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MECHANISMS OF AMYLOID-BETA CYTOTOXICITY IN HIPPOCAMPAL NETWORK FUNCTION- RESCUE STRATEGIES IN ALZHEIMER'S DISEASE

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Mechanisms of Amyloid-Beta Cytotoxicity in Hippocampal Network Function- Rescue strategies in Alzheimer's Disease

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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I would like to dedicate this thesis work especially to my grandfather, other family members, friends and all well-wishers.

Science never solves a problem without creating ten more.
Sir George Bernard Shaw

ABSTRACT

The origin and nature of cognitive processes are strongly associated with synchronous rhythmic activity in the brain. Gamma oscillations that span the frequency range of 30–80 Hz are particularly important for sensory perception, attention, learning, and memory. These oscillations occur intrinsically in brain regions, such as the hippocampus, that are directly linked to memory and disease. It has been reported that gamma and other rhythms are impaired in brain disorders such as Alzheimer's disease, Parkinson's disease, and schizophrenia; however, little is known about how these oscillations are affected.

In the studies contained in this thesis, we investigated a possible involvement of toxic Amyloid-beta (Aβ) peptide associated with Alzheimer's disease in degradation of gamma oscillations and the underlying cellular mechanisms in rodent hippocampi. We also aimed to prevent possible Aβ-induced effects by using specially designed molecular tools known to reduce toxicity associated with Aβ by interfering with its folding and aggregation steps.

Using electrophysiological techniques to study the local field potentials and cellular properties in the CA3 region of the hippocampus, we found that Aβ in physiological concentrations acutely degrades pharmacologically-induced hippocampal gamma oscillations *in vitro* in a concentration- and time-dependent manner. The severity of degradation also increased with the amount of fibrillar Aβ present.

We report that the underlying cause of degradation of gamma oscillations is Aβ-induced desynchronization of action potentials in pyramidal neurons and a shift in the equilibrium of excitatory-inhibitory synaptic transmission. Using specially designed molecular tools such as Aβ-binding ligands and molecular chaperones, we provide evidence that Aβ-induced effects on gamma oscillations, cellular firing, and synaptic dynamics can be prevented. We also show unpublished data on Aβ effects on parvalbumin-positive baskets cells or fast-spiking interneurons, in which Aβ causes an increase in firing rate during gamma oscillations. This is similar to what is observed in neighboring pyramidal neurons, suggesting a general mechanism behind the effect of Aβ.

The studies in this thesis provide a correlative link between Aβ-induced effects on excitatory and inhibitory neurons in the hippocampus and extracellular gamma oscillations, and identify the Aβ aggregation state responsible for its toxicity. We demonstrate that strategies aimed at preventing peptide aggregation are able to prevent the toxic effects of Aβ on neurons and gamma oscillations. The studies have the potential to contribute to the design of future therapeutic interventions that are aimed at preserving neuronal oscillations in the brain to achieve cognitive benefits for patients.

LIST OF SCIENTIFIC PAPERS

I. **Kurudenkandy FR**, Zilberter M, Biverstål H, Presto J, Honcharenko D, Strömberg R, Johansson J, Winblad B and Fisahn A. Amyloid-beta-induced action potential desynchronization and degradation of hippocampal gamma oscillations is prevented by interference with peptide conformation change and aggregation. *The Journal of Neuroscience*. 2014;34(34):11416-25

II. Honcharenko D, Bose PP, Maity J, **Kurudenkandy FR**, Juneja A, Floistrup E, Biverstål H, Johansson J, Nilsson L, Fisahn A and Stromberg R. Synthesis and evaluation of antineurotoxicity properties of an amyloid-beta peptide targeting ligand containing a triamino acid. *Organic and Biomolecular Chemistry*. 2014;12(34):6684-93.

III. Cohen SI, Arosio P, Presto J, **Kurudenkandy FR**, Biverstal H, Dolfe L, Dunning C, Yang X, Frohm B, Vendruscolo, Johansson J, Dobson C.M, Fisahn A, Knowles T.P.J and Linse S, A molecular chaperone breaks the catalytic cycle that generates toxic Abeta oligomers. *Nature Structural Molecular Biology*. 2015;22(3):207-13.

IV. Poska H, Haslbeck M, **Kurudenkandy FR**, Hermansson E, Chen G, Kostallas G, Biverstål H, Crux H, Fisahn A, Presto J and Johansson J. Dementia related Bri2 BRICHOS is a molecular chaperone that efficiently inhibits Aβ42 toxicity in Drosophila. *Biochemical Journal.* 2016 *(Epub ahead of print).*

CONTENTS

LIST OF ABBREVIATIONS

Aβ	Amyloid-beta
ACSF	Artificial cerebrospinal fluid
AD	Alzheimer's disease
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP	Action potential
APP	Amyloid precursor protein
ATP	Adenosine triphosphate
CA	Cornu ammonis
CCK	Cholecystokinin
CNS	Central nervous system
DG	Dentate gyrus
EEG	Electroencephelogram
E_m	Resting membrane potential
EPSC	Excitatory postsynaptic current
EPSP	Excitatory post synaptic potential
FSN	Fast-spiking interneuron
GABA	γ-aminobutyric acid
IN	Interneuron
ING	Interneuron gamma
IPSC	Inhibitory post synaptic current
IPSP	Inhibitory post synaptic potential
I-V	Current-voltage
KA	Kainic acid/kainate
LFP	Local field potential
LTP	Long term potentiation
LTD	Long term depression
MS	Medial septum
NFT	Neuro fibrillary tangles
PING	Pyramidal interneuron gamma
PN	Pyramidal neuron
PV	Parvalbumin
Ri	Input resistance
SC	Schaffer collateral
V_h	Holding membrane potential

1 LAYPERSON'S INTRODUCTION

The mammalian brain is one of the most intriguing multifunctional organs to have evolved over several million years. It enables us to move, see, perceive, understand, memorize, and decide; actions which allow for the achievement of favorable outcomes. The dynamism and multiplicity with which the brain works is intriguing. However, our understanding of brain functions and disorders are limited due to the brain's complexity. Research on levels ranging from single proteins to neurons and neuronal networks attempts to link molecular and cellular behavior found in the brain to specific behaviors.

Neurons are the primary components of the brain and number over 100 billion, producing electrical and chemical signals that are critical for information processing. Neurons are connected to one another and organized in specialized circuits and networks where information processing takes place. Information processing in these circuits gives rise to the perception of the external world, attention, and the ability to act voluntarily. It is therefore essential to understand how individual neurons function, how they are organized into signaling pathways, and how they communicate with each other through chemical and electrical signals. Signals from multiple neurons can synchronize, producing activity with rhythmic properties, called *oscillations*. An analogy for neuronal network oscillations and their role in cognition is an orchestra. In an orchestra, individual musicians play in coordination to produce music. If a few players play asynchronously, the correct music is not produced. Similarly, neurons in networks have to work in a coordinated fashion to enable various cognitive tasks. Such oscillations can be classified into unique frequency bands associated with specific behaviors. One of the well-studied oscillations is the gamma rhythm, which is important for learning, memory and cognition. An understanding of the way neurons and neuronal oscillations function can enable us to identify mechanisms underlying neurodegenerative disorders and find possible cures.

The hippocampus is an important part of the cortex, known for its role in spatial navigation and memory. It is also one of the main areas affected during neurodegenerative disorders such as Alzheimer's disease (AD), affecting memory formation, consolidation and retrieval. The hippocampus has a highly structured laminar architecture with unique neurons in each layer, coordinated neuronal networks, and intrinsic properties. Consequently, the hippocampus has the ability to generate oscillations, making it an interesting area of study concerning neuronal communication in healthy individuals and AD patients. The oscillatory properties of the hippocampus and other regions of the cortex are dependent on a network of connected neurons which mutually excite and inhibit each other. The balance of excitation and inhibition in neuronal networks is delicate and, if disturbed, may result in abnormal brain functioning.

Advancements in science have enabled the removal of specific regions of the brain, such as the hippocampus, to study them in isolation. While maintaining the regions in artificial laboratory conditions akin to physiological conditions, it is possible to study electrical signals propagated between neurons in local circuits, as well as in wider networks, using electrophysiological techniques.

Brain disorders such as AD are a growing concern for society as they place a burden on affected individuals and their relatives, and require many resources for care. Individuals with AD show poor memory formation and it has been reported that neuronal oscillations are altered in AD patients. Considering the hippocampus' role in memory formation and its relevance in AD research, this thesis examines chemically-induced hippocampal gamma oscillations in an AD experimental model.

Amyloid-beta (Aβ) is a peptide that misfolds and aggregates in AD and has been implicated in triggering and propagating the disease. This misfolded peptide has the ability to self-assemble and polymerize into larger forms - fibrils - that are variably toxic for neurons. Aβ is first released as a monomer with an unstructured and/or α-helical secondary peptide structure. Over time, these monomers can change their secondary structure to a β-sheet form, which can then aggregate to form oligomers (believed to be the most toxic), proto-fibrils, and fibrils. The latter can then form the plaques typical of AD.

We acutely exposed hippocampal brain slices to highly purified Aβ and studied its effect on gamma oscillations and neuronal characteristics. We report that Aβ, especially oligomeric and fibrillar forms cause a significant reduction in the power or strength of gamma oscillations in the CA3 region of the hippocampus. The effect is dependent on the concentration and exposure time of Aβ. Further investigation into individual neurons that contribute to gamma oscillations revealed that Aβ causes i) a change in the electrical properties of neurons and ii) an imbalance in the excitation-inhibition relationship within the network.

In an attempt to prevent these aberrations, we used molecular tools known to specifically bind to Aβ and prevent its cytotoxic effects. The molecular tools included i) designed Aβ-binding ligands, known to stabilize Aβ in its α-helical secondary structure, thereby favoring these forms over a β-sheet secondary structure; and ii) naturally occurring molecular chaperones containing the BRICHOS domain that act late in the Aβ polymerization process, delaying the formation of fibrils. When these molecular tools were applied, we observed that Aβ failed to disrupt gamma oscillations, electrical properties of pyramidal neurons (PNs) or the balance of excitation and inhibition between neurons.

This thesis offers insights into changes in cellular and network mechanisms caused by Aβ in an AD model and suggests methods to maintain normal gamma oscillations using specially designed molecular tools. Further studies of the described mechanisms in mouse models of the

disease, including behavioral assessments, are necessary for the clinical translation of the results.

2 INTRODUCTION

After 17 weeks of prenatal development, the human brain begins to show neuronal activity. The electrical signals generated represent the status of the brain and the entire body. Neurons are the source of these signals, receiving stimuli via dendrites, processing these signals internally, and transmitting them across their axons as action potentials (APs) over both short and long distances. The transmission of APs is a critical aspect of information processing in the brain. Early in the development of neurons, networks and specialized microcircuits with other neurons are readily formed. Information processing in the brain happens at every conceivable level, including the network level in the hippocampus, the focus of this thesis. In a network, individual neurons are connected to each other, forming microcircuits, influence each other's output, and as such function interdependently. These microcircuits are well connected at various levels of organization. For example, a microcircuit in the hippocampus receives input from other microcircuits nearby, projections from the septum, brainstem, and long-range projections from other cortical areas.

Information processing at the neuronal level relies heavily on synaptic currents generated at synapses in response to connected neurons' APs. The resting membrane potential (E_m) of neurons can change dynamically depending on the type of synaptic transmission they receive. These transmissions can be either excitatory or inhibitory in nature, leading to excitatory postsynaptic potentials (EPSPs) or inhibitory postsynaptic potentials (IPSPs), respectively. These primary transmembrane potentials integrate and, upon reaching a threshold, generate an active output or action potential, AP (the neuron "fires"). The synchronous activity of a group of neurons and subthreshold membrane oscillations can give rise to rhythmicity in the local field potential (LFP) (1-3). These rhythmic events or oscillations are believed to be critical for information processing in the brain (4).

