Let's not forget: Peptidases in Alzheimer's disease

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THE STORM BEFORE THE QUIET: NEURONAL HYPERACTIVITY AND Aβ IN THE PRESYMPTOMATIC STAGES OF ALZHEIMER’S DISEASE

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ABSTRACT

Neuronal activity directly promotes the production and secretion of Aβ. Interestingly, neuronal hyperactivity can be observed in presymptomatic stages of both sporadic and familial Alzheimer's disease (AD) and in several AD mouse models. In this review, we will highlight recent evidence for neuronal hyperactivity before or during the onset of cognitive defects in mild cognitive impairment. Furthermore, we review specific molecular mechanisms through which neuronal hyperactivity affects Aβ production and degradation. With these data, we will provide more insight into the two-faced nature of neuronal hyperactivity: does enhanced neuronal activity during the presymptomatic stages of AD provide protection against the earliest disease processes, or is it a pathogenic contributor to AD?

INTRODUCTION

Sporadic Alzheimer's disease (AD) is the most common form of dementia. Although major risk factors for AD have been identified - most notably aging and the ApoE ε4 allele - , it remains unknown why people develop AD, and there is no cure for AD. Neuropathologically, AD is characterized by extracellular plaques containing aggregated forms of the Aβ peptide, and neurofibrillary tangles consisting of hyperphosphorylated tau. Additionally, the loss of functional synapses correlates with cognitive decline (Arendt, 2009). The progression of AD can be divided into 7 so-called Braak stages (Braak and Braak, 1991). In Braak stages 0-II, subjects are cognitively unaffected. In these stages, AD neuropathology is practically absent in brain areas important for memory and cognition: the prefrontal cortex is free from tangles but may contain some (diffuse) plaques, whereas a few tangles may be observed in the hippocampus. In stages III and IV (mild cognitive impairment), plaque density dramatically increases in the prefrontal cortex and significant tangle pathology becomes apparent in the hippocampus. In fully demented patients (Braak stages V and VI dementia), plaques and tangles can be observed throughout most brain areas.

The discovery that familial, early onset AD (fAD) correlates with several mutations in the genes encoding for presenilin 1 (PSEN1), presenilin 2 (PSEN2), or the amyloid precursor protein (APP) has focused much of the AD research field on the Aβ peptide - a peptide that is formed after sequential β- and γ-secretase-mediated cleavage of APP - as cause for AD. Indeed, these mutations cause an increase in the brain levels of Aβ and shift the ratio between Aβ₄₀ and Aβ₄₂ - the two dominant Aβ species - towards the more aggregate-prone Aβ₄₂ species (Shen and Kelleher, 2007). Interestingly, subsequent research into the properties of Aβ has revealed that excessive levels of Aβ inhibit activity-dependent synaptic plasticity, the basis for learning and memory (Koffie et al., 2011;
LaFerla et al., 2007). For example, infusion of Aβ in rats transiently impairs cognitive function (Cleary et al., 2005), and acute neuronal overproduction of Aβ blocks synaptic plasticity (Kessels et al., 2013; Wei et al., 2010). These unique synaptotoxic properties, together with its accumulation in both familial and sporadic AD, established Aβ as a key player in AD.

In contrast, the normal physiological role of Aβ is poorly understood. Aβ is continuously produced throughout life in the healthy brain, and endogenous Aβ is required for synaptic plasticity and memory (Puzzo et al., 2011). The effects of Aβ on synaptic function are dose-dependent: picomolar concentrations of Aβ enhance learning and memory, while nanomolar concentrations block learning and memory (Puzzo and Arancio, 2012). Interestingly, neuronal activity directly promotes the production and secretion of Aβ (Cirrito et al., 2008, 2005), suggesting that neuronal activity and plasticity and Aβ together form a negative feedback loop. Thus, learning and memory require a precisely tuned balance between synaptic activity and plasticity on one hand, and Aβ levels on the other hand (Fig. 1). Disturbances in the balance between Aβ and neuronal activity may initiate a pathogenic cascade of events that eventually results in cognitive decline and dementia.

In this review, we will discuss how increased neuronal activity during the initial, presymptomatic stages of AD affects the levels and synaptotoxic properties of Aβ. We will highlight recent evidence that demonstrates that neuronal hyperactivity is observed during or even before mild cognitive impairment (MCI). Furthermore, we review specific molecular mechanisms through which neuronal hyperactivity affects Aβ production and degradation. With these data, we will provide more insight into the two-faced nature of neuronal hyperactivity: does enhanced neuronal activity during the presymptomatic stages of AD provide protection against the earliest disease processes, or is it a pathogenic contributor to AD?

It should be noted that in this review, we specifically consider the situation where neuronal hyperactivity already exists. As schematically depicted in Fig. 1, this does not imply that neuronal hyperactivity – by increasing Aβ production and secretion - is the cause of increased Aβ, as it can also be that neuronal hyperactivity is compensatory response to the first synaptotoxic effects of increased Aβ levels. It remains at this moment unclear whether neuronal hyperactivity precedes the buildup of synaptotoxic Aβ levels, or vice-versa. We therefore focus our discussion on the modulatory role of neuronal hyperactivity on Aβ levels.
1. NEURONAL HYPERACTIVITY DURING THE EARLIEST STAGES OF AD AND IN AD MOUSE MODELS

During the last decade, an increasing amount of evidence has been gathered that shows that the typical decrease in neuronal activity associated with advanced Alzheimer’s disease (AD) is preceded by a period of neuronal hyperactivity in brain areas relevant to AD. Interestingly, this can already be observed in the earliest stages of AD, prior to plaque formation.

