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Bakalářská práce

Vápníková signalizace u gliových buněk v progresi Alzheimerovy choroby

Calcium signaling in glial cells during Alzheimer's disease progression

Eliška Waloschková

Školitelka: Ing. Miroslava Anděrová, CSc.

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V Praze, 15.05.2015

Podpis

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Abstrakt

Alzheimerova choroba je neudegenerativní onemocnění postihující celou centrální nervovou soustavu včetně gliových buněk. Mechanismy tohoto onemocnění stále nejsou zcela objasněny, přesto současný výzkum naznačuje, že spolu se známými charakteristickými znaky Alzheimerovy choroby, jako je hromadění amyloidu β a hyperfosforylovaného tau, by důležitým rysem jak v neuronech, tak v gliových buňkách, především v astrocytech a mikrogliích, mohla být dysregulace vnitrobuněčné vápníkové homeostáze. Gliové buňky hrají důležitou roli jak ve zdravém mozku, tak během progrese Alzheimerovy choroby. Jejich hlavní funkce, jako například podpora neuronů a udržování synapsí, jsou během této nemoci narušeny. Současný výzkum naznačuje, že narušená vápníková signalizace gliových buněk vyvolaná během Alzheimerovy choroby, by eventuálně mohla podporovat nesprávnou činnost těchto buněk a zvýšit jejich zánětlivou reakci, tudíž ovlivňovat neurony a zůsobit poškození mozku. Je pravděpodobné, že probíhající zánětlivá reakce a zhoršená vápníková signalizace se navzájem ovlivňují a následně urychlují progresi Alzheimerovy choroby.

Klíčová slova: Alzheimerova choroba, gliové buňky, astrocyty, mikroglie, oligodendrocyty, NG2 glie, polydendrocyty, vápníková homeostáze, vápníková signalizace

Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder affecting the entire central nervous system including glial cells. The mechanisms of this disease are not yet entirely clear, although recent studies suggest that among the known hallmarks of AD, such as accumulation of amyloid β and hyperphosphorylated tau, dysregulation of intracellular calcium homeostasis is proposed to be a significant feature both in neurons and glial cells, namely astrocytes and microglia. Glial cells play an important role both in healthy brain and during AD progression. Their major functions, such as supporting neurons or maintaining synapses, are impaired during this disease. Recent findings suggest that aberrant glial calcium signaling activated during AD, could possibly promote the malfunction of these cells and increase their inflammatory response, thus affecting neurons and causing brain damage. It is likely, that the ongoing inflammation and the impaired calcium signaling affect one another, consequently enhancing the progression of AD.

Key words: Azheimer's disease, glial cells, astrocytes, microglia, oligodendrocytes, NG2 glia, polydendrocytes, calcium homeostasis, calcium signaling

Contents

Shortcut list

AD	Alzheimer's disease
AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid
APP	amyloid precursor protein
ATP	adenosine triphosphate
Aβ	beta-amyloid
CNS	central nervous system
CSF	cerebrospinal fluid
DAG	1,2-diacylglycerol
DICTs	damage-induced Ca^{2+} transients
FAD	familial form of Alzheimer's disease
GABA	gamma-aminobutyric acid
GPCRs	G-protein coupled receptors
IL-1β	interleukin-1 beta
IP3	inositol triphosphate
IP3R	inositol triphosphate receptor
LPS	lipopolysaccharide
MCI	mild cognitive impairment
mGluR3	metabotropic glutamate receptor type 3
mGluR5	metabotropic glutamate receptors type 5
NFTs	neurofibrillary tangles
NLRP3	nucleotide binding and oligomerization domain-like receptor family pyrin domain containing 3

NMDA	N-methyl-D-aspartate
NO	nitric oxide
PHFs	paired helical filaments
PIP2	phosphatidylinositol-4,5-bisphosphate
PS1	presenilin 1
ROS	reactive oxygen species
RyR	ryanodine receptor
TNF-α	tumor necrosis factor alpha
3xTg-AD	triple-transgenic Alzheimer's disease model

Introduction

Alzheimer's disease (AD) represents the most common form of dementia among the elderly. According to the Delphi consensus study in 2010 an estimated 24 million people had dementia with 4,6 million of new cases every year. The number is expected to double every 20 years up to 81 million in 2040 (Ferri et al., 2010). In 2013 there was approximately 35 million patients affected by AD (McGeer & McGeer, 2013). Among other risk factors of AD age is one of the most relevant. With the increasing life span of today's population, AD has become a significant health issue. The increasing number of cases shows the exponential growth of its prevalence with higher age. The fact that there is no known cure or prevention of AD together with the high costs of healthcare for AD patients, this disease has become a major public health concern of today's society.

The aim of the thesis is to give an overview about the major pathological features of AD together with the main mouse models used for research of this disease, glial cells as major participants in the central nervous system (CNS), their functions and impairment during normal aging and AD progression. Importantly, the thesis also focuses on calcium signaling and homeostasis in glial cells, their disruption during AD and the impact on the nervous system.

Alzheimer's disease

Alzheimer's disease is a form of dementia which mostly impacts people older than 65 years of age. Dementia as an overall term is defined as a sum of symptoms such as declined cognitive and functional abilities, deterioration of memory and in late stages of AD the inability to preform everyday activities caused by major neuronal loss in the brain tissue. Ultimately, AD patients lose their ability to walk or even swallow and the final stages of disease lead to impairment of all basic functions of human body, eventually being fatal. During such late stages of AD the patients are bed-bound and entirely dependent on 24/7 care (Association, 2014).

There are two known forms of AD: familial AD with an earlier onset and sporadic AD which represents 99% of all cases (Bekris, Yu, Bird, & Tsuang, 2010). Familial AD is caused by mutations in one of the 3 known genes, including genes for the amyloid precursor protein,

the presenilin 1 and presenilin 2 proteins (Bekris et al., 2010). People having mutations in one of these genes are certain to develop AD. Individuals with this familial form incline to express AD symptoms before 65 years of age, however, the phenotypes of the familial and sporadic forms are practically identical.

The revised criteria and guidelines for diagnosing AD proposed by the National Institute on Aging (NIA) and the Alzheimer's Association in 2011 recognize three stages of this disease; preclinical AD, mild clinical impairment (MCI) and dementia. In the first stage (preclinal AD), individuals do not show noticeable AD symptoms such as memory loss or cognitive impairment but early brain changes such as beta-amyloid (Aβ) accumulation can be identified (Sperling et al., 2011). Such changes may begin 20 years before the first observable symptoms of AD occur (Villemagne et al., 2013). Mild cognitive impairment (MCI) is the second stage of AD, in which individuals express slight, but recognizable, changes in thinking abilities, nevertheless, not yet affecting their performance of everyday activities (Albert et al., 2011). Dementia due to AD is the third and final stage. This stage leads to major cognitive impairment, which causes the inability to preform basic daily routines (McKhann et al., 2011). Nevertheless, an additional research is needed to validate these 2011 criteria, hence they yet cannot be used for clinical diagnosis (Association, 2014).

Pathophysiology

The neuropathology of AD is characterized by extracellular accumulation of beta-amyloid (Aβ) in the form of Aβ-plaques (also called senile or neuritic plaques) and the appearance of neurofibrillary tangles (NFTs).

