum. During baseline recording fEPSP is evoked by a biphasic, voltage constant pulse of 0.05 ms per half cycle with an A-M Systems Isolated Pulse Stimulator 2100 and digital-analog converter (CED micro 3, Cambridge Electronics Design, Cambridge, UK). The recorded signals are amplified with a differential amplifier (Institute development & A-M Systems Model 1700), filtered with a Bessel filter and converted to a digital signal by CED micro 3. Afterwards the signal is directly transmitted to the computer program PWIN (Institute development). The data is obtained by the measurement of the slope. For LTP induction a stimulus of 100Hz and pulse duration of 0.1ms per half cycle is used.

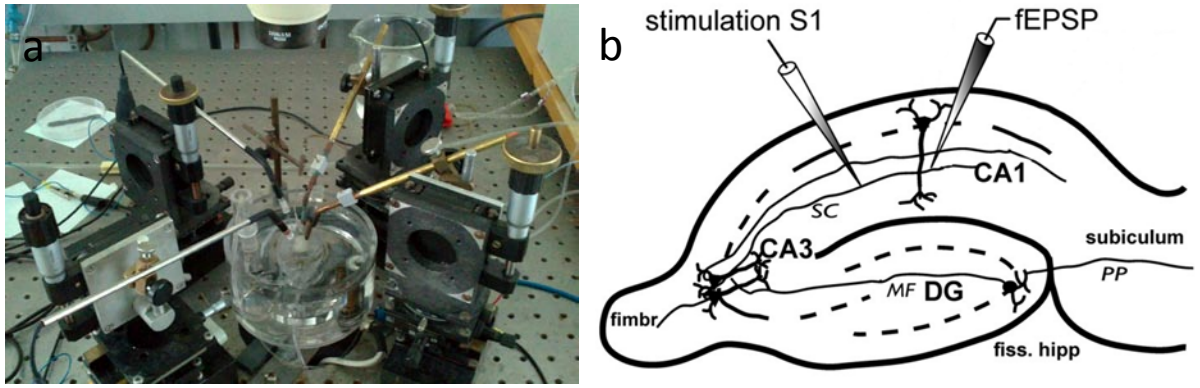

Figure 9: Electrophysiological measurement set-up. a) Submerged-type recording chamber. On the left-side are the glass electrodes and on the right the stimulation electrodes. **b)** Positioning of the stimulation and glass electrode in the CA1 area of the hippocampus.

2.5. Statistic Analysis

To determine significance of the differences between the two groups are an independent-sample Student's T-Test and a Two-way ANOVA of repeated measurements performed. In the graphs the standard error of the mean (SEM) is given by error bars. To calculate the statistics SPSS 17 is used.

3. Results

The effect of Aβ peptides was investigated using the most toxic species Aβ1-42 (El-Agnaf *et al.*, 2000). As shown by Klein oligomer species of the peptide, called ADDL, are the most potent in causing the damage seen in AD (Klein, 2002). To prepare the ADDLs a similar protocol was used. For the experiments a total of 10 hippocampal slices for the control group and 7 for the ADDL group were used. Those derived from 5 and 4 mice, respectively.

The effect of ADDL 1-42 on basal synaptic transmission can be seen in the Input-Output curve. As shown in figure 10, there is a significant difference in signal intensity between the control and ADDL 1-42 group at higher stimulus intensities (2.5V stimulus strength: ADDL 2.37 ± 0.25 mV/ms; Control 3.87 ± 0.29 mV/ms).

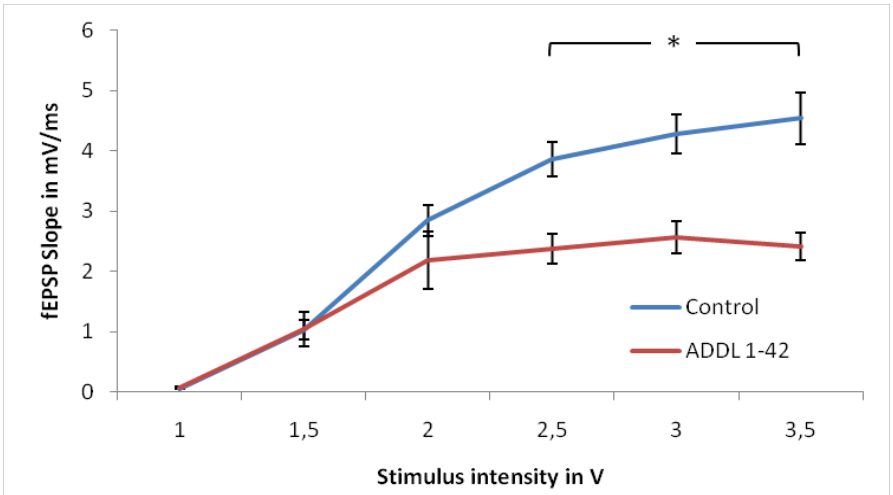

Figure 10: Pooled Input-Output Curve showing the effect of 500nM ADDL 1-42 on basal synaptic transmission. The fEPSP slope at increasing signal intensities is studied in four month old male mice: control n=10 (5 mice), ADDL 1-42 n=7 (4 mice); *$p < 0.05$ in Student's T-Test.