1.1 Neuronal hyperactivity in the presymptomatic Alzheimer’s disease brain

1.1.1 fMRI evidence for neuronal hyperactivity in sporadic mild cognitive impairment

The majority of experimental evidence for neuronal hyperactivity in the brain of patients with MCI has been gathered by functional magnetic resonance imaging (fMRI) measurements that assess brain activity levels during memory tasks. Using this technique, a substantial number of studies have reported that MCI is associated with hyperactivation of multiple brain areas compared to age-matched controls (e.g. Celone et al., 2006; Dickerson et al., 2005, 2004; Hämäläinen et al., 2007; Kim et al., 2012; Kircher et al., 2007), for a review see Pihlajamäki et al., 2009). Although fMRI measurements do not reach neuronal resolution, increased synaptic function indeed forms the basis for elevated levels of regional metabolic, blood flow or BOLD-signal changes in the healthy brain (Logothetis et al., 2001). In the MCI and/or AD brain however, pathological changes such as reactive gliosis and vascular changes may provide additional sources to the observed fMRI signal. Thus, when we refer to “neuronal hyperactivity” based on fMRI evidence, we cannot exclude that metabolic changes from non-neuronal sources also contribute to the observed changes. fMRI studies of Bassett et al. demonstrated that asymptomatic offspring (50-75 years of age) of autopsy-confirmed late-onset, sporadic AD cases showed more intense and extensive activation in the frontal and temporal lobes including the hippocampus during memory encoding, compared to age-matched controls. These findings were corroborated by data showing that MCI was associated with hyperactivity of the hippocampus (Putcha et al., 2011). Importantly, the increase in hippocampal activity correlated with atrophy in cortical regions vulnerable to AD, such as the medial temporal lobe, suggesting a relationship between hyperactivity and atrophy in these functionally connected brain areas. Also in amnestic MCI (aMCI), a task dependent on verbal working memory induced a higher activation in the parietal and frontal lobes during the maintenance phase in the aMCI group compared to matched controls (Bokde et al., 2010). The authors hypothesized that the activation differences may
be indicative for a compensation mechanism counteracting the first AD associated neuropathological alterations.

In contrast, other studies explain neuronal hyperactivity rather as an early pathological event that directly contributes to memory deficits in MCI. For example, Yassa et al. suggested that functional hyperactivity may be an indication of aberrant memory encoding: they observed an increase in neuronal activity in CA3/dentate gyrus areas in aMCI compared to age-matched healthy adults, which was associated with a functional deficit in pattern separation (Yassa et al., 2010). Bakker et al. provided experimental support for the hypothesis that early hyperactivity is linked to functional impairment. In this study, aMCI patients that were treated with a low dose of the anti-epileptic levetiracetam (which reduces hippocampal activation) showed significantly improved task-related memory performance compared to aMCI patients that received a placebo treatment (Bakker et al., 2012). It should be noted that some studies (e.g. Johnson et al., 2006; Machulda et al., 2003; Petrella et al., 2006) actually observed lower levels of neuronal activity in MCI. This apparent discrepancy may in part be due to subtle differences in disease stages in the included cohorts. For example Celone et al. observed a reduction in hippocampal activity in patients with

![Figure 1](image-url)  
**Figure 1 - The feedback loop between synaptic activity and Aβ in the normal and Alzheimer's disease brain.**  
In the normal physiological situation, Aβ levels and synaptic activity and plasticity are tightly coupled (left panel). In this system, Aβ acts as a “brake” to prevent excessive synaptic activity: when neuronal activity levels increase too much (bottom left), a physiological increase in Aβ can desensitize synapses and restore normal synaptic activity levels. On the other hand, a reduction in synaptic transmission due to slightly elevated Aβ levels can be overcome by increasing neuronal activity levels. However, more severe disturbances in the feedback loop between neuronal activity and Aβ levels can trigger a self-amplifying pathogenic cascade that ultimately results in Aβ accumulation and synaptic dysfunction, as observed in AD (right panel). For example, if Aβ levels rise above a pathological threshold and start to accumulate, a physiological increase in synaptic activity is not sufficient anymore to restore normal synaptic transmission levels. Neuronal hyperactivity may temporarily overcome the Aβ-induced block in learning and memory, but could in the long run exacerbate Aβ accumulation and overload the synaptic machinery, leading to the first AD symptoms: Aβ aggregation and synaptic dysfunction.
late aMCI and early AD, while less impaired aMCI patients showed increased hippocampal activity (Celone et al., 2006). Secondly, enhanced metabolic activity in a particular brain area may be a compensatory response to decreased activity in another brain area. In addition, functional activation strongly depends on performance and task difficulty (reviewed in Prvulovic et al., 2005).