The formation of amyloid plaques is a result of extracellular Aβ deposition, which is a product of alternative splicing of the amyloid precursor protein (APP). APP is cleaved by specific secretases into Aβ(1-40) or Aβ(1-42), the latter being more prone to self-aggregation (Citron et al., 1996). Aβ deposits form the core of the complex radial structure of amyloid plaques. This core is surrounded by dystrophic neurites and on the periphery several microglia and reactive astrocytes are commonly encountered (Dickson, 1997). Although considered as a substantial factor causing neuronal loss during AD, amyloid plaques can be found in the cortex of normally aging elderly subjects in absence of any signs of dementia, thus it is only one of the numerous factors triggering the disease (Maccioni, Muñoz, & Barbeito, 2001).

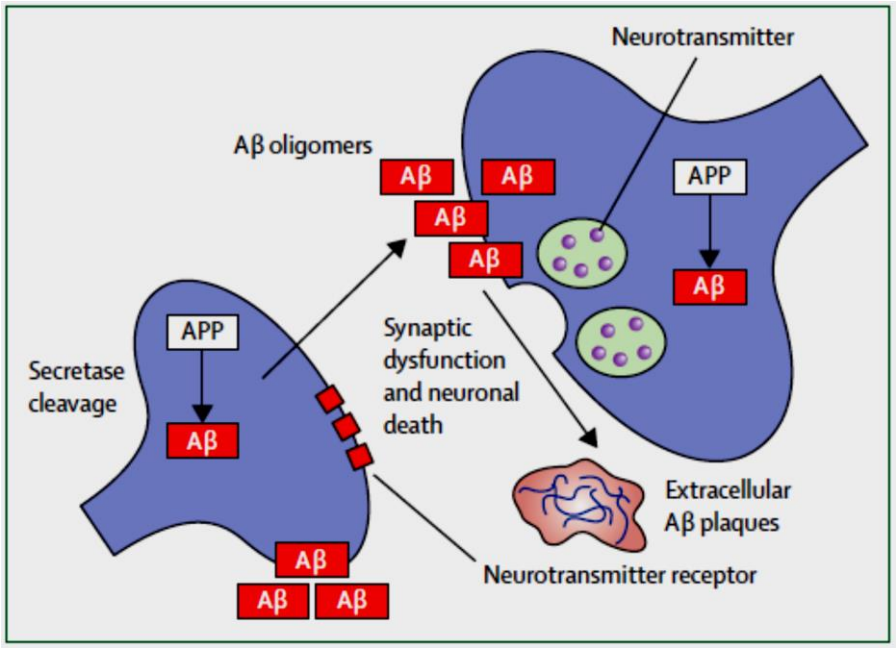

Figure 1 – The amyloid cascade hypothesis. Amyloid precursor protein (APP) is cleaved into Aβ forming intracellular and extracellular deposits, the latter forming Aβ plaques (Ballard et al., 2011).

Neurofibrillary tangles (NFTs) are composed of clustered structures of paired helical filaments (PHFs), which can be found mainly in the regions of the hippocampus, entorrhinal cortex, and amygdala. These abnormal structures form a compact filamentous network which is generated by self-aggregation of hyper-phosphorylated forms of the tau protein that belongs to microtubule-associated proteins. In a healthy brain the tau protein participates in the assembly of microtubules, their stabilization and bridging with other cytoskeletal filaments. Therefore, it plays a major role in cytoskeletal stabilization and consequently axonal morphology. The hyper-phosphorylation, which is caused by diverse protein kinase and phosphatase systems, results in structural and conformational changes of the tau protein and affects its ability of proper binding with tubulin and microtubular assembly (Dickson, 1997) .

Even though neurofibrillary tangles and amyloid plaques are the major pathological hallmarks of AD there are several other factors considered as possible causes of neurodegeneration such as estrogen levels or oxidative stress (Maccioni et al., 2001).

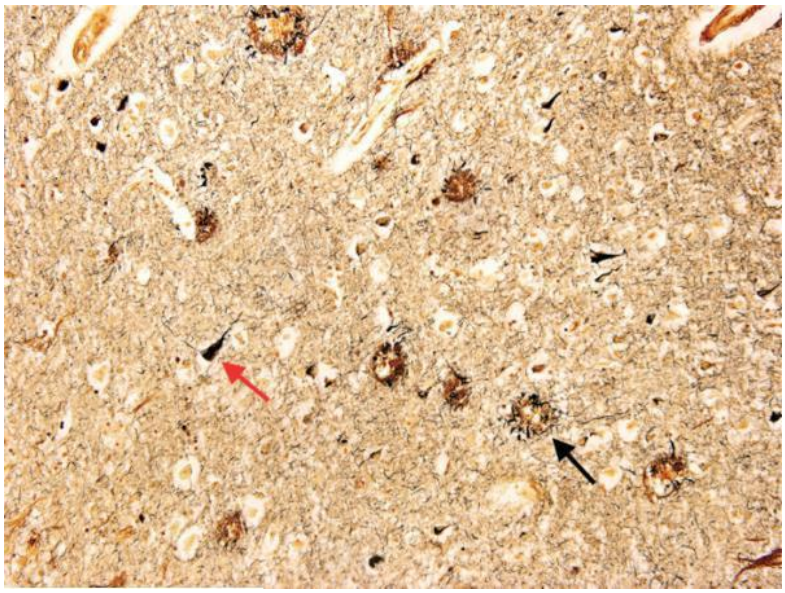

Figure 2 – Amyloid plaques (black arrow) and neurofibrillary tangles (red arrow) stained in brain tissue of an AD patient. Visualized by Bielschowski staining, 40x magnified. (Perl, 2010)

Transgenic mouse models

Transgenic mouse models are the key tool to current research in AD. Although various forms of these models have been generated, none of them expresses complete AD symptoms. The finding of mutations causing the familial form of AD (FAD) greatly improved the transgenic AD mouse modeling. Before this discovery there were attempts of overexpressing wild-type APP in transgenic mice, however there were no amyloid plaques or NFTs observed (Elder, Sosa, & Gasperi, 2010).

After the finding of FAD mutations new animal models were developed on the basis of overexpressing mutated transgenes typical for FAD onset. The first model generated using this approach is termed PDAPP (Games et al., 1995). Mice in this line exhibit age-related amyloid plaques with dense cores, dystrophic neurites, reactive astrocytes and activated microglia. However, the most widely studied transgenic AD model has been the Tg2576 mouse line overexpressing the Swedish FAD mutation (Hsiao et al., 1996). Despite the extensive amyloid deposition, none of these mouse lines displayed neuronal loss or NFTs. In order to enhance neuronal loss double transgenic models have been developed combining the presenilin 1 (PS1) and APP mutations (Holcomb et al., 1998).

In addition, new mouse models have been produced by combing FAD mutations with mutant forms of tau, which are found in a different form of dementia and result in formation

of NFTs (Lewis et al., 2001). The triple transgenic mouse model (3xTg-AD) characterized by overexpression of APP, tau and PS1 transgenes expresses both amyloid plaques and NFTs functionally leading to age-dependent synaptic loss (Oddo et al., 2003).

Transgenic mouse models of AD currently play a substantial role in developing potential immunotherapies for AD treatment. The following table contains an overview of transgenic mouse models of AD (Table 1).

Table 1 - Neuropathological features of the main transgenic mouse models of Alzheimer disease.