Neurons have special properties, which enable them to resonate and oscillate at multiple frequencies (5). The timing of this activity is critical for information processing in neuronal networks (5). For effective cortical information processing to happen, a pattern of coherence among neuronal groups is required (6). This coherent neuronal activity is sensitive to synaptic inputs and modulates rhythmic excitability, which determines spike output (6). The rhythmic spike output provides temporal windows for neuronal communication and it is widely viewed that coherently oscillating neuronal activity is important for cortical information processing (5-7). It has been shown that systemic functions associated with neuronal network activity are critically dependent on synaptic modifications at the command of pre- and postsynaptic spiking that occurs within a critical window of tens of milliseconds (8). The rhythmic neuronal patterns or neuronal oscillations form an important interface between cellular activity and larger systemic functions such as cognition and behavior.

2.1 The hippocampus and its relevance to health and disease

The hippocampus, part of the limbic system, is a seahorse-like structure in the medial temporal lobe that is well connected to other subcortical areas and the contralateral hippocampal region. The hippocampus has a profound role in the consolidation of short- to long-term memory, cognition and spatial navigation (9-12). It is among the most well studied areas of the mammalian brain and has provided a wealth of information concerning neurons and neuronal networks in health and disease. One of the most intriguing case studies of the 20th century was the epileptic patient Henry Molasion who underwent surgery to remove his medial temporal lobes. Following the surgery, the role of the hippocampus in new, declarative memory formation became evident (13). Moreover, the Nobel Prize in Physiology or Medicine in 2014 was awarded to John O'Keefe, May-Britt Moser, and Edvard Moser for demonstrating how a group of neurons in the hippocampus, called place cells, and a group of neurons in the entorhinal cortex (EC), called grid cells, form a physiological substrate for spatial navigation. Given the role of the hippocampus in memory formation and its intrinsic ability to generate neuronal oscillations associated with various activities, it is an ideal brain region to study such rhythmic activity.

The hippocampal formation consists of the EC; the dentate gyrus (DG); *cornu ammonis* (CA) regions CA1, CA2, and CA3; and the subiculum (***figure 1***). These regions are each special and differ slightly structurally and functionally. The PNs of layer II of the EC send axonal projections to excite granule cells of the DG via the perforant pathways. The granule cells forward the excitatory drive to PNs in the CA3 region via mossy fibers. The PNs of the CA3 region, through axonal projections known was Schaffer Collaterals (SC), send an excitatory drive to the PNs of CA1, forming the trisynaptic circuit/pathway. Additionally, there is a dense associative network connecting the PNs of CA1, forming the main hippocampal output back to the EC via the subiculum. The hippocampus is abundantly rich in a variety of interneurons (INs), such as FSNs, axo-axonic INs, oriens–lacunosum molecular INs, and bistratified INs, all of which have unique parts in modulating neuronal oscillations. As discussed, INs have a profound role in oscillogenesis and their firing pattern is primarily synchronized through excitatory synaptic inputs projected from the local collaterals of PNs (14).

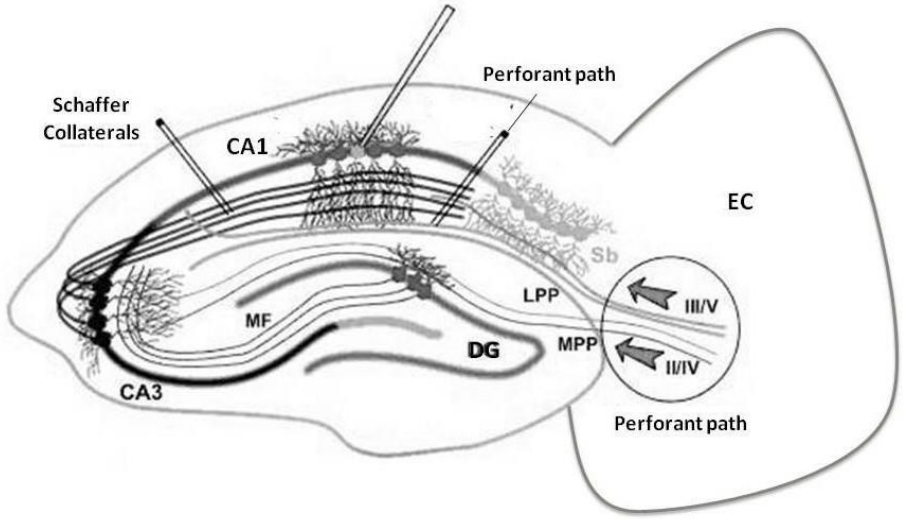

Figure 1. *A hippocampal brain slice. The highly interconnected laminar architecture of the hippocampus possesses intrinsic properties suitable for the generation of neuronal oscillations. The prominent regions are (clockwise) the cornu ammonis region 1 (CA1), entorhina cortex (EC), dentate gyrus (DG), and cornu ammonis region 3 (CA3). The supporting structures are the lateral perforant path (LPP), medial perforant path (MPP), mossy fibers (MFs), and Schaffer Collaterals (SC). Figure adapted from (15).*

The CA1–3 regions of the hippocampus have a similar architecture with the outermost region being the alveus, consisting of white myelinated fibers. Further inwards is the stratum oriens which is rich in various types of INs. Next, the stratum pyramidale consists of a group of glutamatergic principle cells (PNs) in CA1, with their axons projecting to CA2 and CA3. The stratum radiatum can be observed inside the curve of the pyramidal cell layer, and it is rich in INs as well as axonal projections from CA3. The innermost layer is the stratum lacunosum-moleculare, where the apical dendrites of PNs and axonal projections from EC terminate (16). The region CA3 is particularly interesting as it has been shown to consolidate episodic and contextual memories (17, 18), while CA1 is believed to play a role in spatial memory (19). For the purpose of this study, we focus on the region CA3 of the hippocampus where the most prominent gamma oscillation recordings are observed.

It is clear that the hippocampus is relevant to studies relating to neurodegenerative disorders. The cortex and the hippocampus in particular are the earliest brain regions affected by AD (20). Histological examinations of the hippocampus have revealed that it is vulnerable to AD pathology (21) and longitudinal MRI studies have shown that substantial loss of hippocampal volume is observed in AD subjects (22-25). Examinations of hippocampal regions CA1-CA3 for neuronal density in AD patients compared to healthy individuals of the same age have shown that CA1 and CA3 regions are particularly prone neuronal density loss (26). The hippocampus is relevant for studies relating to AD and sub-regions CA1 and CA3 are of particular interest given their role in generating gamma oscillations and implication in AD pathology**.**

2.2 Oscillations in neuronal networks

Simple, synchronous neuronal activity can be observed as a result of the excitation and inhibition of cellular membranes' electric potential in individual neurons. These are maintained through a dynamic balance of inward and outward currents (27). Larger, synchronous neuronal activities, such as neuronal oscillations, make use of local excitatory and inhibitory connections between neurons, whereby the excitatory synaptic currents increase the occurrence of APs. These are regulated by inhibitory synaptic currents, which maintain a dynamic balance between the local excitation and inhibition (1). The firing of neurons with respect to an ongoing gamma oscillation may provide a plausible mechanism for information processing in neuronal networks (28). The amplitude and frequency of oscillations are continuously modulated by stimuli received by the network and the behavioral state of the subject.

Neuronal oscillations in the mammalian brain exhibit a wide range of frequencies - 0.05 Hz – 500 Hz - associated with different states of the brain (5). They are widely observed in the central nervous system (CNS), such as in cortices, the cerebellum, olfactory bulb, thalamus, hypothalamus, hippocampus and others (27, 29-31). In particular oscillations in the hippocampus are important and have been extensively studied for their role in various aspects of cognition, memory, and navigation (32, 33). The three primary oscillations in the hippocampus are theta (4–10 Hz), gamma (30–80 Hz) and sharp wave ripples (>80 Hz) (34-36). Theta and gamma oscillations in the hippocampus have been implicated in many aspects of neuronal processing; during hippocampus-dependent behaviors (37), modulating synaptic plasticity (38, 39) and neuronal network function (5, 40, 41). Oscillations in different frequency bands can co-exist temporally and interact with each other, representing various behavioral states. For example, gamma oscillations have been shown to be nested within theta oscillations and are important for working memory, the representation of spatial sequences and long-range synchronization between brain regions (7, 42).

In the hippocampus the continuous discharge of INs has been demonstrated, whereas PN activity is suppressed by short-term synaptic inhibition (43, 44). Following this suppression, population discharge of PNs takes place again and a new oscillatory cycle is initiated. Interneurons also play a role in controlling Ca^{2+} spike generation in dendrites (14) and help in the generation of gamma oscillations in hippocampal subfields (45).

Our primary interest is to study gamma oscillations between 30–80 Hz that are associated with cognitive functions such as sensory perception, visual attention, exploring environment novelty, spatial navigation and memory (46-49). Moreover, we explore how these gamma oscillations are altered in neurodegenerative disorders such as AD. It has been reported clinically that gamma oscillations are disturbed or disrupted in several brain disorders associated with cognitive decline, such as AD (46, 47, 50), Parkinson's disease (51), and schizophrenia (52). This thesis focuses on possible mechanisms by which gamma oscillations are disturbed in an

AD model by studying individual neurons and networks that generate gamma oscillations and preventive and/or rescue measures that can be used to sustain normal gamma rhythms.

What drives gamma oscillations in neuronal networks?

It is well established that gamma oscillations are critically dependent on GABAergic inhibitory synaptic transmission (53, 54). Interneurons with their widespread axonal plexus perform the synchronous inhibition of multiple neurons, such as PNs, in their network (54). Interneurons are highly diverse and studies have shown that different subtypes of INs contribute distinctly to neuronal network dynamics (55). Interneurons can be classified into groups based on different criteria, including their expression pattern of calcium-binding proteins (56). Among them, parvalbumin-expressing (PV+) perisomatic-targeting basket cells or FSNs and cholecystokinin-expressing (CCK+) IN subtypes have been shown to be particularly relevant to neuronal oscillations (56, 57). In structures such as the neocortex and hippocampus, phasic inhibition is regulated by a heterogeneous population of fast-spiking INs (FSNs) or basket cells (1, 7). These FSNs fire in synchrony with or, in other terms, are phase-locked to gamma oscillations and are important for generating the oscillations both *in vivo* (30) and *in vitro* (58). Currently, there are two popular mechanistic explanations for the generation of gamma oscillations: The Pyramidal-Interneuron Network Gamma (PING) model and the Interneuron-Network gamma (ING) model (53) (***figure 2***). The PING model posits that excitatory PNs provide feedback excitation and INs provide a phasic drive to pace the oscillation of the network, suggesting that reciprocal connections between INs and PNs are responsible for oscillations. This is supported by *in vivo* and *in vitro* findings which show that PNs fire synchronously in the gamma frequency and their activity precedes FSN firing (4, 59, 60) and that the selective ablation of AMPA receptors on FSNs weakens gamma oscillations (61), respectively. The ING model suggests that a group of INs alone in a network is sufficient to establish and maintain gamma oscillations. The rationale behind this model is that INs, by virtue of mutual inhibition, are capable of generating oscillations. The ING model is supported by studies where tonic excitation of INs induced gamma oscillations which continued to maintain oscillations even after the excitatory synaptic transmission was blocked (58, 62). The requirements for ING model generation of gamma oscillation are mutual synaptic connections among INs in the network, tonic firing, and GABA receptor-mediated conductance.