1.1.2 fMRI evidence for neuronal hyperactivity associated with genetic factors for AD

Neuronal hyperactivity is not only described in sporadic forms of AD, but is also associated with genetic components of the disease. Multiple studies, using a number of memory paradigms, have shown that carriers of the Apolipoprotein E gene (ApoE) variant ε4 show enhanced activation of multiple brain areas, already when still cognitively unaffected (reviewed in Bookheimer and Burggren, 2009). Interestingly, carriers of the ApoE ε4 variant have a 10-30 times higher risk of developing late-onset sporadic AD (Corder et al., 1993). Also non-demented carriers of a clusterin (CLU) allele that recently was identified as a risk factor for AD (Harold et al., 2009; Lambert et al., 2009), show higher activity levels in the frontal and posterior cingulate cortex and the hippocampus, particularly during working memory tasks, as compared to subjects with the protective allele (Lancaster et al., 2011). Finally, Quiroz et al. compared hippocampal activation in young, healthy carriers of the E280A mutation in the presenilin 1 gene (PS1) with matched controls. The presymptomatic PS1 mutation carriers performed equally well as the control group on an encoding task, but this was associated with an increased activation of the right anterior hippocampus (Quiroz et al., 2010).

1.1.3 Cellular and molecular indications for neuronal hyperactivity in MCI

We have observed indications for early neuronal activation in control and MCI brains using cellular and molecular biological techniques. Using Golgi apparatus size as a measure for neuronal metabolic activity, Dubelaar et al. reported that neurons in the nucleus basalis of Meynert, the main source of cholinergic projections to the neocortex, are metabolically hyperactive during MCI (Dubelaar et al., 2006). In addition, we have recently conducted a genome-wide gene expression study to elucidate the transcriptional alterations associated with the development and progression of AD in the prefrontal cortex. This study revealed that in non-demented controls (Braak stage I and II), the expression of a large number of genes involved in synaptic activity and plasticity is increased, which is followed by a decrease in later Braak stages (Bossers et al., 2010) (Fig. 2, middle panel). Examples of genes that follow this pattern of regulation are the synaptic activity-dependent genes Arc and EGR1, BDNF and NPTX2. In a subsequent study in the same tissue, we analyzed
The storm before the quiet

Figure 2 - Patterns of synaptic activity, Aβ accumulation and Aβ degradation during the course of Alzheimer's disease.

In the earliest, presymptomatic stages of AD (Braak stages I-II), Aβ starts to accumulate inside neurons (top panel). At the same time, neuronal activity levels increase (middle panel) but IDE-mediated degradation of intraneuronal Aβ is decreased (bottom panel). In the symptomatic stages of the disease (Braak III and later), Aβ levels rise above a physiological threshold, and plaques start to accumulate, intraneuronal Aβ disappears, and synaptic gene expression is decreased. Although IDE activity slightly recovers at Braak III, IDE activity again decreases at later disease stages. These parallel events may drive disease progression to more severe stages of AD, leading to the cognitive defects as observed in AD patients. Top and middle panel adapted from Bossers et al. (2010), bottom panel adapted from Stargardt et al. (2013).

miRNA changes during the progression of AD. This study revealed that the expression pattern of miR-132, which promotes synaptic activity and plasticity, also increases in the earliest nonsymptomatic Braak stages, and decreases during the symptomatic stages of the disease (Lau et al., 2013). These findings may be a representation of neuronal hyperactivity on a molecular level. Interestingly, the increase in synaptic activity and plasticity gene and miRNA expression coincided with an increase in intraneuronal Aβ levels (Fig. 2, top and middle panels). These data suggest that during the course of AD, Aβ and neuronal activity levels are linked in the human brain. As these studies
were performed on human postmortem material, the exact temporal relationship between increased neuronal metabolism, elevated expression of synaptic activity and plasticity gene expression and the buildup of intraneuronal Aβ levels remains unknown: we cannot conclude if Aβ triggers a (perhaps compensatory) increase in synaptic activity, or that neuronal hyperactivity directly causes the buildup of intraneuronal Aβ.

1.1.4 Epilepsy and AD

There is accumulating evidence for a link between neuronal hyperactivity disorders and AD. Chronic epilepsy (which is characterized by episodes of neuronal hyperactivity) is associated with an increased risk of AD, and cognitive decline and memory problems similar to those in AD have been described in epileptic patients, most notably in patients with early onset temporal lobe epilepsy (reviewed in Thom et al., 2011). Also, the occurrence of the classical pathological hallmarks of AD is increased in epileptic brain areas. A postmortem analysis of the brains of 138 chronic epilepsy patients revealed that intermediate levels of neurofibrillar pathology (Braak stages III/IV) were found more often in middle-aged epileptic brains as compared to age-matched controls (Thom et al., 2011), and the incidence of senile plaque pathology is increased in brain biopsies of epileptic patients (Mackenzie and Miller, 1994). Finally, epileptic activity is observed in a subset of sporadic AD patients and in many pedigrees with familial AD, and aggravates the disease course (Palop and Mucke, 2009). e.g. aMCI patients with epilepsy develop cognitive defects at a younger age than non-epileptic aMCI patients (Vossel et al., 2013).