Mouse model	Gene (mutation)	Intraneuronal Aβ	Parenchymal Aβ plaques	Hyperphosphorylated Tau	Neurofibrillary tangles	Neuronal loss	Synaptic loss	CAA	Primary reference
PDAPP	APP (V717F)	-	Yes	Yes	No	No	Yes	-	Games et al. 1995
Tg2576	APP (K670N/M671L)	Yes	Yes	-	-	No	No	-	Hsiao et al. 1996
TgCRND8	APP (K670N/M671L, V717F)	-	Yes	-	No	No	-	-	Chishti et al. 2001
APP/PS1	APP (K670N/M671L), PS1 (M146L)	-	Yes	-	-	-	-	-	Holcomb et al. 1998
APP23	APP (K670N/M671L)	-	Yes	Yes	No	Little	Yes	Yes	Sturchler-Pierrat et al. 1997
Tg-SwDI	APP (E693Q, D694N)	-	Yes	-	-	-	-	Yes	Davis et al. 2004
APPDutch	APP (E693Q)	-	Little	-	-	-	-	Yes	Herzig et al. 2004
APPDutch/PS1	APP (E693Q), PS1 (G384A)	-	Yes	-	-	-	-	Little	Herzig et al. 2004
hAPP-Arc	APP (E693G, K670N/M671L, V717F)	-	Yes	-	-	-	-	Little	Cheng et al. 2004
Tg-ArcSwe	APP (E693G, K670N/M671L)	Yes	Yes	-	-	-	-	Yes	Lord et al. 2006 Knobloch et al. 2007
APPArc	APP (E693G)	-	Yes	-	-	-	-	Yes	Rönnbäck et al. 2011
TAPP	APP (K670N/M671L), Tau (P301L)	-	Yes	-	Yes	-	-	-	Lewis et al. 2001
3xTg-AD	APP (K670N/M671L), Tau (P301L), PS1 (M146V)	Yes	Yes	Yes	Yes	-	No	-	Oddo et al. 2003
APP_{SL}/PS1	APP (K670N/M671L, V717I), PS1 (M146L)	Yes	Yes	-	-	Yes	Yes	-	Wirths et al. 2002
APP/PS1KI	APP (K670N/M671L, V717I), PS1 (M233T/L235P)	Yes	Yes	-	-	Yes	Yes	-	Casas et al. 2004
5xFAD	APP (K670N/M671L, I716V, V717I), PS1 (M146L/L286V)	Yes	Yes	-	-	Yes	Yes	-	Oakley et al. 2006

CAA = cerebral amyloid angiopathy; Dash (-) = not reported.

Table 1 – Overview of main transgenic AD mouse models with their neuropathological features. (Schaeffer, Figueiro, & Gattaz, 2011)

Glial cells

For a long time glial cells were considered as the supportive cells of the central nervous system (CNS), however the current glia-oriented research reveals that glial cells are essential for proper neuronal functioning of neurons. Although they do not directly take part in synaptic interactions and electrical signaling, glial cells support the defining of synaptic contacts and signaling abilities. Among other the core supportive roles of glial cells are maintaining the ion/neurotransmitter homeostasis in CNS, providing physical support to neurons during neural development, responding to injury, participating in the blood-brain barrier, regulating the uptake of neurotransmitters in synapses, exchanging metabolites with neurons and insulating nervous pathways. Unlike neurons glial cells have retained the ability of mitosis and as stem cells they participate in gliogenesis and neurogenesis (Rodríguez & Verkhratsky, 2011), also their membrane comprises various ion channels, receptors and transporters, such as voltage-dependent K^+, Cl^- or Ca^{2+} channels or transporters for glutamate or K^+ uptake.

The four known types of glial cells in the CNS are astrocytes, microglia, oligodendrocytes and NG2 glia also called synantocytes.

Astrocytes

Besides their structural function astrocytes play a key role in providing suitable environment for neurons and they participate in neuronal synaptic connections. Astrocytes express functional neurotransmitter receptors and transporters, thus strongly contributing to neurotransmitter homeostasis. Other major functions of astrocytes include the formation of the blood-brain barrier, regulating the cerebral blood-flow and metabolic supply for neurons (Magistretti, 2006). Furthermore, astrocytes have the ability to integrate and respond to excitatory external inputs by generating Ca^{2+} oscillations and to release gliotransmitters, thus bridging neurons, vascular cells and distant glia (Volterra & Meldolesi, 2005).

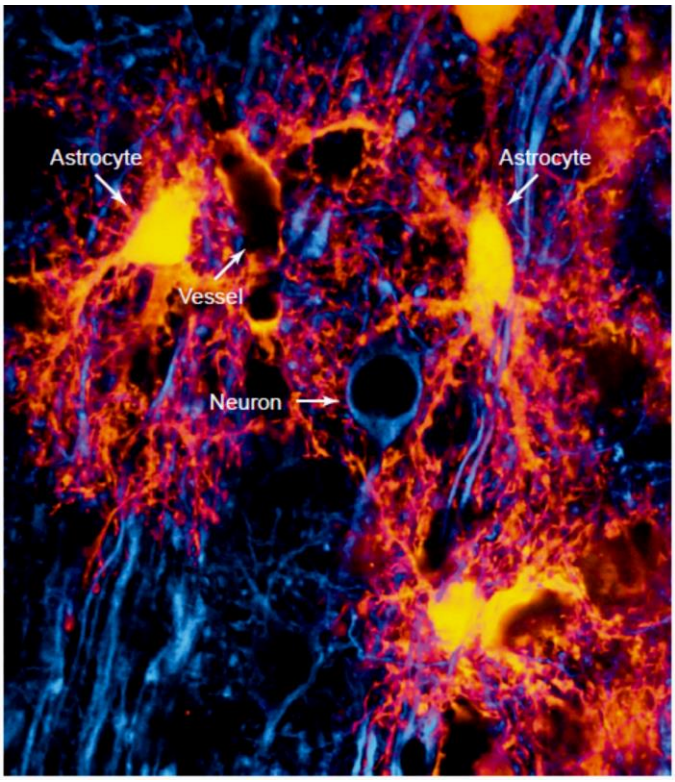

Figure 3 – Organization of astrocytes and neurons in rat cortex. Neurons are labeled blue by MAP-2 and astrocytes are labeled yellow by eGFP (Nedergaard, Ransom, & Goldman, 2003).

Morphologically astrocytes are divided into two major types. Fibrous astrocytes have long unbranched processes and are found primarily in white matter. Protoplasmic astrocytes are located mainly in gray matter and their processes are short and highly branched (Miller & Raff, 1984). During the process of aging the ability of astrocytes to protect neurons is diminished. During aging the Ca^{2+} homeostasis is disrupted in astrocytes and the Ca^{2+} signaling dynamics are noticeably faster due to decreased mitochondrial Ca^{2+} uptake. Astrocytes of aged CNS are also more sensitive to oxidative stress, which further contributes to the aging process (Lin et al., 2007).

In AD the occurrence of amyloid plaques is associated with the presence of reactive astrocytes. This specific glial reaction caused by acute or chronic brain insults is generally known as reactive astrogliosis during which astrocytes undergo morphological and functional changes. In severe cases the newly proliferated astrocytes form a glial scar in the brain tissue (Sofroniew, 2009). Via glutamate transporter reversal or enhanced gliotransmission, reactive astrocytes increase neurotoxic molecules, such as glutamate, in extracellular space, and resulting glutamate cytotoxicity leads to neuronal loss. In AD reactive astrocytes lose their

neuroprotective functions and more importantly, they even contribute to the progress of the disease. Likewise, neurotransmitter homeostasis and metabolic exchange can be impaired due to changes in reactive astrocytes (Heneka et al., 2010). In proximate astrocytes Aβ deposits cause spontaneous Ca^{2+} signaling and changes in Ca^{2+} oscillations. Reactive astrocytes also participate in neuroinflammation by releasing proinflammatory molecules such as cytokines and reactive oxygen species (ROS) (Verkhratsky et al., 2010). Astrocytes distant to amyloid plaques become atrophic as a result of losing their processes. This leads to synaptic loss, which underlies cognitive impairment in early stages of AD (Yeh et al., 2011).