Although ING models can be replicated *in vitro* and *in vivo*, a neuronal network model for the generation of gamma oscillations that relies on the PING model is a more plausible mechanism as excitatory neurons are an integral part of cortical networks. Undoubtedly, inhibitory INs are essential for the generation of gamma oscillations as they pace these oscillations in the neuronal networks and different types are able to modulate the firing of APs at preferred phases of the oscillations (2, 58). As the targets of FSNs are the somata of PNs, they are able to exert a strong inhibitory effect on the initial axon segment. Additionally, INs, through gap-junctions, modulate highly coordinated firing in other neurons (63, 64) and express the rapidly

deactivating potassium channel Kv3.1, which has an important role in regulating every cycle of gamma oscillations (57, 65). As PV+ FSNs have been shown to play an important role in the generation of gamma oscillations (66-68) it is necessary to study the role of these inhibitory INs and their influence on PNs to understand typical cortical information processing, as well as changes in cortical information processing during neurodegeneration.

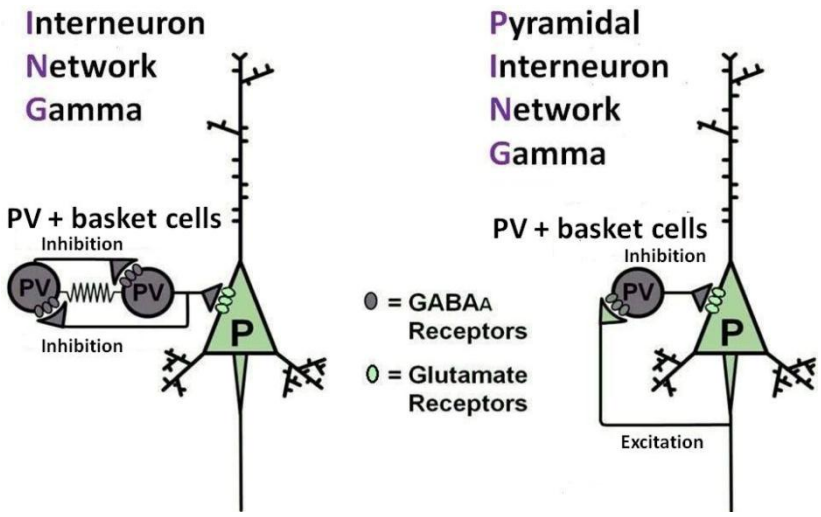

Figure 2. *The two working models of gamma oscillations. I) Interneuron-Network gamma (ING) model, according to which a network of GABAergic parvalbumin-expressing (PV+) fast spiking interneurons (FSNs) or basket cells mutually inhibit each other to produce gamma oscillations, although a minimal involvement of glutamatergic pyramidal neurons can be observed. II) Pyramidal-Interneuron Network Gamma (PING) model, according to which both glutamatergic pyramidal neurons and GABAergic PV+ FSNs or basket cells, by virtue of mutual excitation and inhibition, produce gamma oscillations. Figure adapted from (69).*

In vitro hippocampal gamma oscillations

While studying *in vivo* gamma oscillations is more relevant for clinical applications, *in vitro* models of oscillations allow for easier control of variables that modulate oscillations and provide better access to individual cells. One of the key aspects of reliably verifying *in vitro* models of gamma oscillation is to replicate the observed features and mechanistic insights *in vivo. In vitro* models of gamma oscillation have shown that PNs have a low firing probability during ongoing gamma oscillations (58, 60, 70). These findings have been verified for *in vivo* models (59, 71). Similarly, *in vitro* studies have shown that perisomatic targeting PV+ FSNs or basket cells are essential for generating gamma oscillations (58, 72). These reports were also verified *in vivo* using optogenetic activation of PV+ INs (73). *In vitro* models arguably provide a prototype for *in vivo* physiological manifestations, however they have some limitations, as discussed in the conclusion of this thesis. Gamma oscillations are likely the most studied *in vitro* neuronal oscillation in the hippocampus and can be evoked through bath applications of carbachol (60) or kainic acid/kainate (KA) (74, 75) (***figure 3***). Carbachol generates gamma oscillations by activating the cholinergic systems, while KA triggers a generalized

depolarization which generates a sufficient excitatory drive to enable neuronal firing and synchronization in the gamma frequency band (74, 75).

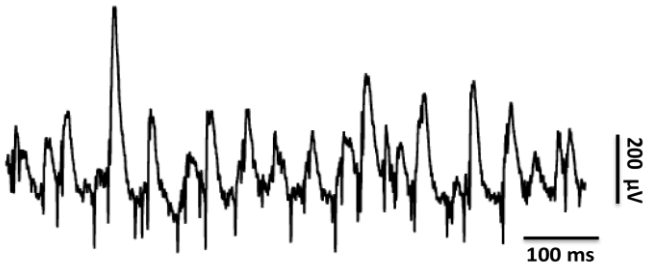

Figure 3. *In vitro kainic acid/kainate (KA) induced gamma oscillations based on the chemical activation of KA receptors in a mouse hippocampal slice preparation. Figure adapted from (**paper III**).*

Many studies have investigated the mechanisms behind the generation of hippocampal gamma oscillations, looking at the cell types involved, their role in regulating the rhythm, and various factors influencing their functioning. In CA1, gamma oscillations have been observed originating from fast gamma oscillations propagated from the EC via the tempero-ammonic pathway, and also from slow gamma oscillations originating from CA3 via the SC (42, 60, 76, 77). The EC has been shown to be vital for spatial navigation (78) and SC inputs from CA3 to CA1 have been shown to be important for memory consolidation (79). Some regions of the hippocampus have been shown to be intrinsically oscillogenic, such as the CA3 region (44, 59, 80-82). Furthermore, a recent study has shown that CA1 is also capable of generating intrinsic gamma oscillation in response to a theta input in addition to inheriting activity from the projections received from CA3 and EC (83). Therefore, gamma oscillations in the hippocampus are thought to be an emergent property of the local circuitry within the hippocampus (60, 62, 75, 83, 84). Studying the properties of oscillations within sub-regions of the hippocampus can provide great insight into normal brain functioning and brain disorders.

2.3 Alzheimer's disease and network dysfunction

Alzheimer’s disease is undoubtedly a global health priority given its burden on health systems and economies. Not only is AD the most common form of dementia in the elderly, it is also estimated to affect individuals who actively participate in the workforce (85). Alzheimer’s disease is progressive in nature and affected individuals may live for a long time with clinical symptoms. Signs and symptoms related to AD can be classified as pre-dementia, early, moderate, and advanced phases. Symptoms associated with AD are noticeably poor cognitive performance, learning and spatial navigation impairment and, in some cases, seizures and epileptiform activity (86, 87). Episodes of low cognitive function and heightened erratic neuronal activity are seen across various stages of AD and it is important to understand the

underlying mechanisms that cause them. Pathological changes in the brain may begin up to 20 years before clinical AD symptoms manifest (88), by which time the cellular pathology is potentially too severe for any significant treatment. Current medications for AD can only alleviate symptoms and do not slow or stop the progression of AD. Diseases such as type-2 diabetes and cardiovascular diseases are reported to occur concomitantly with AD and may increase the AD pathogenic process at a molecular level (89-91).

Although AD was described more than a century ago, the molecular mechanisms underlying it remain largely unclear. Early pathological changes in AD are observed in the transentorhinal and EC, then spreading to the hippocampus, adjacent allocortical areas, and finally the neocortex (92). The severity and symptoms of the disease are associated with the aforementioned anatomical targets (92). Other important features of AD include cortical and subcortical atrophies that cause ventricular enlargement and the hypofunction of cholinergic neurons of the basal forebrain with the associated cognitive deficits (93, 94).

Cortical electroencephalogram (EEG) recordings of AD patients have been reported to vary over a wide range of frequencies. A shift from fast to slow waves in spontaneous EEG recordings is typical, such as an increase in delta- and theta-frequency oscillations and a decrease in alpha- and beta-frequency (95-97). Numerous other studies suggest that there are alterations in gamma oscillations recorded in AD patients who have been clinically diagnosed as lacking long-term memory formation (98-101). Magnetic field tomography recordings showing reduced thalamocortical coherence of gamma oscillations in AD patients (46) and magnetoencephalography (MEG) recordings showing a loss in gamma-band synchronization in AD patients (50) have been reported. Electroencephalogram studies in rodent models of AD have suggested that degeneration of cholinergic neurons in the nucleus basalis (102, 103) contributes to the cognitive symptoms and the slowing of the EEG.

Aberrations observed in AD, such as disturbed gamma oscillations, may be attributed to the two abnormal protein structures occurring in AD: i) intracellular neurofibrillary tangles (NFTs) that consist of hyperphosphorylated tau proteins; and ii) extracellular deposits of Aβ in the form of toxic soluble oligomers, fibrils, and plaques that may interfere with mechanisms driving neuronal oscillations. These two misfolded proteins are well known to cause the most significant loss of neurons and synapses (104, 105). The majority of AD research has focused on these two hallmarks and provided generous insight into how they influence neuronal function in AD. In particular, soluble oligomeric forms of Aβ have gained importance in AD research owing to their heightened cytotoxicity and effects on synaptic functions (106-110). Several studies have directly linked acute exposure to Aβ to loss of functions such as neuronal oscillations in various brain regions (108, 109, 111-114). An inquiry into this misfolded protein's potential role in network dysfunctions will greatly aid the design of therapeutic interventions aimed at preventing and/or rescuing neuronal dysfunction. The studies included in

this thesis aim to understand aberrations in neuronal network dynamics associated with acute Aβ exposure and investigate methods for their prevention.

2.4 Amyloid-β peptide

Histopathological examinations show that the brains of individuals with AD are characterized by a large number of extracellular deposits of Aβ, mostly in the cortical neuropil and blood vessel walls (115). The observation of extracellular Aβ is consistent with the fact that Aβ is an extracellular proteolytic cleavage product of the transmembrane amyloid precursor protein (APP), prevalent in the synapses of neurons (116). The sequential proteolytic processing of APP by β- and γ-secretase produces Aβ peptides of 36–43 amino acids in length and releases them into the extracellular space (117-119). Extracellular deposition of Aβ is well established and *in vitro* studies using extracellular applications of biochemically identified and isolated oligomeric forms of Aβ in physiological concentrations (120, 121) have shown induced alterations in synaptic plasticity and synapse formation (106, 122, 123). Apart from extracellular Aβ, careful examination of the lumens of multivesicular bodies and other types of intracellular vesicles has revealed the presence of intraneuronal Aβ (124-126). The existence of intracellular Aβ may occur through the secretory and endosomal pathways associated with the proteolytic cleaving of APP which provides an entry point to the cellular interior and the endosomal/lysosomal pathway, reported to cause lysosomal leakage (127). The existence of intraneuronal Aβ is associated with the disruption of cytosol and the interruption of mitochondrial function (128) Moreover, it is a pathological driver for AD progression (129). Therefore, it is important to discern whether observed synaptic dysfunctions and neurotoxicity principally arise from intracellular Aβ and/or extracellular Aβ.