1.2 Neuronal hyperactivity in animal models for AD

The majority of mouse models for AD are based on genetic mutations that cause rare, presenile familial forms of AD. Neuropathologically, the introduction of these genes in mice is usually associated with increased levels of the Aβ peptide, a shift towards higher Aβ42/Aβ40 ratios, plaque formation and memory deficits. Interestingly, an increasing number of publications report increased neuronal excitability and neuronal hyperactivity in several AD mouse models (see below), which suggests that indeed neuronal activity and Aβ levels are linked and that the balance between these two is disturbed already early in the disease. Similar to neuronal hyperactivity in the earliest AD stages in humans, it remains unclear whether hyperactivity in AD mouse models represents a compensatory mechanism or is an early pathological event that directly contributes to memory deficits and the development of AD.
1.2.1 Neuronal hyperactivity is a prominent feature in multiple AD mouse models

In a series of seminal papers, Palop, Mucke and colleagues investigated the molecular mechanisms underlying spontaneous nonconvulsive seizure activity in cortical and hippocampal networks in a mouse model that overexpresses a mutated form of human APP (hAPPFAD) (Palop et al., 2007). The investigators demonstrated functional impairment in the inhibitory interneuron circuit in these mice that resulted in aberrant excitatory activity. Restoring the inhibitory synaptic activity to normal levels reduced epileptic activity, memory deficits and even premature mortality (Verret et al., 2012). In addition, they showed that application of the anti-epileptic drug levetiracetam reversed abnormal spike activity, hippocampal remodeling, and learning and memory deficits in these mice (Sanchez et al., 2012). It is striking to note that the same drug also reversed memory deficits in aMCI patients (Bakker et al., 2012), adding further support to the hypothesis that neuronal network hyperactivity may causally contribute to cognitive impairment in both aMCI and AD mouse models.

This view is further supported by data from the double-transgenic APP23xPS45 mice, overexpressing the APPswe and PS1G384A mutations (Sturchler-Pierrat et al., 1997). These mice show neuronal hyperactivity exclusively near Aβ plaques, as was demonstrated by in vivo two-photon calcium imaging (Busche et al., 2008). Hyperactivity of neurons in the visual cortex of these mice was coupled to defects in neuronal tuning for the orientation of visual stimuli (Grienberger et al., 2012). Interestingly, the fraction of hyperactive neurons was already dramatically increased in the CA1 area before the onset of plaque pathology, compared to control mice. Treatment with a γ-secretase inhibitor reversed the neuronal hyperactivity and reduced soluble Aβ levels, and infusion of soluble Aβ was sufficient to induce neuronal hyperactivity (Busche et al., 2012). As intraneuronal Aβ accumulation precedes plaque formation in AD brains (Bossers et al., 2010; Gyure et al., 2001; Wirths et al., 2001), it would be of interest to investigate whether intraneuronal Aβ levels were increased in the population of hyperactive neurons compared to those exhibiting normal levels of activity. As the aforementioned mouse AD models are all based on the overexpression of transgenic forms of APP, they are not directly suitable to study the hypothesis that neuronal hyperactivity by itself can alter Aβ levels to such an extent that it triggers the pathogenic cascade associated with AD. It is however striking to see that interaction between Aβ and neuronal activity appears to play an essential modulatory role in the phenotypes observed in these mice: cognitive defects can be rescued by reversing neuronal hyperactivity (Sanchez et al., 2012; Verret et al., 2012), and neuronal hyperactivity can be reduced by interfering with Aβ production (Busche et al., 2012). As such, these models clearly suggest that the interaction between Aβ and neuronal activity may play an important role in the development of AD.
1.2.2 Increasing synaptic activity and plasticity can protect against Aβ

Despite a lot of data showing neuronal hyperactivity as a pathological event, there are also many studies that indicate that increasing neuronal activity and plasticity protects against Aβ-related pathological changes in the brain. Cognitive stimulation by means of environmental enrichment - which increases synaptic transmission and plasticity (Eckert and Abraham, 2013) - has been shown to be beneficial for learning and memory in both mice and rat AD models, not only by stimulating clearance of Aβ, but also by modulating APP processing. For example, rats housed in an enriched environment show lower amounts of C-terminal fragments of APP, resulting in decreased Aβ plaque load compared to rats in a normal, not enriched environment (Briones et al., 2009). A similar effect was demonstrated in the APP/PS1 mouse model; environmental enrichment of these mice reduced Aβ levels and deposition compared to mice in standard housing conditions, which coincided with increased expression of synaptic activity genes (Lazarov et al., 2005). In line with this, early cognitive stimulation of Tg2576 mice not only reduced soluble and oligomeric Aβ levels, but also led to increased levels of hippocampal post-synaptic markers and proteins involved in synapse formation (Gerenu et al., 2013). The beneficial effects of environmental enrichment on memory appear to be independent of physical exercise (Birch et al., 2013), which may explain why physical exercise programs in demented patients did not reveal clear cognitive improvements (Eggermont et al., 2009; Littbrand et al., 2011).