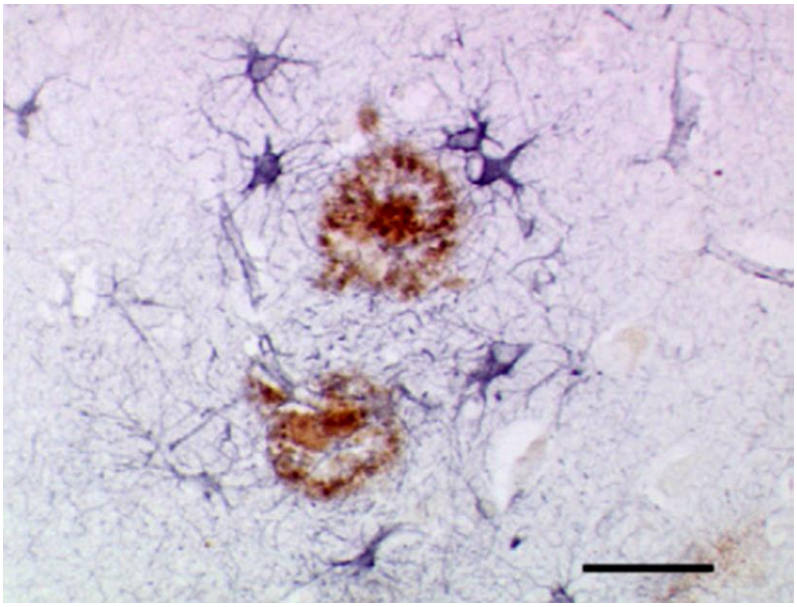

Figure 4 – Activated astrocytes surrounding Aβ plaques in a double immunostained brain section of a 70 year old AD patient. Astrocytes are stained by antibody against glial fibrillary acidic protein (blue) and Aβ by 6E10 (brown) (Heneka et al., 2010).

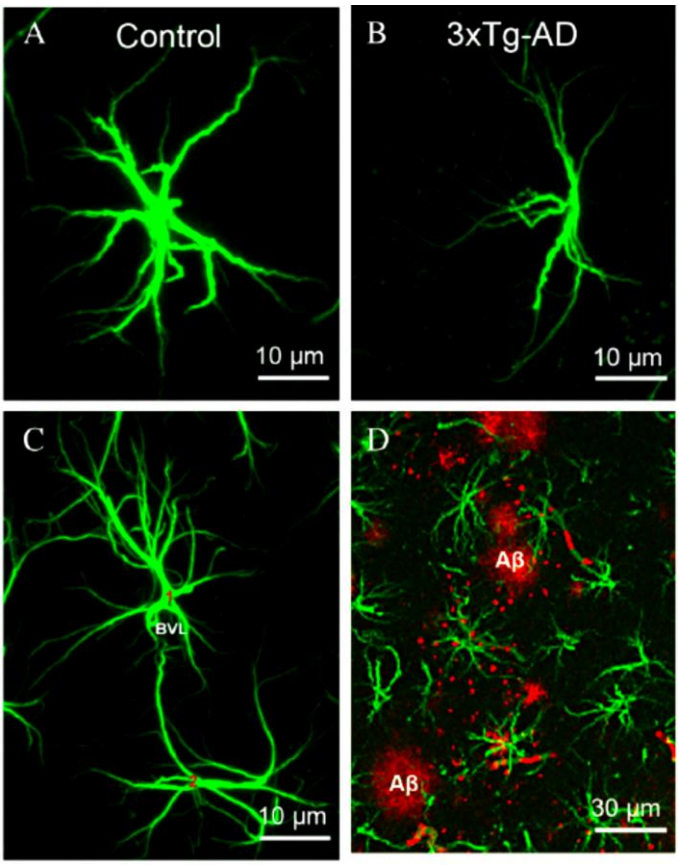

Figure 5 – Confocal micrographs from 12 months old mouse models comparing astrocytes under physiological conditions/aging (A,C) and astrocytes from 3xTg-AD mouse models (B,D). The images show atrophy of astrocytes distant from amyloid plaques by losing processes (B) and astrocytes undergoing astrogliosis in the vicinity of Aβ plaques (labeled red) (D) (Heneka et al., 2010).

Microglia

Microglia represent the core effectors of the immune system in the CNS. They function as local macrophages, hence in their activated state microglia have the ability of phagocytosis and they are able to proliferate and migrate to injured or infected locations. Activated microglia also release proteolytic enzymes, chemokines, cytokines, complement proteins and various other molecules affecting astrocytes and other microglia during neuroinflammation (Ransohoff & Perry, 2009).

Resting microglia in the mature brain have a relatively small cell soma with a number of thin branched processes (J J Rodríguez et al., 2010). Each microglial cell in its non-activated state scans the microenvironment of its anatomical domain. Importantly, these domains are strictly bounded and non-overlapping (Heneka et al., 2010).

Microglia undergo various changes during aging. The total number and density of microglia increase with age, perhaps to compensate their impaired protective functions. Senescent microglia have decreased motility and ability of migration, altered production and release of pro-inflammatory factors and impaired ability of degrading phagocytized material (Mosher & Wyss-Coray, 2014).

Microglia in AD can have neuroprotective functions in the early stages of the disease due to their ability to phagocytize Aβ. However during the progression of AD the struggle of microglia to digest the massive quantity of Aβ possibly promotes the neuroinflammation processes (Lue, Walker, & Rogers, 2001). In later stages of AD, uncontrolled inflammation caused by over-activated microglia result in promoting Aβ aggregation and neuronal loss. Moreover, pro-inflammatory cytokines released by microglia can also modify specific kinases leading to hyperphosphorylation of the tau protein and generating NFTs (Solito & Sastre, 2012).

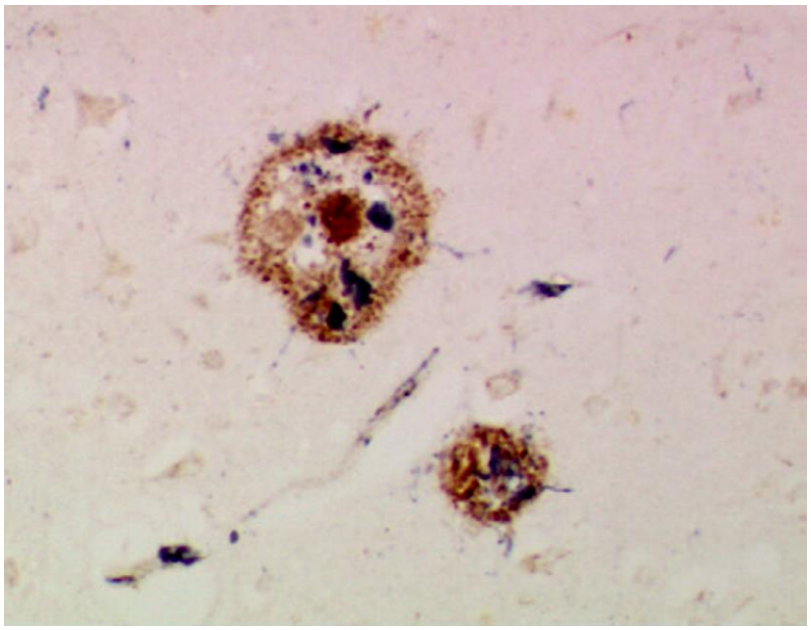

Figure 6 – Activated microglia in the vicinity of Aβ plaques. Aβ stained brown and microglial cells stained blue by CD68 (Heneka et al., 2010).