Of the two main isoforms of Aβ associated with AD, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$, $A\beta_{1\text{-}42}$ in particular seems to be most active in AD, as it shows the strongest propensity for spontaneous aggregation and is more fibrillogenic (130). Both isoforms have been identified in senile plaques in the brains of patients with AD (131). Studies have shown that Aβ aggregates have deleterious effects on brain function and are associated with early cognitive decline in AD (132). One of the properties of Aβ peptides is the propensity to self-assemble or polymerize, forming dimers, trimers, and higher-order oligomers, which are variably toxic in each state and cause neuronal death (133, 134) (***figure 4***). There are also recent reports that the surface of the fibrillar form of Aβ provides a catalytic substrate for monomeric forms of Aβ to form deleterious oligomers (135). This is known as the secondary nucleation theory and is discussed in greater detail in the results. The aggregated, water-soluble oligomeric forms of $A\beta_{1\text{-}42}$ have gained importance in research for their cytotoxic effects (126, 136), direct link to the affected synaptic function (132, 137), and other pathologic relevance for AD. The mechanism of Aβ-induced toxicity has been studied extensively and several perspectives have been put forth, such as the activation of inflammatory effects through interaction with the membrane (138),

induction of oxidative stress (139) by forming metal-Aβ complexes (140, 141), disruption of receptors' function by binding to them (142), forming of membrane pores that lead to alterations in ionic homeostasis (143-145), and modification of DNA structures through attachment (146).

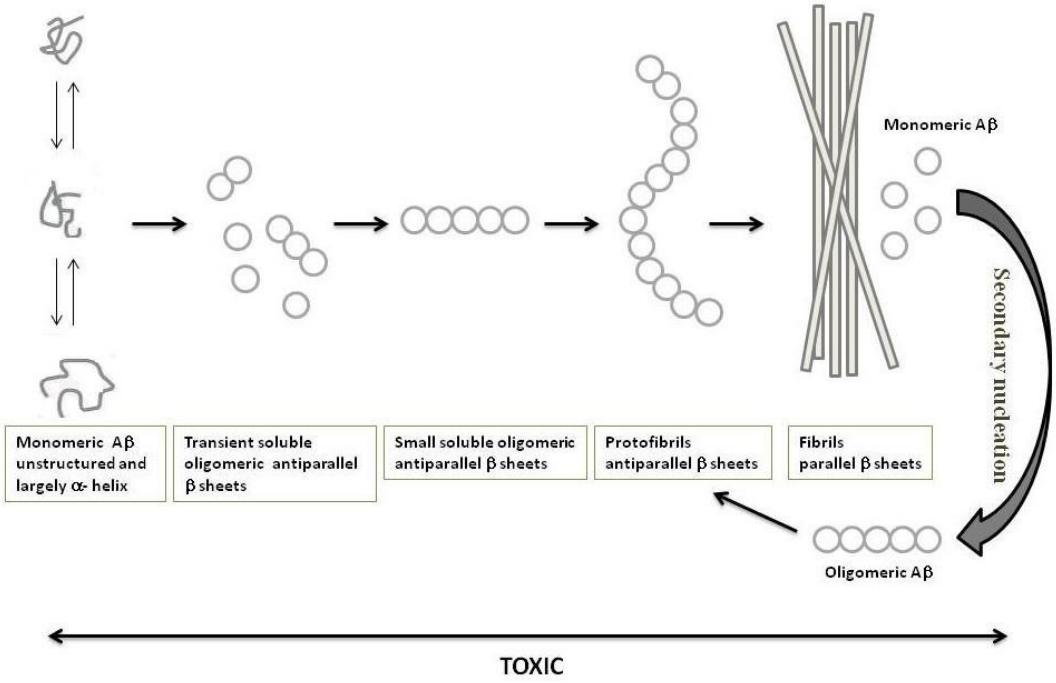

Figure 4. Amyloid-β *polymerization steps: from monomeric form to transient soluble oligomeric, small oligomeric, protofibrillar, and fibrillar forms. The secondary nucleation theory suggests that an interaction of monomeric Aβ on the fibrillar surfaces produces more oligomeric forms, making Aβ polymerization a cyclic event. Figure adapted from (147).*

Aβ in neuronal networks

The cellular pathology that underlies network dysfunction during AD, especially neuronal oscillations, is not well understood and is an exciting area for exploration. An important question for AD research is how Aβ assemblies affect different classes of neurons and synapses (***figure 5***). Determining the answer will substantially help in predicting the effects of Aβ on neuronal circuit function (148, 149) in relevant brain areas such as the hippocampus.

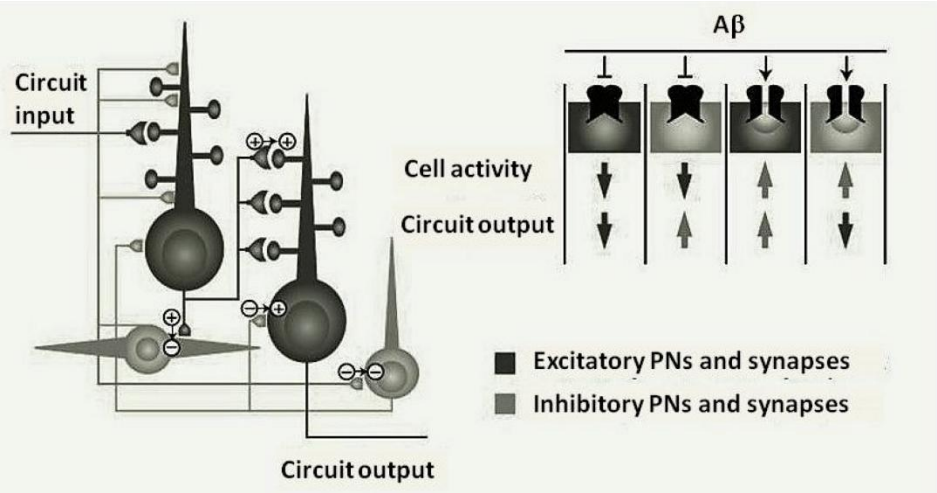

Figure 5. Amyloid-β *is reported to cause abnormal patterns of neuronal activity in microcircuits and neuronal networks. Amyloid-β is known to differentially affect the functioning of pyramidal neurons (PNs) and inhibitory cells (INs) and their synapses, causing an imbalance in network activity. Figure adapted from (150).*

There have been reports that Aβ forms ion-permeable pores in the neuronal membrane, thereby causing alterations in cellular homeostasis, leading to cell death (151-153). This may be concurrent with reported mechanisms wherein Aβ is suggested to cause aberrant synaptic changes and degeneration.

Examinations of the brains of patients with AD have consistently revealed abnormalities in synapses: quantitative ultrastructural and immunohistochemical examinations from post mortem studies (154-158) have reported the loss of a significant number of synaptic boutons in the neocortex and hippocampus of AD patients compared to age-matched healthy individuals. Several studies have reported that synthetic Aβ oligomers can impair the molecular processes involved in long-term potentiation (LTP), leading to its significant disruption in hippocampal slices (159-162). It has also been reported that oligomeric Aβ, extracted from the cerebral cortex of brains from individuals with AD, depresses LTP and enhances long-term depression (LTD) in rat organotypic hippocampal cultures (107).

In other studies, Aβ has been shown to change calcium homeostasis, leading to heightened neuronal excitability. This has been confirmed by many studies in which Aβ peptides have been shown to increase the cellular influx of calcium, causing excitation (163-166) and resulting in pre- and postsynaptic modifications (167). *In vivo* studies in human amyloid precursor protein (hAPP)-expressing mice have reported anatomical and biochemical alterations of the DG that bear resemblance to rodent models of epilepsy or neuronal overexcitation (168, 169). Similarly, video EEG recordings in freely behaving hAPP-expressing mice have shown cortical and hippocampal epileptiform activity (169-172). Studies have also shown that Aβ-induced neuronal hyperexcitability is responsible for the epileptic phenotype in AD mice models (86, 170). A clinical study has reported that hippocampal

hyperactivity is associated with increased chances of cognitive dysfunctions in patients with mild cognitive impairment and targeting hippocampal hyperactivity has therapeutic potential (173).

Moreover, in electrophysiological studies focusing on two glutamatergic synapses in the hippocampus, the CA3-SC-CA1 synapse and EC-perforant path-DG granule cell synapse, Aβ has been reported to suppress transmission strength and short/long-term plasticity (149). It is interesting to note that many studies have also reported that Aβ causes a marked reduction in neuronal network rhythmic activities. An example is the focal injection of Aβ into the hippocampus of rats, resulting in a decrease of theta oscillations along with a decline in cognitive performance (174). Similarly, acute exposure to Aβ reduced rhythmic activity to theta frequencies for GABAergic neurons (175) and glutamatergic neurons (153), wherein Aβ is suggested to have caused a functional loss of network activity rather than a structural deficit following the exposure. Oligomeric forms of Aβ have also been demonstrated to slow sensory-induced theta rhythms (176), where the intraventricular injection of Aβ was directly associated with a slowing of oscillatory activity induced by sensory stimulation. This is in agreement with alterations of evoked theta oscillations in patients (177). Gamma oscillations have been shown to form long-term memory in rodents (178). Furthermore, in slices from human amyloid precursor protein (APP) Swedish (SWE) mutation (TAS10) mice, hippocampal gamma oscillations have been shown to be suppressed (179). The administration of oligomeric Aβ has also been shown to impair KA-induced beta oscillations (180). Consistent with these findings, *in vitro* hippocampal oscillations have been shown to decrease in aging mice (181). This leaves us with the question whether Aβ affects neuronal function in different ways, either by acting as an excitotoxin that aberrantly heightens excitability and/or acting as a suppressor of neuronal and network function.

An important aspect to examine is whether Aβ causes dysfunction in INs and, if so, what the mechanisms are. One hypothesis is that Aβ impairs the synaptic function of INs more than PNs at the network level, whereby disinhibition and overexcitation occurs, rather than suppression (150). A similar effect can be expected if Aβ suppresses excitatory-inhibitory synapses more than excitatory-excitatory synapses (150). It has been noted that Aβ causes the impairment of PV+ IN activity by decreasing sodium channel activity, leading to epileptic seizures (182). Interestingly, the same study also showed that Aβ-induced epileptiform activity in hAPP mice are correlated with reduced gamma activity (182). Another study with Sprawley-Dawley male rats demonstrated that KA-induced epileptiform spikes desynchronized and reduced fast gamma activity through an “anti-binding” mechanism (183). The heightened, aberrant excitability of individual neurons likely results in the disruption of firing rates and synaptic properties that underlie the strength of gamma oscillations.

Clinically documented comorbid cases of AD and epilepsy have been reported (184-186). There is a strong relation between the occurrence of epilepsy and early-onset AD, particularly,

autosomal, dominant pedigrees suggest the potential role of high Aβ levels and cytopathology (150). Aβ has also been shown to increase met-enkaphalin levels in the hippocampus and EC, which could suppress inhibitory IN activity by activating μ-opoid receptors (187). It can thus be hypothesized that in AD and AD animal models compensatory inhibitory mechanisms that can delay and diminish excitotoxic processes may become dysfunctional, eventually leading to the loss of synapses and neuronal death. However, compelling and direct evidence is lacking to prove whether Aβ overly and/or moderately affects the neuronal activity of INs. A strong reason exists to examine in detail the underlying molecular mechanisms of Aβ-induced aberrant neuronal functions and postsynaptic currents (EPSCs and IPSCs). This thesis attempts to fulfill this task.