The seemingly contradictory effects of synaptic activity on Aβ-associated neuropathology as outlined in sections 1.2.1 and 1.2.2 may be attributable to the level of neuronal hyperactivity. In animal experiments, environmental enrichment and cognitive training enhance neuronal excitability, synaptic transmission and plasticity within physiological levels. Epileptiform activity on the other hand is a pathological increase in excitability, activity and plasticity. It is also conceivable that the stage of the disease in part defines if neuronal hyperactivity protects against Aβ or aggravates the pathological processes that are already initiated. Finally, the age of onset of neuronal hyperactivity can severely impact the functional outcome. Aged neurons exhibit specific changes in dendritic morphology, cellular connectivity, Ca²⁺ homeostasis, and immediate early gene expression (reviewed in Burke and Barnes, 2006). Collectively, these age-related changes may render neurons less resilient during periods of neuronal hyperactivity. For example, as intracellular Ca²⁺ levels are increased in aged neurons, additional Ca²⁺ influx during prolonged periods of synaptic hyperactivity can increase their vulnerability to neurodegeneration (Hajieva et al., 2009).
2. NEURONAL HYPERACTIVITY MODULATES Aβ LEVELS AND SECRETION

The data in section 1 reviews evidence that neuronal hyperactivity and Aβ accumulation are linked during the earliest stages of AD in both humans and AD mouse models. To gain a more mechanistic understanding of the impact of neuronal hyperactivity on the development and progression of AD, we now discuss how neuronal hyperactivity affects the production and degradation of Aβ.

2.1 Neuronal hyperactivity increases Aβ production via the endocytic pathway

2.1.1 Neuronal hyperactivity increases the production of Aβ by enhancing clathrin-mediated endocytosis

During synaptic transmission, neurotransmitters are released by fusion of the synaptic vesicles with the plasma membrane. The size and molecular composition of the presynaptic membrane is held constant by a continuous recycling of the membrane after vesicle fusion. The clathrin-mediated endocytic pathway mediates this recycling process and also recycles, modifies and degrades receptors and other integral membrane proteins after neurotransmitter release (Cataldo et al., 2000). When neurons become hyperactive, elevated levels of synaptic activity result in an increase in the frequency of vesicle fusion events. Consequently, the rate of endocytosis needs to be enhanced to maintain the normal size of the synapse. On the postsynaptic side, synaptic activity-dependent changes in the molecular makeup of the postsynaptic membrane are believed to underlie long term potentiation and –depression (Kennedy and Ehlers, 2006). Notably, the amount of AMPA receptors is a strong determinant of synaptic strength, and both AMPA and NMDA receptor trafficking is mediated through endocytosis (Malinow and Malenka, 2002; Newpher and Ehlers, 2008; van der Sluijs and Hoogenraad, 2011).

Interestingly, it has become apparent that synaptic activity-dependent clathrin-mediated endocytosis in particular is required for Aβ production (reviewed in Wu and Yao, 2009). Synaptic activity increases the amount of APP-containing vesicles moving to dendritic synapses and the amount of APP localized at the plasma membrane. After vesicle fusion, APP-containing membranes are internalized by endocytosis (Tampellini et al., 2009). Both β-secretase and γ-secretase accumulate in endosomes, where their activity is enhanced due to the mild acidic pH (Frykman et al., 2010; Huse et al., 2000). Particularly in these endosomes, there is a continued close interaction between APP and β-secretase. Indeed, β-secretase cleaved C-terminal APP fragments (β-CTF) are present in endosomes (Kinoshita et al., 2003; Rajendran et al., 2006;
The opposite effect was also observed: decreasing neuronal activity significantly lowered β-CTF levels. Importantly, Holtzmann and coworkers demonstrated that the inhibition of clathrin-mediated endocytosis was sufficient to immediately lower Aβ levels in vivo, and established that the majority of the Aβ pool in the brain is generated through synaptic activity-dependent endocytotic mechanisms (Cirrito et al., 2008). Thus, the increase in neuronal activity in the earliest stages of AD may increase Aβ production through synaptic activity-dependent clathrin-mediated endocytosis.

2.1.2 Endocytosis is disturbed in the earliest stages of AD

There is a growing amount of evidence that suggests that the endocytic pathway is becoming dysfunctional during these earliest stages of AD, causing aberrances in the endocytic machinery that may amplify the increase in activity-dependent Aβ production. Cataldo et al. observed abnormally large endosomes in neurons in the entorhinal cortex, hippocampus and prefrontal cortex of the pre-AD cases (nondemented elderly exhibiting the earliest AD-like pathological changes restricted to the entorhinal cortex and hippocampus), as well as in pre-dementia Down Syndrome patients (Cataldo et al., 2000). Endosomal enlargement could already be detected in the absence of extracellular Aβ accumulation or neurofibrillary tangles and thus precedes the formation of these inclusions in sAD brains. A potential molecular basis for these structural changes in endosomes in pre-clinical AD was discovered by Ginsberg et al, who performed a microarray analysis on laser-dissected CA1 hippocampal neurons and detected a specific increase in genes regulating early and late endocytosis (Rab-4, Rab-5 and Rab-7) (Ginsberg et al., 2010). Indeed, Rab-5 overexpression in murine L cells stably transfected with human APP695 resulted in abnormally enlarged endosomes, resembling the endosome morphology observed in pre-AD patients and led a 2-fold increase in endosomal β-CTF levels and a 2.5-fold increase in secreted Aβ levels (Grbovic et al., 2003).