Oligodendrocytes

Besides the well-known function of myelination of neuronal axons oligodendrocytes have several other important roles. They are responsible for sodium channel clustering along axon segments necessary for generating action potentials and also appear to be essential for proper axonal transport and microtubule levels. Furthermore, oligodendrocytes provide trophic support for neuronal somas (McTigue & Tripathi, 2008).

Morphologically oligodendrocytes are cells with many branched processes and upon terminal differentiation they extend numerous parallel processes to ensheathe and myelinate neuronal axons (Osterhout, 1999).

During aging several changes in myelin sheaths occur, such as forming dense cytoplasm regions and so called "balloons". These changes both indicate the signs of myelin degeneration. On the other hand, surprisingly, myelin production appears not to be impaired during aging (Peters, 2003).

During AD progression oligodendrocytic membranes might be aberrated by Aβ due to its ability to damage membranes rich in cholesterol (Roth et al., 2005). This would result in diminishing the capability of oligodendrocytes to form myelin sheaths, thus affecting the conduction of axonal signals and eventually leading to neurodegeneration (Horiuchi et al., 2012). Furthermore, by losing the trophic support from oligodendrocytes neurons become more vulnerable to damage and neuroinflammation (Roth et al., 2005).

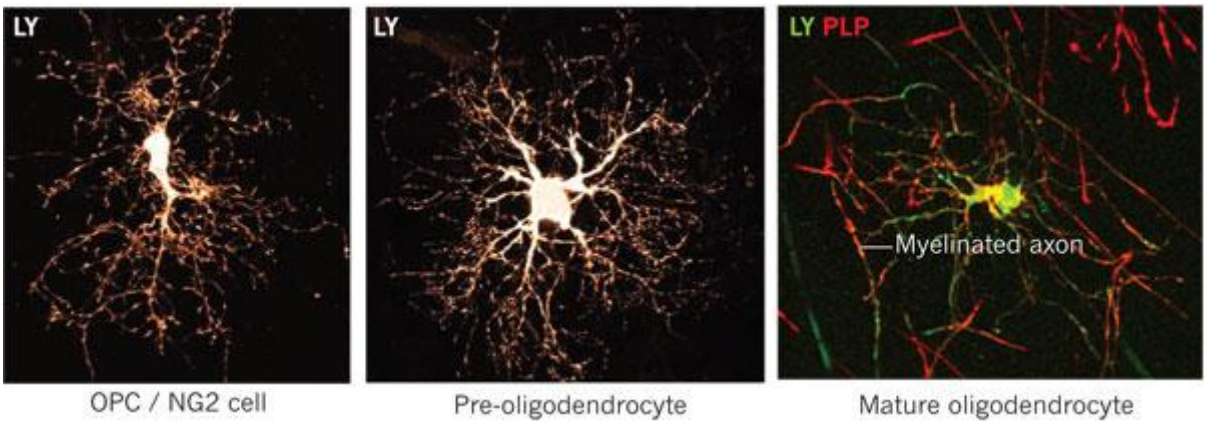

Figure 7 – The development of oligodendrocytes from NG2 cells (Nave, 2010).

NG2 glia/polydendrocytes

Synantocytes, also referred to as polydendrocytes or NG2 cells, are a recently discovered type of glial cells with the function of progenitor cells. Synantocytes are able to generate oligodendrocytes, astrocytes (Zhu, Bergles, & Nishiyama, 2008) and possibly even neurons (Rivers et al., 2008). They also might participate in stabilizing synapses and guiding axonal growth (A. M. Butt et al., 2005). The number of synantocytes increases during injury and infection due to their capability of proliferation, thus being able to produce new astrocytes and oligodendrocytes (Levine, Reynolds, & Fawcett, 2001).

Synantocytes are morphologically described as cells with a central soma and numerous processes slightly overlapping between nearby synantocytes. In gray matter the processes integrate with neuronal synapses.

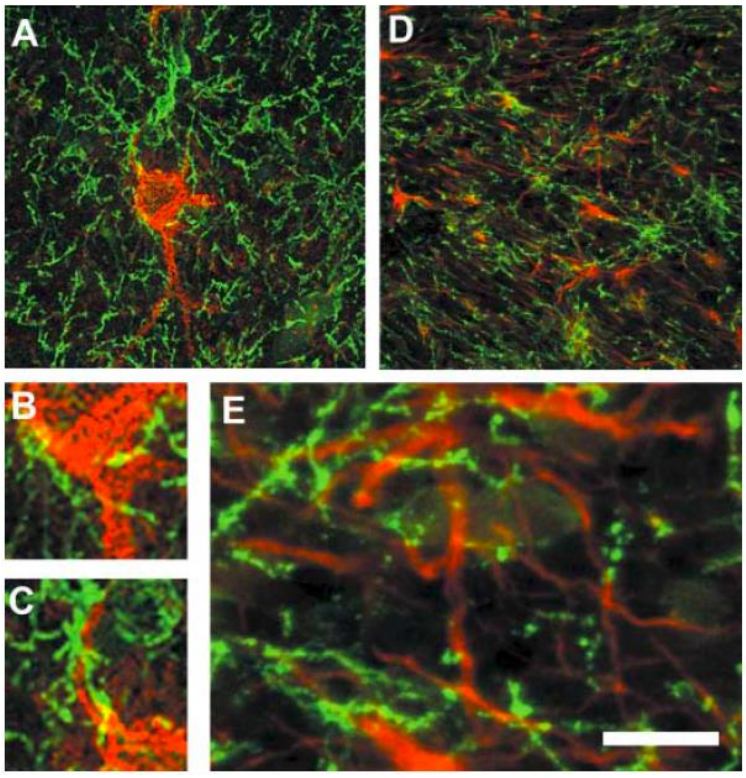

Figure 8 – Immunofluorescence images showing NG2 glia in contact with neurons and astrocytes. Double immunofluorescence labelling NG2 (green), neurons (red) with calbindin (A-C) and astrocytes (red) with glial fibrillary acidic protein (D-E). Scale 50 μm (A,D) and 12,5 μm (B,C,E) (A. M. Butt et al., 2005).

Synantocytes play a key role in remyelination (Levine & Reynolds, 1999), hence their diminished capacity for regenerating oligodendrocytes is proposed to be a critical factor causing myelin loss in the aging brain (A. Butt, 2014).

In the early stages of AD, synantocytes display atrophic changes, furthermore, with the progression of the disease they appear to become hypertrophic and are closely related to Aβ deposits. Importantly, the proliferation of synantocytes might be impaired, thus inducing enhanced myelin loss in AD (A. Butt, 2014).

Calcium signaling during Alzheimer's disease

Calcium homeostasis in the nervous system is essential for appropriate functioning of the brain. Therefore, dysregulation of calcium homeostasis and thus disruption of calcium signaling is proposed to be one of the common proximal causes of neurodegeneration and neuronal dysfunction during aging and AD (Khachaturian, 1994). According to the "calcium hypothesis of brain aging and Alzheimer's disease", the dysregulation of intracellular calcium homeostasis characterizes one of the earliest hallmarks of AD (Khachaturian, 1994). Disturbance in neuronal calcium signaling occurs preceding to the advancement of histopathological markers and clinical symptoms (LaFerla, 2002); however, it is not yet clear if dysregulation of calcium homeostasis could be the primary cause of AD-related pathological features.