2.5 Therapeutic interventions aimed at Aβ-mediated neurotoxicity in neuronal networks

Since AD is a slowly progressing and dynamic disorder, different mechanisms may guide different stages of the disease. For this reason, therapeutic interventions should be aimed at mechanisms at various stages of the disorder. One current therapeutic measure aims to maintain the level of Aβ production by inhibiting the enzymatic steps (β- and γ-secretase activity) required to cleave APP to Aβ. Another approach is to enhance clearance of Aβ (188). However these measures failed to produce successful clinical trials results even though they showed successful results in pre-clinical investigations (189-191). It is, however, uncertain whether these strategies in fact lower levels of Aβ to the extent that would protect the neuronal network. Detrimental effects of Aβ on adult granule cells have been shown to be prevented by inhibiting $GABA_A$ receptors during early stages of adult neurogenesis or by enhancing glutamatergic signaling during later stages of maturation (192). A potential method to counter Aβ-induced aberrant network excitability and hypersynchronization is yet to be discovered and the effects of antiepileptic drugs on AD patients are not yet understood (86, 150, 182). The other pathogenic hallmark of AD, endogenous tau, may also influence the detrimental effects of Aβ through interactions or synergistic actions, as they are both compartmentalized in neurons (193, 194). As Aβ can form toxic oligomers and higher fibrillar forms, Aβ polymerization inhibitors have been suggested to protect neuronal network functions. Ligands designed to bind and stabilize the 13–26 region of Aβ in an α-helical conformation have been shown to reduce Aβ toxicity in cell cultures and hippocampal slice preparations (114). It has also been shown that KCNQ channel dysfunction is associated with decreased medial-septum theta oscillations (153). D. Melanogaster expressing human $Aβ_{1-42}$ in the CNS, when exposed to these ligands exhibited a prolonged life span, increased locomotor activity, and reduced neurodegeneration, suggesting that stabilizing of the Aβ α-helix may counteract polymerization to toxic assemblies by shifting equilibrium between the α-helical and β-sheet secondary peptide conformation towards the α-helical structure (114). For several decades, certain molecular chaperones have been known to play an important role in aiding *in vivo* folding of newly synthesized proteins in

their native states, trafficking to specific cellular locations, and the efficient assembly of molecular subunits to multimeric structures (195-197). Amyloid fibrils are found in a number of diseases and it has been shown that the C-terminal domain of the lung surfactant protein C (SP-C), ProSPC (CTC), has anti-amyloid effects (198, 199). It was suggested that CTC functioned as a chaperone that acted preferentially against amyloid fibril formation (200), thus influencing later stages of the Aβ aggregation sequence, limiting fibril formation. Similarly, Bri2 BRICHOS, another molecular chaperone that is ubiquitous in the CNS, has been shown to prevent Aβ-induced cellular and network changes and may have potential as a future therapeutic intervention tool in AD (201, 202).

3 AIMS

The overall goal of the studies included in this thesis is to understand the cellular-, synaptic-, and network-level changes caused by Aβ in mouse hippocampus *in vitro,* identify the cytotoxic step/steps in the Aβ aggregation process that causes these changes, and attempt to prevent these changes using Aβ-binding ligands and chaperone peptides that delay Aβ aggregation into toxic forms such as oligomers and fibrils.

I) Examination of acute effects of different Aβ assemblies on major hippocampal cell classes and network function: How are glutamatergic and GABAergic neurons affected by different Aβ species and what are the consequences for the functional dynamics in the hippocampal network?

II) Identification of the exact cytotoxic step in the Aβ peptide (mis)folding sequence and investigation of the folding and aggregation mechanisms responsible.

III) Assessment of strategies aimed at preventing and/or rescuing Aβ-induced cellular and network changes. Use of Aβ-binding ligands and molecular chaperones known to prevent/delay Aβ fibrillization.

4 MATERIALS AND METHODS

This section provides a detailed account of the rationale behind the materials and methods used in the studies contained in this thesis.

4.1 Ethical Considerations

All experiments were performed in accordance with ethical permits issued to Dr. André Fisahn by the regional ethical board in Sweden (*Norra Stockholms Djurförsöksetiska Nämnd*). Experiments were carefully considered to maximize results with a minimum number of animals used. This was done by, for instance, sharing brain tissue from one animal amongst several researchers in the lab. Though we minimized animal use, the question as to whether or not animals need to be used for scientific experiments should be addressed. As our experiments focused on neuronal network functions which cannot be elicited in cell lines or non-animal based systems, we were limited to using animals with complex and well-established neuronal networks that can approximate human brain functions. Neuronal oscillations, as discussed, are an integral part of normal brain functioning and are also affected by neurodegenerative disorders. They can be pharmacologically elicited *in vitro* in brain areas such as the hippocampus, septum, and other cortical regions. This *in vitro* approach offers several advantages for addressing our aims such as: 1) reduced long-term animal suffering when compared to *in vivo* recordings; 2) relatively fewer experimental animals are used, as the tissue samples obtained can be used for multiple experiments per brain and shared between several investigators; 3) good access to neuronal networks and individual neurons for LFP and intracellular recordings; 4) direct pharmacological manipulations and stable long-term recordings of gamma oscillations; 5) the possibility to study neuronal networks from an area of interest in isolation.

4.2 Animals

Our model for AD studies is the acute exposure of Aβ to both quiescent and activated hippocampal slices of C57BL/6 mice of either sex (postnatal 14–30 days) in all experiments. The use of experimental mice gives us several advantages as the majority of AD research has centered on mice models of various types, including transgenic types, which show a great level of AD-like phenotypes (169, 172, 182, 193, 203). Genetically-modified mice that are green fluorescent protein (GFP)-labeled for PV+ (204, 205) also simplify finding and recording the activity of PV+ FSNs in neuronal network dynamics (182, 183). For the purpose of the enclosed projects, wild-type and PV+ mice were used. Acute exposure of Aβ to hippocampal

slices is a common way of studying electrophysiological properties that are associated with cells and neuronal networks (179). $A\beta_{1-42}$ was the only form of Aβ used in all our experiments and is referred to as simply 'Aβ' in this thesis.

4.3 Tissue preparation and recordings

All animals were anesthetized using isoflurane (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane) before decapitation. The brain was dissected and kept in a cold artificial cerebrospinal fluid (ACSF) that was modified for dissection (please refer to the enclosed papers for the various ACSF compositions). Horizontal sections with a thickness of 350 µm were cut from the ventral hippocampi of both hemispheres using a Leica VT1200S vibratome (Leica Microsystems) and were immediately transferred to a humidified, oxygenated interface chamber and allowed to recover for a minimum of 1 hour. For experiments exclusively aimed at examining gamma oscillations, slices were transferred to submerged chambers which either had Aβ (Aβ slices) of a desired concentration and exposure time or not (control slices). Recording of gamma oscillations were performed in the interface-styled recording chamber (***figure 6***). All experiments were performed with parallel controls from the same animal brain hippocampal preparation.

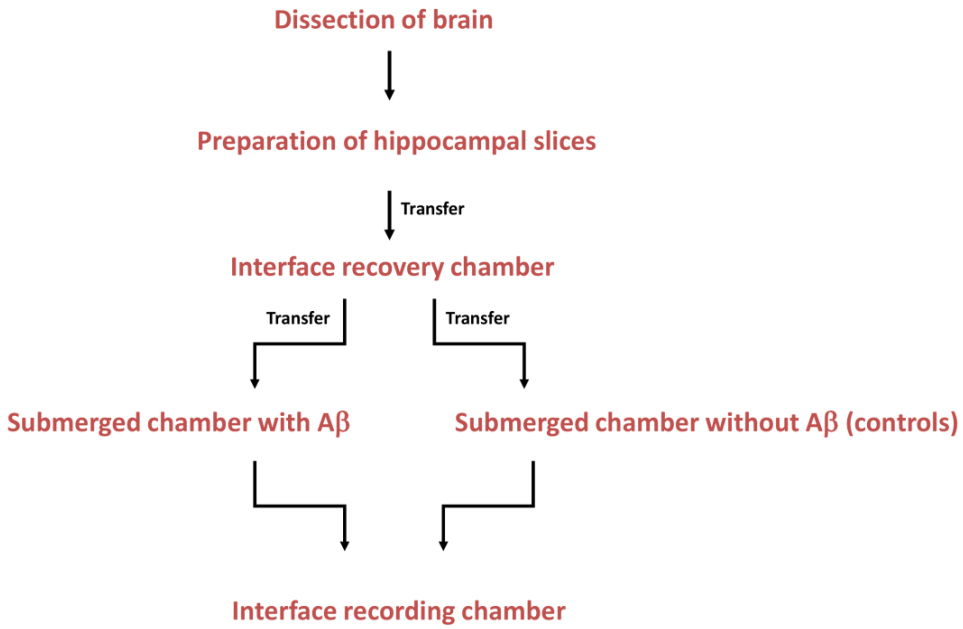

Figure 6. *A schematic representation of experimental procedure to compare the unpaired data sets of Aβ-exposed and control gamma oscillation recordings in an interface chamber.*

Local field potential recordings

Local field potential recordings were carried in an interface recording chamber in which slices were superfused at 34°C with ACSF modified for the optimum recording condition (please refer papers for composition) at a rate of 4–7 ml/min and continuously bubbled with 95% oxygen and 5% carbon dioxide to ensure adequate oxygenation for slice health. Gamma oscillations were elicited by superfusing KA (100 nM) for at least 20 min to allow slices to stabilize; recordings were carried out in hippocampal area CA3 stratum pyramidale with ACSF-filled borosilicate glass microelectrodes pulled to a resistance of 3–5 Ω. It should be noted that *in vivo* CA3 gamma oscillations have a peak frequency of ~30–40 Hz, however, *in vitro*, the peak frequency of gamma oscillations is usually somewhat lower owing to its dependence on the recording temperature (206). Electrophysiological recordings at room temperature for a long period of time cause early cell death, therefore the recording temperature is often kept around 34°C. Gamma oscillation experiments examining the effects of Aβ-binding ligands and molecular chaperones on various forms of Aβ were designed to pre-incubate Aβ and the molecular tool in the submerged chamber, similar to the method shown in ***figure 6***, before transfer to the interface recording chamber. The idea being that these molecular tools act on Aβ at different temporal points in its folding and polymerization process (***figure 7***).