For the investigation of the effect of ADDL on LTP the same slices are used as for the Input-Output curve. In these slices LTP is induced after a forty-five minute baseline by three high frequent stimuli (100Hz, 1s, Pulse duration 0.1 ms per half cycle). The measurement is continued for four hours, so that transcription and protein synthesis take place. These conditions allow study of both, the effect of ADDL on early and late phase of LTP. Figure 11 shows the significant difference in fEPSP slope in percent of baseline between control and ADDL 1-42 group (1min after tetanus: ADDL 248.36 ± 23.72 %, Control 291.84 ± 17.93 %; 240min after tetanus: ADDL 89.64 ± 9.46 %, Control 124.28 ± 5.09 %)

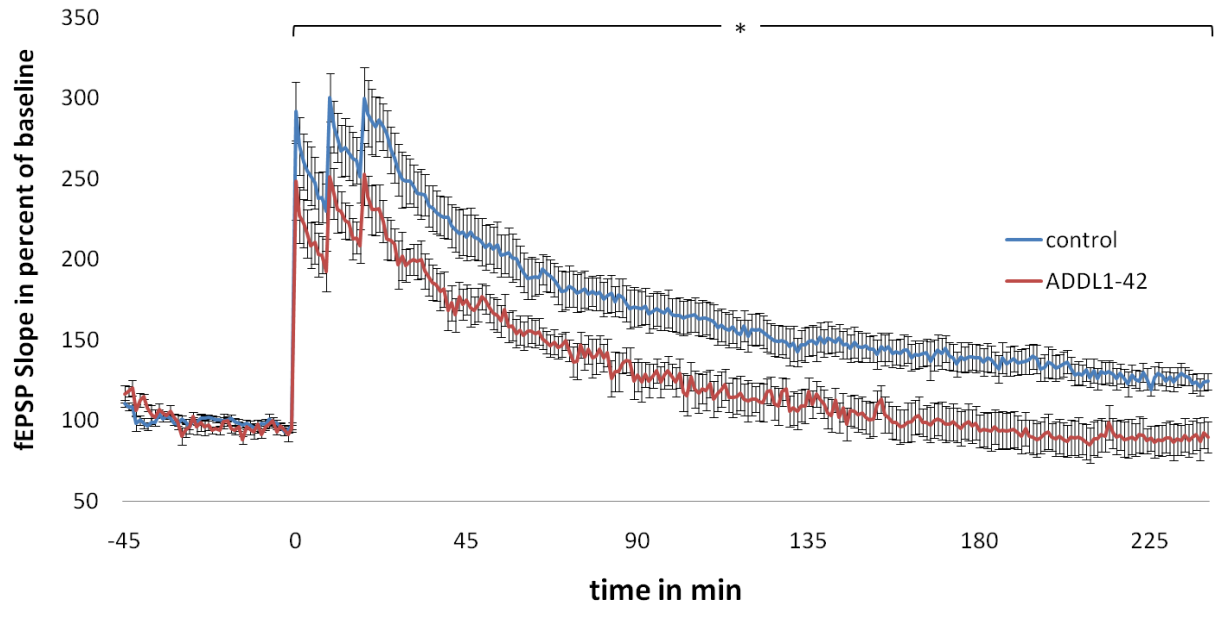

Figure 11: Effect of 500nM ADDL1-42 on long-term potentiation. The fEPSP slope in percent of baseline is studied in four month old male mice: control n=10 (5 mice), ADDL 1-42 n=7 (4 mice); Long-term potentiation is induced at 0, 10 and 20 with three high frequent stimuli (100Hz, 1sec, pulse duration 0,2). *$p < 0.05$ in two-way ANOVA of repeated measurements.

4. Discussion

A key symptom of AD is the inability of acquire new memory (Stahl, 2008), an effect that is also seen in bilateral hippocampus lesions (Scoville and Milner, 1957). This makes the hippocampus an important region to study the pathology of AD. Several studies have implicated that LTP, a process involved in plasticity, plays a major role in memory formation (Bliss and Lomo, 1973; Dash *et al.*, 2004; Reymann and Frey, 2007). Especially LTP in the SC pathway in the hippocampus is investigated intensively. It is though that this process is disrupted by Aβ, a peptide that is found to increasingly aggregate and form plaques in brains of AD patients. Several laboratories provided evidence that the oligomers of Aβ1-42 are the most potent in causing the damage observed in AD patients (El-Agnaf *et al.*, 2000; Klein, 2002; Roher *et al.*, 1996; Wang *et al.*, 1999).

Acute hippocampal slices of mice were used to investigate the effect of ADDL 1-42 on LTP in the SC pathway. The results showed a significant decrease in fEPSP in percent of baseline between the control and ADDL group (figure 11). Comparable results are also shown by several other research groups although some stimulated other hippocampal areas (Adekar *et al.*, 2010; Chen *et al.*, 2000; Wang *et al.*,

2002). In order to confirm that the decrease is indeed caused by ADDL, Adekar *et al.* administrated an antibody against it (Adekar *et al.*, 2010). The impairment of LTP was rescued in these experiments (Adekar *et al.*, 2010) and also resulted in enhanced improvement of the memory performance (Zhang *et al.*, 2011), indicating the possibility of an immunotherapy in AD.

Besides the disruption of LTP, an impairment of basal synaptic transmission was also found in the conducted experiments (figure 10). Therefore the change in LTP could also be caused by impairment of basal synaptic transmission, which could mean that ADDL had no effect on the LTP. However by using the fEPSP in percent of baseline, the curve is corrected for some decrease in excitability. Furthermore the stimulus intensity used was below 2V where no significant difference was detected. Therefore it can be assumed that the effect seen in figure 11 is due to LTP impairment. In the dentate gyrus normal synaptic transmission is found to be unaffected by ADDL, only a decrease in short-term plasticity was observed (Wang *et al.*, 2002). This could either mean that there is a difference between the two hippocampal regions or that due to distinction in the protocols different results were observed. Further investigation would be necessary.

In summary, this work showed the disruption of normal synaptic transmission and LTP in acute hippocampal slices caused by Aβ 1-42 oligomers.

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