Intracellular calcium signaling affects many crucial physiological functions including gene expression, initiation of apoptosis, cell differentiation or neurotransmitter release (Berridge, Bootman, & Roderick, 2003), therefore calcium homeostasis must be strictly regulated. Importantly, any disruption of this balance may result in cell degeneration and dysfunction. Furthermore the hypothesis suggests that dysregulation of the intracellular calcium homeostasis might lead to age-related functional changes in brain cells, hence aberrant calcium signaling could be a common sign of aging and AD (Khachaturian, 1994).

Recent studies suggest that dysregulation of calcium homeostasis does not reflect only to neurons, but inflicts all cell types of the nervous system (McLarnon, Choi, Lue, Walker, & Kim, 2005). The inflammatory response of astrocytes and microglia is significantly affected by aberrant calcium signaling activated during AD, consecutively resulting in impaired calcium homeostasis of neurons and thus affecting proper brain functions. These interactions

between altered calcium signaling and the inflammatory responses of glial cells are thought as one of the factors increasing the progression of AD (Brawek & Garaschuk, 2014). For example, activated glial cells produce pro-inflammatory cytokines, which affect neuronal calcium signaling by modifying their voltage-sensitive Ca^{2+} channels, N-methyl-D-aspartate (NMDA) receptors, inositol triphosphate receptors (IP3R), ryanodine receptors (RyR) and other Ca^{2+}-dependent receptors (Halassa & Haydon, 2010; Sama & Norris, 2013). Moreover the production and release of the cytokines itself is regulated by Ca^{2+} signaling (Lee et al., 2012).

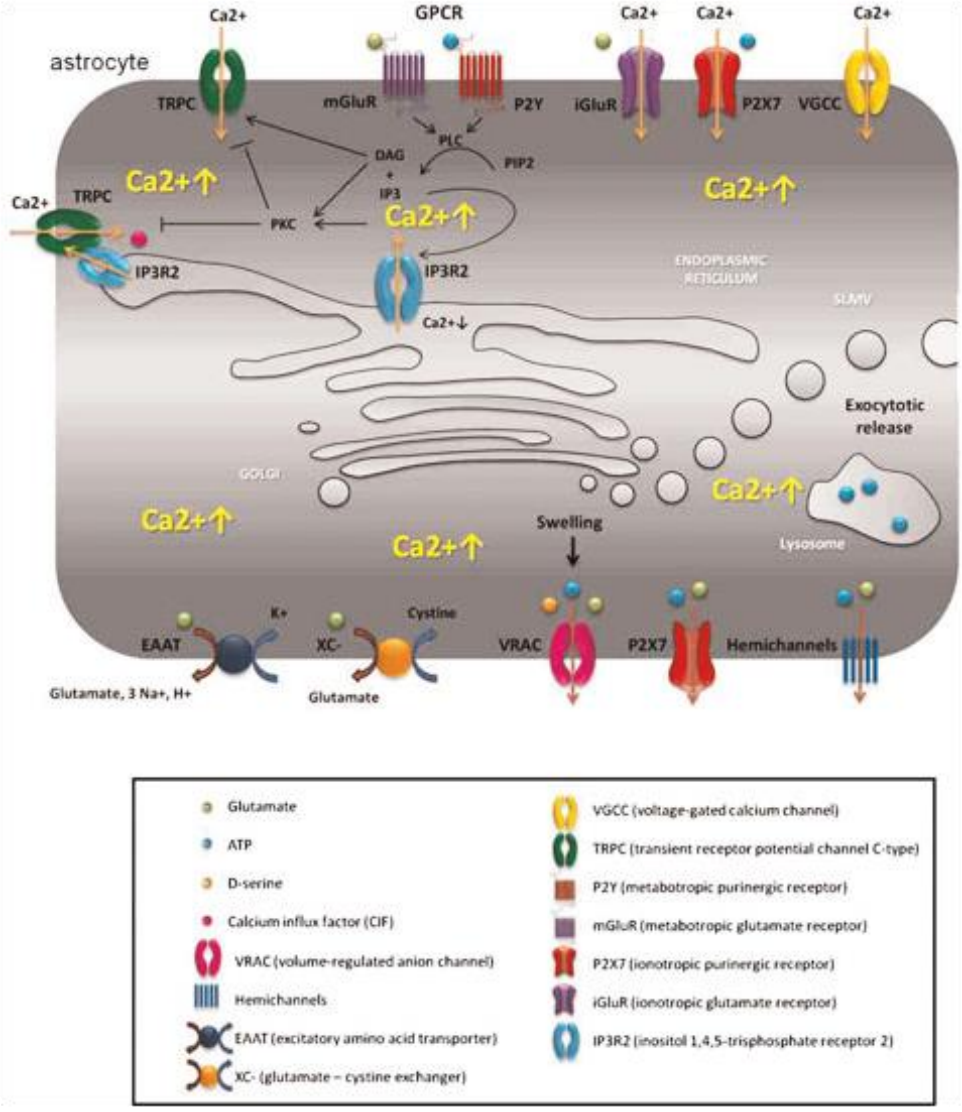

Figure 10 – Scheme showing various calcium channels expressed on surface of an astrocyte responsible for maintaining calcium signaling and homeostasis. Calcium release from internal stores is triggered mainly by the phospholipase C pathway activated by G-protein coupled receptors (GPCRs). Phosphatidylinositol-4,5-bisphosphate (PIP2) breaks down into 1,2-diacylglycerol (DAG) and inositol triphosphate (IP3), which may trigger calcium release from the endoplasmic reticulum by activating IP3 receptors on its surface. P2Y purinergic receptors and metabotropic glutamate receptors type 3 and 5 (mGluR3, mGluR5) are the major types of GPCRs in astrocytes (Achour et al., 2010).

Impaired calcium signaling in astrocytes

In astrocytes calcium affects many important physiological functions including responses to neurotransmitters (Fields, 2000; Haydon, 2001), regulation of gene expression (Neary, 2000) and the release of ATP, glutamate and other gliotransmitters (Jeftinija et al., 1996). Furthermore, astrocytes can impact neurons by generating intercellular calcium waves that transfer signals, which can modify their activity and survival (Blanc, Bruce-Keller, & Mattson, 1998). During AD, calcium signaling of astrocytes is impaired or altered by their activation in the vicinity of Aβ deposits (Mattson & Chan, 2003).

In a sub-group of cultured astrocytes, exposure to Aβ(1-42) causes Ca^{2+} to enter the cells from the extracellular space, thus increasing basal intracellular calcium concentration ($[Ca^{2+}]_i$). Plaque-associated astrocytes also appear to upregulate the expression of inositol-3-phosphate receptors (IP_3R) and glutamate receptor mGluR5 (Grolla et al., 2013; Lim et al., 2013). MGluR5 is a metabotropic receptor, of which activation leads to Ca^{2+} release from intracellular stores. In a healthy adult brain its expression is downregulated (Sun et al., 2013), hence the increase during AD may cause disruption of calcium homeostasis in astrocytes.