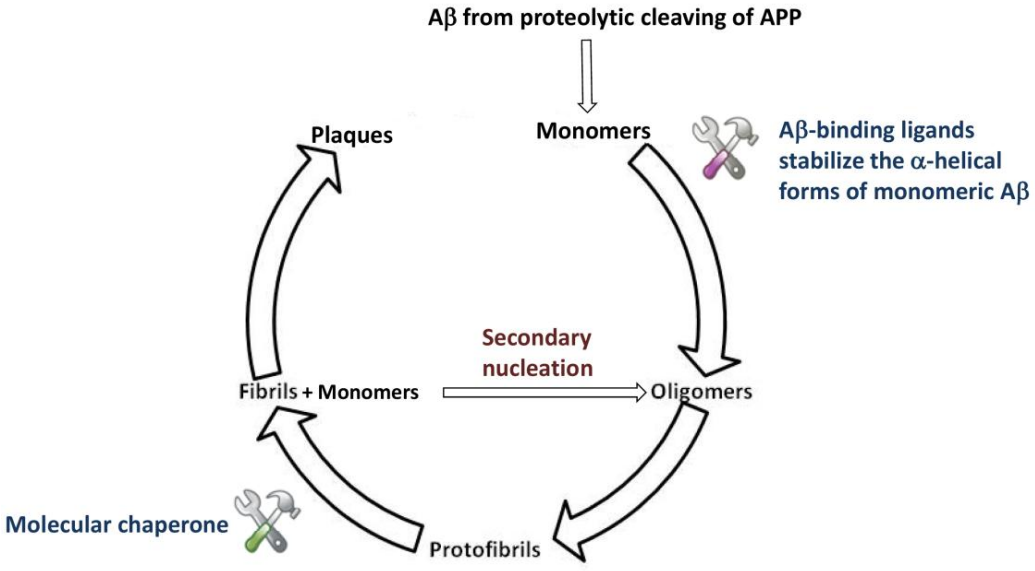

Figure 7. *Prevention strategies of Aβ polymerization. Use of Aβ-binding ligands that stabilize α-helical conformation over β-sheet conformation and molecular chaperones that delay the process of fibrillization.*

Single-cell experiments

Experiments examining single-cell events and concomitant extracellular recordings were carried out in a submerged recording chamber. A K-gluconate based intracellular solution (refer to papers for composition) was used for the single-cell whole-cell patch clamp recordings. ACSF was used as an intra-pipette solution for extracellular LFP and cell-attached recordings. PN and FSN AP firing were recorded using a non-invasive, cell-attached recording mode, with cell-attached configuration obtained after establishing a giga-ohm seal between the recording pipette and cell membrane. APs were detected as capacitance transients in voltage-clamp mode. For experiments examining synaptic currents, whole-cell patch clamp with a K^+ channel-blocking, Cs^+-based, 10 mM Cl^- intrapipette solution was used (please refer to papers for composition). Excitatory postsynaptic currents were recorded at a holding membrane potential (V_h) of -70 mV and IPSCs were recorded at a V_h of 0 mV. For experiments looking at the synaptic properties of FSNs, a gramicidin-based perforated patch recording was used. The main difference between a perforated patch and whole-cell patch clamp is that a perforated patch uses an antibiotic agent, such as gramicidin, which forms small pores in the cellular membrane. The gramicidin patch clamp method has several advantages over the whole-cell method, such as allowing for the equilibration of only monovalent ions between the patch pipette and the cytosol while maintaining endogenous levels of divalent ions, such as Ca^{2+} and signaling molecules (207, 208), making this method mostly non-invasive. The gramicidin-based perforated-patch pipette solution consisted of KCl, NaCl, $MgCl_2$, and KOH (140 mM, 10 mM, 1 mM, and 10 mM, respectively), to which 50 mg/ml gramicidin was added.

4.4 Recording protocol and data analysis

Local field potential recordings in the interface chamber were carried out with a 4-channel amplifier M102 (Electronics laboratory, Faculty of Mathematics and Natural Sciences, University of Cologne, Cologne, Germany) and conditioned using a HumBug 50 Hz noise eliminator. Submerged LFP and patch clamp recordings were carried out using a Multiclamp 700B amplifier where data was sampled at a rate of 10 kHz. A Digidata 1322A digitizer and Clampex 9.6 software (Molecular Devices) were used. The protocols used for spontaneous and activated LFP, PSC, and AP recordings were in gap free current clamp mode. Patch clamp recordings were performed on PNs identified by their firing patterns in response to a series of current injection steps (200 ms); FSNs were identified by GFP fluorescence in slices from PV-GFP mice (204, 205) and confirmed by their firing patterns. Fast-spiking INs were segregated from non-FSNs counterparts by their distinct firing profile and action potential shape.

All data were analyzed using Axograph X (Kagi, Berkely, CA, USA), Kaleidegraph (Synergy software), and IGOR Pro (Wavemetrics). Gamma oscillations/LFP recordings were estimated

as power spectral density plots (from 60 second-long recordings) calculated as averaged Fourier-segments of 8192 points using Axograph X in all the ***papers (I, II, III*** and ***IV***). The power of oscillations was calculated by integrating the power spectral density between 20 and 80 Hz using Kaleidegraph and data are reported as means ± standard error of mean (SEM) in the texts and as median and upper/lower quartile in the figure box plots in ***papers I, II and III***. The power spectral density between 20 and 80 Hz in ***paper IV*** is represented as bar graphs. For unpaired (non wash-in) experiments, Mann-Whitney *U* tests (two-tailed) were used; and for paired (wash-in) experiments of gamma oscillations, Student's *t* tests were used. All levels of significance were reported as * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

For the total quantification of single-cell experiment recordings, total values were averaged over a 5 min period post 15 min of Aβ application compared with the average of a 5 min initial control period in ***paper I***. All data are presented normalized to the average of a 5 min initial control period. Postsynaptic currents (charge transfer, event amplitude, event frequency) were analyzed using custom macros in IGOR Pro and the normalized results represented average values taken over 1 min periods. Paired pulse ratio was defined as the first EPSP slope divided by the second EPSP slope. Student's *t* test was used as the statistical tool for paired data of patch-clamp recordings where *$p<0.05$, **$p<0.02$, and ***$p< 0.01$.

5 RESULTS AND DISCUSSION

5.1 Paper I

Paper I investigates the mechanisms underlying the degradation of gamma oscillations caused by acute application of Aβ and tests potential methods to prevent this degradation.

Study of Aβ effects on gamma oscillations

We initially established the power of a stable, control gamma oscillation in the CA3 region of the hippocampus by washing in 100 nM KA for over 20 min. The power of these oscillations served as the control and was compared with the power of gamma oscillations from slices pre-incubated with high-purity recombinant $A\beta_{1-42}$ (containing a mix of conformation states of oligomeric, pre-fibrillar, and fibrillar Aβ) of varying concentrations (2 nM, 50 nM, 500 nM, 1 μM) for varying lengths of time (15 min, 30 min, 60 min, 120 min and 180 min).

When incubated for 180 min with a physiological concentration of Aβ (50 nM), the power of gamma oscillations was significantly reduced to 20% of the control. Further increases in the concentration of Aβ resulted in proportionally further reductions in the power of gamma oscillations. After trying several concentrations and incubation times of Aβ, we found that incubation with 50 nM Aβ for 15 min significantly reduced the power of gamma oscillations to 55% of the control. We believe a paradigm of "50 nM Aβ for 15 min" is well suited to study cellular and network mechanisms underlying acute Aβ-induced degradation of gamma oscillations. In summary, our findings suggest that the Aβ-induced reduction of gamma oscillation power is incubation time- and concentration-dependent. Similar findings have been reported by our lab before, wherein 1 μM $A\beta_{1-42}$-induced gamma oscillations degradation was observed in the hippocampus (114) and $A\beta_{25-35}$ and $A\beta_{1-40}$-induced the desynchronization of septal theta oscillations (153).

Severity of Aβ effects increases with fibrillization

We then aimed to examine which aggregation state of Aβ causes the most severe effects on a cellular and network level in the hippocampus. Soluble oligomeric forms of Aβ have gained importance in the recent past compared to fully fibrillized forms. We studied the effects caused by purified monomeric Aβ, by a mix of Aβ conformations, and by a fully fibrillized preparation of Aβ. We saw that preparations containing only fully fibrillized Aβ degrade gamma oscillations the most, followed by preparations containing a mix of Aβ conformations. Preparations containing purified Aβ monomeric resulted in the smallest, yet still significant, decrease in the gamma oscillation power. This suggests that as the rate of fibrillization increases, gamma oscillations reduce more strongly. One possible explanation would be that Aβ fibrils act as catalysts for the increased production of toxic oligomeric Aβ species according to recently described secondary nucleation kinetics (135) (***figure 4)***. It has been shown that

when a purely monomeric preparation of Aβ is seeded with an otherwise innocuous amount of fibrillar Aβ, the cytotoxicity of the Aβ preparation is greatly increased because the formation of toxic oligomeric species increases on the catalytic surface of the seed fibrils (135).

Study of Aβ effects at a single-cell level

We tested the hypothesis that a possible desynchronization of AP firing in PNs could be the mechanism underlying Aβ-induced degradation of gamma oscillations. Pyramidal neurons in area CA3 were recorded in cell-attached mode with concomitant LFP recording. We observed that during the wash-in of Aβ there was a steady degradation in the power of gamma oscillations for about 15 min, after which it plateaued. Results revealed that along with this degradation in the power of gamma oscillations there was an increase in the AP firing frequency in PNs. This was paralleled by an increase in AP-gamma phase gaussian half-width, suggesting that Aβ desynchronizes PN firing in relation to the gamma rhythm. It has been shown that desynchronization of AP firing underlies the degradation of neuronal oscillations (153, 209, 210), suggesting it as the most likely mechanism behind the observed Aβ effect on gamma rhythms. It was reported that $A\beta_{25-35}$ induces an increase in input resistance (R_i) associated with membrane depolarization in CA3 PNs, suggesting a possible mechanism behind the increased PN firing we observed. Other studies have reported that Aβ-induced neuronal hyperexcitability underlying AD epileptic phenotype (170) was a result of Aβ-induced depolarization of both the neuronal resting membrane potential (E_m) and GABA-mediated current reversal potential (E_{GABA}), leading to aberrant network activity (86). It has also been reported that acute application of Aβ to hippocampal slices causes a hyperpolarization of AP threshold and a reduction in after-hyperpolarization, leading to increased excitability (211).

Aβ alters balance between excitation and inhibition

Gamma oscillations depend on the tight regulation between inhibition and excitation in neuronal networks. Any change in this equilibrium will alter the characteristics of gamma oscillations. For this reason, we studied the effect of Aβ (1 μM) on EPSCs and IPSCs in CA3 PNs. Our study suggests that both in the quiescent and the activated network (100 nM KA), Aβ acts to increase excitation (EPSCs) while suppressing inhibition (IPSC). The excitation change is based on an Aβ-induced increase of EPSC frequency and amplitude; and the inhibition change is based on an Aβ-induced decrease of IPSC frequency and amplitude. This suggests that Aβ alters the balance of excitation and inhibition independent of the state of the network (activated or quiescent).

A recent *in vitro* study looking at the impact of acute $A\beta_{1-42}$ on inhibitory synaptic transmission in rat somatosensory cortex PNs confirms our results, reporting that Aβ depresses the monosynaptic $GABA_A$ receptor-mediated IPSCs by 60% and indicates the synaptic site of Aβ action (212). It was also shown that Aβ-induced IPSC decline could be prevented with a

$GABA_A$ receptor internalization blocker, suggesting a possible mechanism of Aβ, namely selectively weakening synaptic inhibition via the down-regulation of $GABA_A$ receptors (212). Aβ was also shown to selectively impair IN excitability as well as inhibitory inputs to PN in the prefrontal cortex (213).

Our electrophysiological recordings revealed that I-V (current to voltage) relationships were not associated with a change in cells' R_i or an increase in leak currents (data not presented), leading us to believe there was little or no effect caused by Aβ forming cell membrane pores in our system, as reported in other studies (151, 214, 215). We believe that a more plausible mechanism of Aβ-induced aberrant cellular functioning is the increase in PN firing rate and an increased post-synaptic excitability underlying the observed degradation of gamma oscillations

How exactly the acute application of Aβ induces these electrophysiological changes in PNs is a question which deserves further attention. Two possibilities include; i) Aβ acting on the neuronal synapses, leading to the observed electrophysiological changes and/or ii) Aβ acting onto neurons through certain receptors leading to the observed electrophysiological changes. Acute application of Aβ is reported to cause a deficit in synaptic transmission and plasticity in hippocampal slices (106, 107). Aβ is also reported to affect neurons, by acting on receptors such as nictonic acetylcholine receptors (216), glycine receptors (217), insulin receptors (218), and even internalized into neurons via a lipoprotein pathway (219). More recently, a study has suggested a possible involvement of the caveolin-endocytosis pathway mediating the internalization of Aβ into neurons (220). Once internalized, Aβ could act as a pathological driver for AD, as there are accounts of intracellular Aβ as a major component of Aβ-induced alterations of glutamatergic synaptic transmission and plasticity (221). Both synapse concentrating Aβ and intracellular Aβ are known to cause cell death and ultimately lead to neurodegeneration (104, 108, 222). However, new studies should be designed to examine whether the Aβ-induced electrophysiological changes observed can be attributed to the possibilities discussed above.