Our microarray analysis of human postmortem AD tissue also revealed alterations in endocytic processes during the first presymptomatic stages of AD (Bossers et al., 2010). Specifically, we observed an increase in the expression of clathrin heavy chain and protein kinase C and casein kinase substrate in neurons 1 (PACSIN1), which are both involved in activity-dependent bulk endocytosis (Clayton et al., 2009). At the same time, stonin 2 (involved in endocytic synaptic vesicle protein sorting (Kononenko et al., 2013)) and dynamin 2 (involved in vesicle recycling (Durieux et al., 2010)) were downregulated. These data might indicate a shift in the balance between specific early and late events in endocytosis and vesicle recycling.
In addition, there is increasing evidence that the postsynaptic protein Arc may be linked to endosomal dysfunction in early AD. Wu et al. showed that Arc recruits endophilin2/3 and dynamin to early/recycling endosomes. These Arc-recruited endosomes contain both APP and β-secretase. Arc physically associates with γ-secretase, and Arc expression is required for Aβ production (Wu et al., 2011). Furthermore, the authors showed that Arc expression was significantly increased in the prefrontal cortex of patients with MCI/early AD (Braak III-VI) whereas the occipital cortex, which is less affected by Alzheimer pathology, did not show upregulated Arc expression (Wu et al., 2011). In the APPswe/PS1dE9 mouse model for AD, Arc levels were aberrantly increased in hyperactive neurons near senile plaques in the visual cortex (Rudinskiy et al., 2012). Interestingly, a polymorphism in the ARC gene was recently identified that is associated with a reduced risk for AD (Landgren et al., 2012).

Finally, two recently discovered genetic variations, in BIN1 and PICALM, are associated with an increased risk for AD. Both these genes play a role in clathrin-mediated endocytosis: BIN1 is a regulatory accessory protein that aids in membrane deformation (Doherty and McMahon, 2009) and PICALM mediates assembly of clathrin (Tebar et al., 1999). These data further strengthen the involvement of the clathrin-mediated endocytosis pathway in AD pathogenesis. It remains to be established whether altered clathrin-mediated endocytosis is a primary contributor to AD (through modulation of Aβ levels) or that one of the earliest synaptotoxic effects of Aβ is to perturb clathrin-mediated endocytosis. These data shows that both neuronal hyperactivity and disturbances in the endocytic pathway are already observed in the pre-symptomatic stage of AD. The resulting increase in Aβ production may explain the observations made by us and others that Aβ accumulates inside neurons in the pre-AD and Down syndrome brain and in several AD mouse models, before the onset of plaque pathology (e.g. Bossers et al., 2010; Youmans et al., 2012, reviewed in LaFerla et al., 2007). Whether neuronal hyperactivity is causally linked to disturbances in clathrin-mediated endocytosis (for example by overloading the system), or that it amplifies the effects of alterations already present in the endocytic machinery, is unclear at this moment. In any case, the interaction between neuronal hyperactivity and clathrin-mediated endocytic dysfunction appears to significantly contribute to increased Aβ production and Aβ pathology observed in AD.

2.2 Neuronal hyperactivity and Aβ degrading enzymes

Although the data discussed in section 2.1 suggest that neuronal hyperactivity leads to an increase of Aβ production and secretion, neuronal hyperactivity also triggers compensatory processes to counteract increased levels of Aβ in the brain.
2.2.1 Neuronal activity increases the degradation of extracellular Aβ

Several studies have shown that housing mice in an enriched environment is widely beneficial to brain function. Environmental enrichment stimulates sensory and cognitive functions, social behavior and physical exercise. Exposure to enriched environments leads to multiple physiological changes in the brain, such as increased blood flow, enhanced neuronal activity and plasticity, and increased dendritic branching and spine formation (reviewed in Nithianantharajah and Hannan, 2006). In AD mouse models, environmental enrichment attenuates cognitive defects through Aβ-dependent and Aβ-independent mechanisms, and can even lower brain Aβ levels (reviewed in Xu et al., 2014). Interestingly, environmental enrichment-induced neuronal hyperactivity alters the localization and activity of several Aβ degrading enzymes. One prominent Aβ degrading enzyme, whose activity is regulated by neuronal activity, is the peptidase neprilysin (NEP) (Hafez et al., 2011; Hersh and Rodgers, 2008; Marr et al., 2004). Lazarov and coworkers demonstrated that after environmental enrichment, neuronal activity-dependent NEP activity caused a decrease of Aβ levels in vivo (Lazarov et al., 2005). In this study, cerebral Aβ levels and deposits were markedly reduced in AD mice housed in the enriched environment, compared to AD animals under standard housing. The reduction in Aβ levels was correlated with increased NEP activity in these mice. Furthermore, Tampellini et al. showed that in NEP knock-out neurons or when NEP activity was blocked, synaptic activity failed to reduce intraneuronal Aβ levels (Tampellini et al., 2009). NEP is a type II membrane protein with a catalytic site on the extracytoplasmic side and emerges on the cell surface through the secretory pathway (Hama et al., 2004; Wang et al., 2006). Although overexpression of NEP in primary cortical neurons not only reduced extracellular, but also cell-associated Aβ levels, postsecretory degradation might have contributed to the decreases in cell-associated degradation by altering the equilibrium of intra versus extracellular Aβ (Hama et al., 2004). Other studies confirmed that NEP mainly degrades extracellular Aβ (Malito et al., 2008) and that intracellular Aβ is not affected by inhibiting NEP (Abdul-Hay et al., 2012; Sudoh et al., 2002).