Similarly, spontaneous Ca^{2+} oscillations induced by ATP are largely increased in 3xTg-AD mouse models in comparison with control mice of the same age (Grolla et al., 2013). The data from these studies hence propose that calcium signaling in astrocytes during AD is amplified. Importantly, the Ca^{2+} oscillations evoked by Aβ triggers the death of neurons which have been co-cultured with the astrocytes, thus suggesting that neurotoxicity might be a result of impaired astrocytic Ca^{2+} signaling (Abramov et al., 2003).

In a healthy brain, spontaneous Ca^{2+} oscillations are considered as a form of communication between astrocytic and neuronal networks (Hirase et al., 2004; Nimmerjahn, 2009), hence aging and other pathological processes including AD noticeably alter astrocytic calcium signaling. Higher frequency of spontaneous Ca^{2+} oscillations has been observed in Bergman glia, the astrocytes of the cerebellum, due to aging (Mathiesen et al., 2013). Likewise, the frequency and synchronicity of Ca^{2+} oscillations increase in astrocytes of amyloid-depositing mice (Grienberger et al., 2012; Kuchibhotla et al., 2009). Moreover, higher levels of intracellular calcium deposits occur in astrocytes due to Aβ (Kuchibhotla et al., 2009).

Enhanced Ca^{2+} signaling in reactive astrocytes is likely to affect other glial cells and neurons due to the fact that the release of gliotransmitters and inflammatory factors such as cytokines depends on Ca^{2+} signals (Volterra & Meldolesi, 2005). Gliotransmitters such as ATP, glutamate and D-serine impact neuronal activity, thus increased release of these factors can result in the hyperactivity of neurons during AD (Brawek & Garaschuk, 2014). Moreover, ATP stimulates the activation of astrocytic P2Y1 receptors which are known to induce the release of another gliotransmitter, glutamate (Pascual et al., 2012), and cytokines such as tumor necrosis factor alpha (TNF-α; Domercq et al., 2006), hence acting as a signal of positive feedback. Furthermore, TNF-α evokes the release of glutamate, thus enhanced release of TNF-α significantly amplifies the release of glutamate, which is likely to cause neuronal damage (Bezzi et al., 2001). Importantly, increased release of TNF-α and thus glutamate occurs in astrocytes in the vicinity of activated microglia. On the contrary, the presence of Aβ deposits decreases the glutamate release caused by TNF-α (Rossi et al., 2005), yet the mechanism of this process is not utterly clarified.

Moreover, TNF-α released form reactive astrocytes during AD is assumed to increase neuronal excitability, since mice treated with TNF-α infusions following peripheral inflammation are more susceptible to seizure (Riazi et al., 2008). TNF-α is also thought to enhance the expression of α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) receptors on the cell surface of neurons, which are more permeable to Ca^{2+} (Ogoshi et al., 2005). Hence the higher permeability of AMPA receptors results in extra Ca^{2+} inflow to the cell, therefore possibly affecting gene expression and causing cytotoxicity of neurons (Galic, Riazi, & Pittman, 2012). Similarly, the increased release of TNF-α results in processes such as endocytosis of $GABA_A$ receptors and modifying glutamate uptake in glial cells (Galic et al., 2012), which both affect neuronal activity.

In vivo higher excitability of neurons has been observed also as a result of enhanced release of the cytokine interleukin-1 (IL-1β) from astrocytes (Rodgers et al., 2009), furthermore increased concentration of IL-1β in the brain is linked with the susceptibility to seizures (Allan & Rothwell, 2001).

AD-associated neuronal hyperactivity caused by reactive astrocytes could also be contributed to by changes of extracellular Ca^{2+} levels (Brawek & Garaschuk, 2014). The concentration of extracellular Ca^{2+} decreases due to higher neuronal activity as a result of activating voltage-gated Ca^{2+} channels and ionotropic glutamate receptors causing astrocytes

to release ATP (Torres et al., 2012). Therefore, the increased levels of extracellular ATP induce astrocytic Ca^{2+} waves affecting neuronal activity (Kuchibhotla et al., 2009).

Taken together, the alterations of astrocytic Ca^{2+} signaling can be caused by Aβ-deposits, increased release of gliotransmitters and cytokines such as glutamate, ATP, TNF-α and IL-1β and likewise by lower extracellular calcium levels caused by enhanced neuronal activity. Henceforth the enhanced levels of gliotransmitters and cytokines may result in neuronal hyperactivity, neurodegeneration, seizures and furthermore a significantly stronger microglial and astrocytic inflammatory response.

Impaired calcium signaling in microglia

During AD microglia surround Aβ-plaques shortly after their deposition (Meyer-Luehmann et al., 2008) and similarly to astrocytes become activated (Itagaki et al., 1989). Activated microglia release pro-inflammatory factors which can affect astrocytes and neurons in the vicinity of the plaques, therefore possibly resulting in aberrant Ca^{2+} signaling of these cells (Eichhoff et al., 2011). Recent *in vitro* studies show that during AD impaired Ca^{2+} signaling does not impact merely reactive astrocytes and neurons, however, likewise affects activated microglia. Compared to microglial cells isolated from healthy patients, intracellular Ca^{2+} concentrations occur to be increased in microglia from the AD-affected individuals (McLarnon et al., 2005). Likewise, higher levels of intracellular Ca^{2+} concentration appearing together with a decline of agonist-induced Ca^{2+} signals, has been observed in cultured murine microglia treated with lipopolysaccharide (LPS)-mediated activation (Hoffmann et al., 2003).

According to a recent study comparing the responds of resting and activated microglia to ATP and substance P, activated microglia appear to have enhanced Ca^{2+} signals reacting to these signaling molecules (Seifert et al., 2011). Thus, suggesting that one of the notable features of activated microglia during AD is the increase of Ca^{2+} signaling. Importantly, the concentration of intracellular Ca^{2+} significantly affects the release of pro-inflammatory factors, for instance nitric oxide (NO) and several cytokines are released in response to higher levels of intracellular Ca^{2+}, according to a study of LPS-activated cultured microglial cells (Hoffmann et al., 2003).

Similarly to astrocytes, the release of the pro-inflammatory cytokine TNF-α in microglia also depends on Ca^{2+} signaling. In cultured microglia TNF-α is released as a result

of activating P2 receptors (Ikeda et al., 2013). Importantly, microglial cells initiate the inflammatory-response by releasing pro-inflammatory mediators such as TNF-α, glutamate or ATP, which furthermore affect astrocytic and neuronal activity (Eichhoff et al., 2011).

Due to presence of damaged neuronal cells, Ca^{2+} oscillations known as damage-induced Ca^{2+} transients (DICTs) are induced in microglia (Eichhoff et al., 2011) and such DICTs may be a result of P2Y receptor activity, which is induced by ATP released from damaged cells (Brawek & Garaschuk, 2014). ATP serves as a signaling molecule in both healthy and damaged cells; hence higher levels of extracellular ATP suggest pathological features, such as disrupted cell membrane, inflammation or stress (Trautmann, 2009). During inflammation and activation of microglia the release of ATP is increased due to enhanced Ca^{2+} signaling (Imura et al., 2013). Therefore, higher levels of extracellular ATP might be considered as one of the attributes of AD and aging (Eichhoff et al., 2011). Increased release of ATP during AD results both in basic microglial inflammatory processes (Di Virgilio et al., 2009) and also in amplifying microglial Ca^{2+} signaling. Latter caused by the variety of P2 receptors expressed on their surface (Eichhoff et al., 2011).