Another aspect to examine is whether the Aβ-induced synaptic effects observed may be a consequence of cellular energy metabolism dysfunctions caused by Aβ (223, 224). Impaired glucose utilization, the lowering of cellular adenosine triphosphate (ATP) levels, and brain insulin resistance are also markers of AD (86). Aβ could be acting in a way that results in the loss of cellular ATP content and impairs glucose use. Additionally, Aβ has been shown to impair insulin signaling and neuronal insulin receptors (225, 226). One study has reported that intrahippocampal administration of Aβ resulted in an impaired hippocampal metabolism and insulin signaling that led to a rapid impairment of spatial and working memory (227). It is also possible that the involvement of glucose impairment associated with dysfunctional insulin signaling may be an underlying factor for the observed synaptic effects caused by Aβ. Researchers have found that by supplementing glucose with other energy substrates in an AD mouse model, they were able to prevent Aβ toxicity, hyperexcitability and LTP deficits (86).

Future studies should be designed in a holistic manner to better understand synaptic dysfunctions and the associated dynamics of energy metabolism both *in vitro* and *in vivo* and related to behavioral aspects. This may help in better strategizing therapeutic interventions for AD.

Prevention of gamma oscillation degradation and preservation of excitatory/inhibitory balance using compounds that interfere with Aβ folding and aggregation

As noted in the introduction, Aβ has the propensity to aggregate into larger, fibrillar forms. The secondary peptide conformation of monomeric Aβ changes from its α-helical form to a β-sheet form which can then polymerize into soluble oligomeric forms, then into protofibrils, and finally into the fibrils that accumulate into AD-typical plaques. There are several conformatory states of Aβ associated with its aggregation states. This knowledge is vital to determine the most cytotoxic conformations of Aβ. With recent insight into secondary nucleation mechanisms in the production of toxic oligomeric Aβ, therapeutic strategies aimed at stabilizing the secondary peptide structure of Aβ in its α-helical form and preventing later fibrillization are necessary.

We used two classes of compounds that can interfere with the Aβ folding and aggregation sequence at different temporal points. Dec-DETA is a designed ligand that shifts the equilibrium of co-existing α-helical and the β-sheet secondary peptide conformations of Aβ towards α-helical forms (16, 34). It interferes at an early stage of Aβ folding and aggregation and limits the formation of β-sheets, which are a prerequisite for aggregation. The second group of compounds that we used was a recombinant human BRICHOS domain from a lung surfactant C precursor protein (ProSPC) which delays the aggregation of Aβ later than Dec-DETA by preventing β-sheet-type peptides from aggregating.

When Dec-DETA (250 nM) was incubated with monomeric and mixed Aβ (50 nM), we found that gamma oscillations were preserved at levels similar to the control. However, when Dec-DETA was incubated with fibrillar Aβ, there was only a partial preservation of gamma oscillations. This may be due to the ligand's inability to act fully on the predominantly fibrillized forms in their parallel β-sheet structure. On the other hand side the fact that designed ligands such as Dec-DETA have an effect on fully fibrillized Aβ at all evidences fibril turn over (i.e. breakdown into smaller soluble forms which the ligand can then interact with). When slices were incubated with the second compound, ProSPC BRICHOS (1 µM), and monomeric, mixed, and fibrillar forms of Aβ, similar results were obtained to those with Dec-DETA.

Our next aim was to test whether the preventative actions of these ligands on Aβ-induced gamma degradation were mirrored in cellular characteristics. As discussed concerning altering the excitatory/inhibitory balance with Aβ, we investigated the effect of Aβ (1 µM) in the presence of either compound on IPSCs and EPSCs in CA3 PNs. To do so, EPSCs (V_h = -70 mV) and IPSCs (V_h = 0 mV) were recorded from PNs during KA-induced gamma oscillations.

Analysis showed that both EPSC and IPSC charge transfer remained unaltered. Hence, we conclude that the protective effect of Dec-DETA and ProSPC BRICHOS on gamma oscillation is based on their ability to prevent Aβ toxicity on a cellular and synaptic level.

In summary, gamma oscillations are reduced through the interference by Aβ at its physiological concentrations. The basic mode of Aβ action in the CA3 PNs is to desynchronize the firing of APs and shift the balance between excitation and inhibition. We demonstrate that this kind of Aβ-induced cellular and network changes can be prevented using designed ligands and molecular chaperones that can bind Aβ early in its aggregation sequence, preventing/limiting it from forming toxic forms and interfering with gamma oscillations. The next study was designed to better understand the role of Aβ-binding ligands and molecular chaperones.

5.2 Paper II

Paper II examines whether a novel Aβ targeting, second-generation ligand AEDabDab is more efficient at preventing Aβ-induced degradation of gamma oscillations. In contrast to previously developed first-generation ligands such as Dec-DETA or Pep1b, AEDabDab contains the triamino acid, N_{γ}-(2-aminoethyl)-2, 4-diaminobutanoic (AEDab) acid. The ligand carries an extra positive charge in the side chain. It has been previously shown that peptide-like compounds containing an arginine stabilize the central helix of Aβ in the α-helical conformation, thereby delaying its aggregation into neurotoxic species (114). Moreover, it has been shown that the oral administration of these inhibitors in D. Melanogaster expressing human $A\beta_{1\text{-}42}$ resulted in prolonged lifespans, reduced locomotor dysfunction, and reduced neuronal damage (114). AEDabDab is designed to replace the arginine residue with the AEDab triamino acid for a better interaction with Aβ. This is supported by molecular dynamic simulations of the stability of the Aβ central helix with the new ligand. We hypothesize that the second generation ligands to be more efficient at preventing Aβ-induced degradation of gamma oscillations.

Gamma oscillations were induced by superfusing horizontal hippocampal slices of C57BL/6 mice with 100 nM KA. LFP recordings in the CA3 region were performed both in naïve slices (control) and in slices pre-incubated with Aβ. The studies showed that when Aβ exposed slices were co-treated with Pep1b and AEDabDab separately and compared with control and Aβ slices, AEDabDab greatly prevented Aβ-induced reduction of gamma oscillations and showed a noticeably better ablative effect compared to the first-generation ligand Pep1b.

5.3 Paper III

With ***paper III*** we wanted to expand on previous finding that the molecular chaperone ProSPC BRICHOS prevents or delays the catalytical cycle that produces neurotoxic Aβ oligomers and prevents Aβ-induced degradation of gamma oscillations. BRICHOS is a protein domain with roughly 100 amino acids and was identified in the proteins such as **BRI**, related to familial British dementia, **chondromodulin** associated with chondrosarcoma, and lung surfactant protein C (**ProSPC**) (199, 228). It has been shown previously shown that BRICHOS domain efficiently prevents amyloid fibril formation (198, 199). In this study, our collaborators show that the mode of action of ProSPC BRICHOS is the specific inhibition of the catalytical cycle and limiting of Aβ toxicity by binding to the surfaces of Aβ fibrils and redirecting the aggregation reaction to a pathway that produces minimal formation of toxic oligomeric intermediates. This is verified by monitoring live brain tissue by means of cytotoxicity and electrophysiological findings that we report as a measure for neurotoxicity.

Kinetics of Aβ aggregation

The first part of the study monitored the kinetics of aggregation of Aβ using analytical approaches where the rates of primary nucleation, fibril elongation, and secondary nucleation were predicted. The findings were in agreement with previous reports (135, 229). When Aβ was tested in the presence of an excess of the BRICHOS domain, the data revealed that there was a complete inhibition of secondary nucleation. The findings also reveal that reduction in the rate of primary nucleation, fibril elongation, or secondary nucleation did not significantly affect the total quantity of mature fibrils formed during the polymerization process of Aβ, but had a significant effect in limiting the generation of oligomers during reaction. This analysis confirms that the BRICHOS domain effectively prevents the formation of low molecular weight oligomeric species, thereby dramatically reducing the associated cytotoxicity.

ProSPC BRICHOS site of action

The second part of the study focused on determining which molecular species of Aβ the BRICHOS domain chaperone acted on. To identify this, fibrillar $A\beta_{1\text{-}42}$ produced in the presence and absence of ProSPC BRICHOS was added to solutions of monomeric Aβ. It was observed that pristine fibrils that were not exposed to ProSPC BRICHOS readily enhanced secondary nucleation and rate of polymerization. In contrast, fibrils that were exposed to ProSPC BRICHOS displayed a significant amount of the chaperone bound to the fibril surface during the Aβ polymerization process. This was confirmed with transmission electron microscopy (TEM) visualization and surface plasmon resonance (SPR) studies, where the nature of interactions clearly indicated specific binding of the chaperone to the fibril surface.

By combining kinetic studies and the binding affinities of Aβ and the chaperone, it was clear that the chaperone binds to Aβ fibrils with high affinity and specifically inhibits secondary

nucleation events that likely occur on the fibrillar surface. The study then examined the degree of inhibition of the production of low molecular weight oligomeric species by studying their concentrations during $A\beta_{1-42}$ aggregation in the presence and absence of the chaperone. The different molecular species of Aβ such as monomers, small oligomers (about 14–65 kDa), and large oligomers (about 66–90 kDa) were eluted at different times and their ratio was determined using size exclusion chromatography (SEC). It was observed that the concentrations of oligomeric species were substantially reduced in the presence of the chaperone.

Electrophysiological findings

Our aim was to establish whether a reduction in the population of oligomeric species through the inhibition of the secondary nucleation pathway by ProSPC BRICHOS is sufficient to prevent Aβ-induced degradation of gamma oscillations. Similar to our previous experimental designs, we compared the degree of Aβ-induced degradation of hippocampal gamma oscillations in the presence and absence of ProSPC BRICHOS (1 μM) to control conditions. Our findings revealed that slices, when incubated with soluble monomeric $A\beta_{1-42}$, showed a significant but limited reduction in gamma oscillations as opposed to slices exposed to soluble monomers seeded with a small amount of pre-formed fibrils which showed a much larger reduction of gamma oscillations. This indicates that the toxic species were overwhelmingly produced by the fibril-catalyzed reaction. When the same conditions were used in the presence of ProSPC BRICHOS, we found that there was no degradation of gamma oscillations and the power of oscillations was comparable to control levels. The findings confirm our hypothesis that ProSPC BRICHOS may act to inhibit secondary nucleation of Aβ and that this may be a way of containing its neurotoxicity. This, along with other experiments looking at cell viability and cytoxicity measurements, suggests that the chaperone was able to inhibit toxicity associated with the oligomerization of Aβ.

5.4 Paper IV

In the previous paper, we report that the ProSPC BRICHOS domain may be a novel candidate in the search for AD treatments. However, the expression of ProSPC BRICHOS is restricted to alveolar type II cells, making it unable to interfere with deposition in the CNS (230). In the CNS, two other BRICHOS-containing proteins are expressed: Bri2 and Bri3, also referred to as integral transmembrane protein 2B (ITM2B and ITM2C). It has been shown that Bri2 and Bri3 interact with APP and play a role in its processing in the formation of Aβ peptides (201, 202, 231); and it has been suggested that the fusion protein Bri2 BRICHOS may be a more effective delayer of Aβ fibril formation than ProSPC BRICHOS based on their molar mass (199). In this paper, we test whether the Bri2 BRICHOS domain is a more potent inhibitor of Aβ-induced

cytotoxicity and has a broader mode of action than ProSPC BRICHOS in an *in vitro* electrophysiological study and an *in vivo* model.