In addition to NEP, the matrix metalloproteinase-9 (MMP-9) has received considerable attention for its Aβ degrading capacity (Backstrom et al., 1996; Merlo and Sortino, 2012; Yan et al., 2006) and its ability to elevate sAPPα levels, the product of the non-amyloidogenic pathway of APP processing (Fragkouli et al., 2012). Szklarczyk et al. demonstrated that MMP-9 is regulated by synaptic activity (Szklarczyk et al., 2002). The authors induced a generalized increase in synaptic activity and seizures in rats by intraperitoneal injections of kainate, mimicking neuronal hyperactivity. Kainate administration resulted in upregulation of MMP-9 mRNA and protein levels as well as its enzymatic activity in the dentate gyrus. Furthermore, the neurotrophin NGF also induces MMP-9 expression and α-secretase
activity in PC12 cells in a TrkA-dependent manner (Fragkouli et al., 2011). In addition, Michaluk et al. demonstrated that increased activity of MMP-9 indeed resulted in increased degradation of its substrates (Michaluk et al., 2007). Although this study did not directly evaluate Aβ turnover, Aβ is a substrate of MMP-9, which suggests that the increased activity of MMP-9 would result in enhanced Aβ degradation. Both NEP and MMP-9 only degrade extracellular Aβ (Backstrom et al., 1996; Malito et al., 2008): they therefore merely have an indirect effect on intraneuronal Aβ levels through the balance between intra- and extracellular Aβ pools. An important question that remains to be addressed is: if neuronal hyperactivity both increases the production and degradation of Aβ, why does Aβ start to accumulate inside neurons during the earliest stages of AD (Fig. 2, top panel)?

It is well possible that the balance between the activity-dependent production and degradation of Aβ is shifted towards more Aβ production or less degradation during these stages. Intriguingly, we have observed evidence that suggests that this imbalance may be due to a specific loss of the cytosolic degradation of intraneuronal Aβ during these initial disease stages.

2.2.2 The degradation of intraneuronal Aβ is decreased in the earliest stages of AD

The intraneuronal Aβ pool does not only consist of Aβ trapped in vesicles like endosomes, multi-vesicular bodies and lysosomes, but Aβ is also found in the cytoplasm, probably by passive leakage of Aβ along the secretory pathway or by transport via the ER-associated degradation (ERAD) (e.g. Bückig et al., 2002; Schmitz et al., 2004; Yang et al., 1998, reviewed in Li et al., 2007). Therefore, we investigated whether intracellular Aβ can be cleared by cytosolic peptidases. Using homogenates of human postmortem hippocampal tissue, we found that insulin-degrading enzyme (IDE) is the main peptidase degrading intraneuronal Aβ in the human brain (Stargardt et al., 2013). In addition, we showed that only monomeric Aβ is degraded by IDE and that Aβ oligomerization prevents its clearance by IDE. We further showed that in the human hippocampus, the activity of IDE is decreased already in the earliest Braak stages (stages I and II) (Fig. 2, bottom panel). This decrease in IDE activity is correlated with a decrease in IDE protein levels. In later Braak stages, IDE activity and protein levels recover to roughly baseline levels and decline again in full-blown AD (Braak V and VI). Intriguingly, the initial decline in IDE activity in Braak I-II thus coincides with the period when neuronal hyperactivity is observed (Bossers et al., 2010). In other words, at the moment that activity-dependent Aβ production is increased, intraneuronal Aβ degradation is decreased. Indeed, during these stages, Aβ accumulates inside neurons (Fig. 2, top panel).

Together, these data show that neuronal hyperactivity during the earliest stages of AD might be able to increase the activity of the Aβ degrading enzymes NEP and MMP-9. However, these enzymes
are mainly involved in degrading extracellular Aβ, but not intraneuronal Aβ. In fact, the activity of IDE, which is the main peptidase degrading intraneuronal Aβ, is already significantly decreased in the first Braak stages, the stages at which neuronal hyperactivity and intraneuronal Aβ accumulation is observed. It should be noted that there is as of yet no evidence that IDE activity levels are directly or indirectly linked to neuronal activity. A specific loss of IDE activity could therefore constitute an additional trigger, independent of neuronal hyperactivity, that affect Aβ levels during the initial stages of AD. Importantly however, as reduced IDE activity coincides with hyperactivity, it may disrupt the physiological balance between synaptic activity-dependent production and degradation of Aβ by shifting the balance towards Aβ accumulation.

3. SETTING THE STAGE FOR AD: MULTIPLE CONCURRENT PATHWAYS THAT INCREASE INTRANEURONAL Aβ

In this review, we discussed multiple concurrent events that alter Aβ levels during the initial, nonsymptomatic stages of AD. Firstly, neuronal hyperactivity is observed in cognitively unaffected individuals at risk for AD. Secondly, the increase in neuronal activity requires more clathrin-mediated endocytosis. However, preclinical AD is associated with specific disturbances in clathrin-mediated endocytosis that amplify the activity-dependent production of Aβ and may cause a rise in intraneuronal Aβ levels. Thirdly, the activity of IDE, the main peptidase degrading cytosolic Aβ, is severely decreased. These parallel events may create a sudden and drastic accumulation of Aβ inside neurons, as observed in the brains of presymptomatic AD patients. Eventually, when Aβ levels rise above a physiological threshold and become pathological, patients will progress into the symptomatic stages. At this stage, the synaptotoxic effects of Aβ cause the first cognitive defects and Aβ starts to aggregate into plaques (Fig. 3).