Electrophysiological findings

Much like previous experimental designs, we wanted to test whether Bri2 BRICHOS displayed properties similar to ProSPC BRICHOS in its ability to prevent Aβ-induced degradation of gamma oscillations or, even, if it acted more efficiently. Bri2 BRICHOS and ProSPC BRICHOS (100 nM and 1 µM for both) were separately co-treated with slices exposed to $A\beta_{1-42}$ (50 nM and 1 µM) for 15 min and 180 min. The power of gamma oscillations in these different conditions was then compared with conditions in the absence of the chaperones (Aβ only) and control gamma oscillations. We observed that Bri2 BRICHOS more efficiently prevented Aβ-induced degradation of gamma oscillations compared to ProSPC BRICHOS under the same experimental conditions (for details, please refer to ***paper IV***).

It is notable that both chaperones at a 100 nM concentration failed to prevent Aβ–induced degradation of gamma oscillations when 50 nM Aβ was co-treated with the chaperones for 180 min and when 1 µM Aβ was co-treated with the chaperones for 15 min and 180 min. This indicates that these molecular chaperones may only efficiently act on Aβ at concentrations in a nanomolar range. This could be due to their instability, shedding off the surface of fibrils they interact with and becoming ineffective. Improved chaperone design may be required so that chaperones can act on the Aβ fibril surface for longer periods of time.

Supporting our electrophysiological findings, our collaborators showed that *in vivo* models of D. Melanogaster with transgenic expression of Bri2 BRICHOS in the CNS or the eye efficiently inhibited Aβ toxicity. Following exposure of Bri2 BRICHOS, Aβ diffusely distributed in mushroom bodies, an area involved in learning and memory, ProSPC BRICHOS forms punctuated deposits outside the mushroom bodies. Bri2 BRICHOS also inhibited several steps in Aβ fibrillization pathways and functioned as a more efficient molecular chaperone than ProSPC BRICHOS. This suggests that Bri2 BRICHOS may be a more physiologically relevant chaperone for AD in the CNS and may be a better candidate for AD treatment.

5.5 Aβ-induced depolarization of CA3 Fast-Spiking interneurons (unpublished data)

Fast-spiking interneurons fire synchronously and modulate PN activity through repetitive phasic inhibition (60, 62). The phasic inhibitions that FSNs exert on PNs determine the PN firing rate and phase during an ongoing gamma oscillation (210, 232, 233). It has been shown that the loss of FSN function is directly related to hyperexcitability and reduced gamma oscillations (182). Based on what we have shown in ***paper I,*** where acute application of Aβ resulted in reduced inhibition of CA3 PNs, it was crucial that we also examined any potential

Aβ-induced effect on FSNs, a major pre-synaptic source of PNs' IPSCs. Hence, we investigated the effect of Aβ in FSNs in quiescent and network-activated slices.

GFP-labeled PV+ FSNs were identified and firing rates of APs were recorded in quiescent slices as well as after KA-induced gamma oscillation. Our findings revealed that shortly after bath application of Aβ, the firing rates of FSNs increased dramatically during gamma oscillations, mirroring the Aβ-induced firing rate increase of PNs that we have shown in ***paper I***. However, there was no significant change in the E_m of FSNs in quiescent states after a wash-in of Aβ recorded under perforated-patch clamp conditions, indicating a lack of hyperexcitability we observed in the same cell class during gamma oscillations. Therefore, we repeated the experiments, using synaptic stimulation to activate FSNs to their native firing rate (10 Hz) during ongoing gamma oscillations. It was interesting to observe that after the activation of FSNs, Aβ caused a significant depolarization in line with what was previously reported for DG granule cells and cortical pyramidal cells (86, 170). This was revealed by a shift in the I-V relationship of FSNs. The same stimulation in a control condition (absent Aβ) failed to induce any change in E_m.

To summarize our previous and current findings of Aβ effects in PNs and FSNs, we show that Aβ causes i) an increase in PN and FSN firing rate, ii) an increase of EPSCs and a reduction of IPSCs in PNs, and iii) Aβ's effect on FSNs is activity-dependent.

A recent study has reported that Aβ significantly reduced IPSCs in CA3 PNs by downregulation of $GABA_A$ receptors (212). Hence, the increase in firing rates and reduced IPSCs in PNs could be explained by the Aβ-induced downregulation of $GABA_A$ receptors and Aβ-induced post-synaptic depolarization. The observed Aβ-induced increase in FSN firing rate during a network-activated state and the lack of changes of FSN excitability in quiescent state could be perhaps explained by the activity-dependent depolarization of E_m together with an increased excitatory drive on FSNs exerted by neighboring PNs in an activated-state.

We need to further investigate the mechanisms behind Aβ-induced depolarization of FSN E_m, and how it may be modulated by activity-dependent Aβ toxicity. Studies have shown that FSNs have high energy demands, larger metabolic inefficiencies, and increased vulnerability to metabolic deficits (234, 235). It has been shown that Aβ-induced depolarization of E_m is through energy deprivation by glycolysis breakdown and such deprivation could be prevented by supplementing with alternative energy substrates (86). Cortical FSNs are also relatively abundant in Na^+/K^+ ATPase (236) and K_{ATP} channels (237, 238) compared to PNs. It will be interesting to study whether Aβ could be modulating FSN activity in an activity-dependent manner by causing metabolic aberrations and/or dysfunctional activity of specific channels in FSN and if these aberrations could be corrected by specific therapeutic interventions.

6 CONCLUSION

6.1 Experimental constraints and future directions

Electrophysiological recordings in living brain slices are probably the most widely used method to study the physiology of cortical structures today. Some common types of these *in vitro* studies are concurrent intracellular and extracellular recordings, two-photon calcium imaging, and voltage-sensitive dye imaging of layer-specific physiology of neurons and neuronal networks. All of these techniques have provided insight into the behaviors of cortical structures.

Some of the shortcomings of *in vitro* brain slice preparations are that the slicing action itself damages tissue, the tissues are removed from the life-supporting supply of blood, the long-range axonal inputs to cortical columns in the slices are severed (239), the life span of brain slices is limited, and sensitivity to oxygenation and flow rate of ACSF is evident (223, 240, 241). The effects of decapitation-induced ischemia on the cell viability may be a problem. The artificial bathing medium (ACSF) that we use imperfectly matches compositions of the physiological CSF. The three most concerning factors in our experimental approach are; i) hippocampal slice preparations lacked vital projections from its nearby structures such as the medial septum (MS) which can modulate or contribute to hippocampal rhythmogenesis (242-244), ii) the artificial activation of the neuronal network in isolated hippocampi to induce gamma oscillations do not recreate oscillatory activity that occurs in physiological conditions where external inputs are intact, and iii) the fact that we use young animals owing to easier single-cell recordings to study an age-related disease. Even though these factors are limiting factors, *in vitro* preparations are rapid, use relatively inexpensive animals, allow for direct visualization of slice structures and accurate placement of recording and stimulating electrodes in the desired substructures, and allow for easy pharmacological manipulations.

If substantial and consistent observations are made from *in vitro* studies, certainly the next step is to design *in vivo* studies in intact animals where it is possible to correlate neuronal activity with sensory perception and behavior. Before detailing *in vivo* experimental designs, a number of aspects need to be examined. Given the knowledge we have about Aβ's mode of action on the CA3 PNs and FSNs, it is essential to study the effect of Aβ on other subtypes of INs and PNs in other sub-regions of the hippocampus, such as CA1, the DG, and also structures that project into the hippocampus, such as the MS. These structures should be examined concerning the way they modulate gamma oscillations.

It is important to examine the behavior of various cell types under the influence of Aβ and Aβ-binding molecular tools in a combined septo-hippocampal preparation in both wild-type and mice overexpressing APP with Swedish and/or Arctic mutations (245-247). The projections from the MS to the hippocampus provide vital clues about synaptic transmission and neuronal oscillations in hippocampus (153).

With the latest advancements in optogenetic methods that express channelrhodopsin to form light-gated ion channels in MS, and with the use of laser excitation that influences hippocampal cells, acute application of Aβ and Aβ-induced effects in single cells and neuronal networks in the hippocampus would be an interesting aspect for continued research. If similar findings to those of the current thesis are made, it would also be interesting to use molecular tools that prevent Aβ aggregation and toxicity.

Different forms of Aβ are found in humans (248) and their roles in neuron and neuronal network function are not well understood. An experimental design that recreates the exact mix of these peptides as found in human AD patients with knowledge of the cytotoxic step in Aβ misfolding and the possible prevention/rescue with designed ligands and chaperones that prevent Aβ toxicity would strengthen our findings.

In recent times, simultaneous neuronal recordings across cortical layers are achievable *in vivo* and the ability to replicate our *in vitro* findings *in vivo* will be a great accomplishment. Techniques such as two-photon *in vivo* imaging and calcium imaging provide the opportunity to monitor activities in awake animals and identify neurons for relatively long period of time (249-251). Using such techniques experimental designs that investigate long-term Aβ effects in individual neurons and neuronal networks and cognitive aspects of rodent models of AD will essential for future research. A recent advancement has been deep layer, two-photon imaging using implanted microprisms into the cortex which enable the study of specific neuronal and neuronal network activity (239, 252, 253). This strategy combines optical access of *ex vivo* brain slice preparations and *in vivo* behavioral context and long-range connectivity that is relatively intact. Similarly, there are several *in vivo* studies of neuronal oscillations (254-257) that have given insight into physiologically relevant functions. A combination of these experimental approaches will likely enable us to better understand the acute and long-term effects of Aβ at neuronal and neuronal network levels and also test whether Aβ-binding tools are effective in reducing Aβ-related toxicity.

Another important aspect to study is the metabolomics of AD models, especially in relation to oscillatory functions and examining the dynamics of neurons and neuronal networks. Impaired glucose metabolism, insulin resistance, and neuroinflammation are all associated with AD (258, 259). A recent study using triple-tracer small-animal positron emission tomography (PET) has reported that age-dependent microglial activation is associated with increases in amyloid loads as a sign of Aβ-related neuroinflammation (260). These factors could influence the dynamics of neuronal oscillations; however, this is yet to be experimentally shown. Experiments designed to enhance the availability of energy substrates, ensure well-functioning insulin signalling, and reduce neuroinflammation in AD models could also potentially preserve neuronal oscillations. A holistic approach that ensures reduced Aβ-related toxicity, a well-functioning glucose metabolism, insulin signaling, and reduced neuroinflammation is necessary in our attempt to rescue neuronal oscillations and sustain normal systemic brain functions.

6.2 Concluding remarks

To conclude, the studies contained in this thesis likely serve as a prototype for cognition-relevant gamma oscillations during AD progression in humans. We show that gamma oscillations are reduced when the neuronal network is exposed to pathological Aβ peptides and that Aβ acts by disturbing several intrinsic cellular mechanisms that help generate these oscillations. Two key observed features of the effect are: i) Aβ causes an increase in AP firing properties of both PNs and FSNs and ii) Aβ causes a shift in the equilibrium between excitatory and inhibitory synaptic transmissions in PNs. We also show that such cellular- and network-level aberrations can be prevented using special molecular tools such as Aβ-binding ligands and molecular BRICHOS chaperones, known to prevent Aβ aggregation and toxicity.

We believe our study provides new insights into how gamma oscillations may be affected in AD and suggest ways to preserve them. However, our findings require further confirmation through additional *in vitro* and *in vivo* experimental designs. Additionally, other important aspects, such as the examination of the function of various sub-types of neurons, synapses, and neuronal metabolomics, are necessary to preserve neuronal oscillations and better arrive at a translational research approach in attempts to cure AD.

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