Since Aβ oligomerization initiates rather inside cells than extracellularly, the intraneuronal Aβ pool is an important source for the synaptotoxic oligomeric forms of Aβ (Cleary et al., 2005; Walsh et al., 2002, 2000) and may therefore represent the disease initiating pool of Aβ. A large body of evidence suggest that the synaptotoxic effects of oligomeric Aβ are mainly mediated extracellularly: to a large extent, the intraneuronal Aβ pool is secreted, again through a synaptic activity-dependent manner. This extracellular Aβ interacts with several cell surface receptors located at or near synapses, such as the NMDA, AMPA, cellular PrP and EphB2 receptors and LilrB2 (Benilova and De Strooper, 2013; Kim et al., 2013). Indeed, several studies confirm that the secretion of intraneuronal Aβ and the resulting increase of extracellular, oligomeric Aβ levels have a severe impact on synaptic function and cause learning and memory dysfunctions (Billings et al., 2005; Holscher et al., 2007;
The Storm before The Quiet

Figure 3 - The interplay between neuronal hyperactivity and $\text{A}\beta$ triggers synaptic dysfunction in Alzheimer's disease. In the healthy brain, the levels of synaptic activity, $\text{A}\beta$ production and $\text{A}\beta$ degradation are in balance. During the earliest, asymptomatic stages of AD however, the combined effects of neuronal hyperactivity, increased $\text{A}\beta$ production and a paradoxical decrease in $\text{A}\beta$ clearance by IDE, culminate into the accumulation of $\text{A}\beta$. When $\text{A}\beta$ levels have risen past the disease initiating threshold, synaptic dysfunction becomes so severe that cognitive defects arise and AD for the first time manifests itself clinically.

Shankar et al., 2007). However, intraneuronally located oligomeric $\text{A}\beta$ also directly contributes to AD neuropathology. For example, $\text{A}\beta$ has been observed inside axonal mitochondria, and $\text{A}\beta$ affects axonal mitochondrial movement (Du et al., 2012). It would be very interesting to study if selectively reducing cytosolic $\text{A}\beta$, for example by increasing IDE activity, would be able to prevent the intracellular toxic properties of $\text{A}\beta$ such as mitochondrial dysfunction.

There is still much uncertainty about the exact role of neuronal hyperactivity in the earliest stages of AD: is it one of the first pathological hallmarks of AD or does it counteract the first pathological alterations? The data reviewed here provides evidence for both hypotheses. In the situation where neuronal hyperactivity is a contributing factor to AD, the pathological cascade could for example start with a decrease in IDE activity. This results in an increase in intraneuronal $\text{A}\beta$ levels, which is secreted upon neuronal activity and blocks synaptic transmission. Recent data suggest that interneurons may be particularly vulnerable to $\text{A}\beta$ (Sanchez et al., 2012; Verret et al., 2012). The resulting loss of inhibitory input then causes network hyperactivity, which further increases $\text{A}\beta$ levels and feeds a self-amplifying loop of ever-increasing $\text{A}\beta$ levels and neuronal hyperactivity, which will likely overload the endocytic pathway. In this hypothesis, neuronal hyperactivity is clearly an integral part of the pathological cascade and preventing neuronal hyperactivity – for example by increasing IDE activity and thereby lowering $\text{A}\beta$ levels - may delay or even prevent the progression towards symptomatic stages of AD.

On the other hand, there is substantial evidence that increased synaptic activity and plasticity reduces the risk for AD. Intellectual performance and bilingualism may delay the onset of AD in
humans (Alladi et al., 2013; Stern, 2009; Wilson et al., 2002) and cognitive training reverses some of the cognitive deficits in AD mouse models (section 1.2.2). In this scenario, the initial increase in neuronal activity may act to prevent the onset of cognitive decline. Indeed, our gene and miRNA expression data in the human brain suggest that only when neuronal activity and plasticity is lost, patients develop memory deficits, and decreased levels of EGR1, Arc and miR-132 – all essential modulators of synaptic activity, plasticity and memory – may be the molecular correlates of cognitive decline. Restoration of for example EGR1 levels may therefore represent a therapeutic strategy to reverse memory loss in MCI and/or AD.

Based on the data reviewed here, we cannot unequivocally identify which of these two hypotheses has a dominant effect on the development of AD symptoms. This review does however highlight the central role of neuronal hyperactivity in presymptomatic AD and deepens our insight into the exact molecular mechanisms through which neuronal hyperactivity and Aβ levels are connected. These combined data warrant further studies to decipher the exact roles of specific molecules including - but not limited to - IDE, EGR1 and miR-132 in the initial pathogenic cascade of AD. In the long run, such experiments may open up new avenues for the development of preventive treatments of this devastating disease.

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