Importantly, also the expression of various P2 receptors is enhanced in activated microglia. Upregulation of the $P2X_7$ receptor has been observed in human Aβ-associated microglia from individuals with AD, as well as in cells isolated from the brains of Tg2576 mice (Parvathenani et al., 2003) and likewise in hippocampal microglial cells of AD mouse models treated with Aβ(1-42) injections (McLarnon et al., 2006). In summary, increased Ca^{2+} signaling in microglia both agonist-induced as well as spontaneous might possibly be viewed as an important hallmark of aging and likewise various types of dementia including AD, together with the fact that higher levels of extracellular ATP have a key role in this pathological feature (Brawek & Garaschuk, 2014).

In addition, increased extracellular ATP levels can cause activation of a protein complex known as the nucleotide binding and oligomerization domain-like receptor family pyrin domain containing 3 (NLRP3) inflammasome as a result of enhanced intracellular calcium release caused by the activation of $P2X_7$ receptors (Murakami et al., 2012). This particular inflammasome is responsible for the release of pro-inflammatory cytokines such as IL-1β and IL-18 by activating caspase-1, which causes the cleavage of these cytokines into their mature forms, thus, contributing to the inflammatory response of microglial cells (Franchi et al., 2009). Likewise, the NLRP3 inflammasome is also activated by Aβ (Halle et

al., 2008) and interestingly, enhanced activation of NLRP3 can be observed in microglia found in the vicinity of Aβ-plaques in an AD mouse model (Heneka et al., 2013). Assuming the fact that NLRP activation is prompted by releasing Ca^{2+} from intracellular stores of the cell, together with the possible impact of Aβ-deposits, activated microglia during AD are thought to show higher levels of intracellular Ca^{2+}. Henceforth, enhanced activation of NLRP3 could lead to increased release of the cytokines affected by this inflammasome, and consequently, it may result in the hyperactivity observed in neurons during AD (Galic et al., 2012).

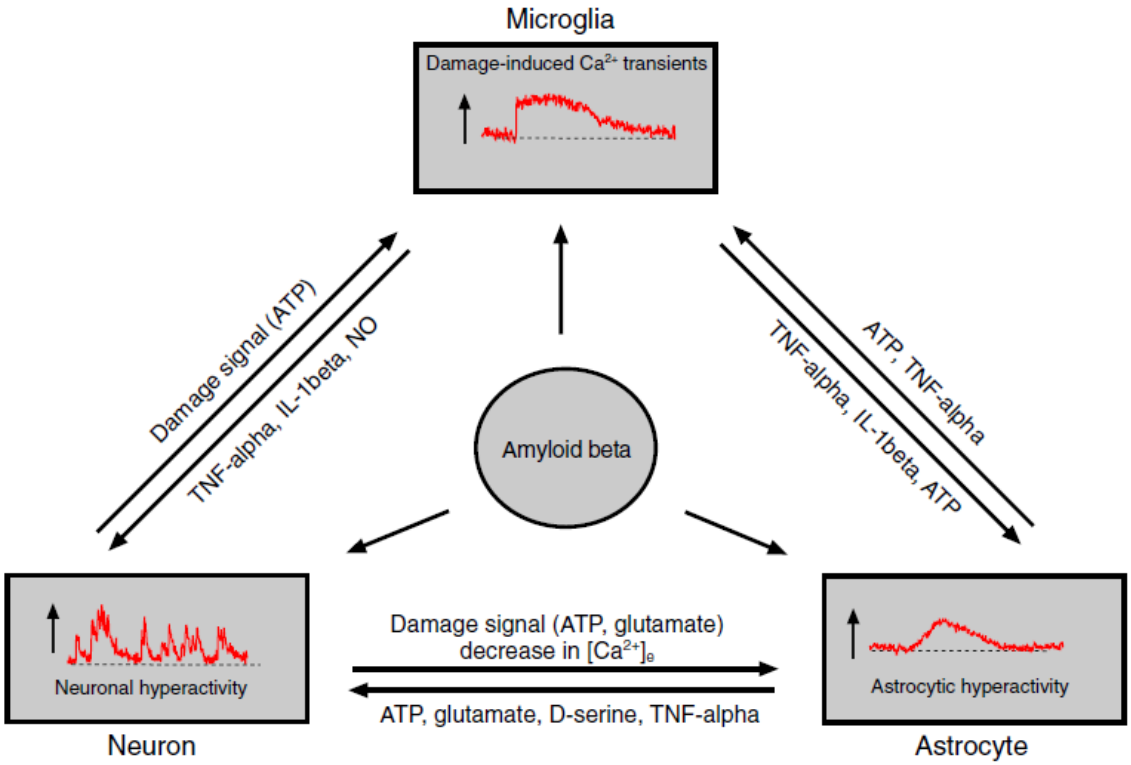

Figure 11 – Illustrative scheme of network wide calcium homeostasis dysregulation during AD showing how astrocytes, microglia and neurons affect each other in the presence of Aβ deposits (Brawek & Garaschuk, 2014; Eichhoff et al., 2011).

Oligodendrocytes and NG2 glia

The mechanisms of impaired calcium signaling in oligodendrocytes during AD are not yet entirely clear, hence recent studies suggest that aberration of calcium homeostasis in oligodendrocytes can contribute to axon demyelization and moreover glutamate induced cell death. A recent study shows that increased resting intracellular calcium levels occur in PS1 mutant mice compared with wild-type mice. Furthermore, oligodendrocytes isolated from

these mouse models are more prone to Aβ and glutamate damage. Degeneration of oligodendrocytes triggered by calcium homeostasis disruption may lead to axonal dysfunction and contribute to cognitive impairment during AD (Pak, Chan, & Mattson, 2003). Generally, oligodendrocytes express ionotropic glutamate receptors, especially Ca^{2+}-permeable AMPA and NMDA receptors and their activation in the presence of increased glutamate levels might result demyelization. NMDA receptors are specifically present in the myelinating processes of oligodendrocytes, in which the small intracellular space may result in a large increases in intracellular ion concentration in response to receptor activation and thus lead to disintegration of myelin and oligodendrocytic processes (Káradóttir et al., 2005).

Until now, there are no data about polydendrocyte pathophysiology in AD progression, which is not that surprising because these cells were discovered quite recently and ongoing polydendrocyte-oriented research is mostly focused on their functions in CNS under physiological conditions or their differentiation potential following various types of CNS injuries. Recent findings of Nielsen and colleagues (Nielsen et al., 2013) revealed that both oligomeric and fibrillar Aβ(1-42) induced changes in NG2 cell morphology and that in vitro exposure to fibrillary Aβ(1-42) decreased the NG2 concentrations in both cell lysates and supernatants. Comparing to non-demented individuals they also found significantly decreased levels of soluble NG2 in the cerebrospinal fluid (CSF) from clinically diagnosed AD patients and that the CSF NG2 levels significantly correlated with the core AD biomarkers Aβ(1-42), T-tau and P-tau.

Conclusion

Glial cells in a healthy brain sustain various crucial roles contributing to the right functioning of the central nervous system. For instance, they maintain synaptic contacts, provide support for neurons, insulate nervous pathways, participate in the blood-brain barrier and preserve homeostasis in the CNS. Importantly, glial cells play a major role in the immune response to brain damage or disease by triggering inflammation processes. Most of these essential functions are impaired during AD especially the inflammation response. Glial cells, primarily astrocytes and microglia, are affected in the presence of Aβ deposits by hyperactivation, causing increased inflammation and possibly neuronal damage or death.

A significant feature of AD-related malfunctioning of glial cells is dysregulation of their calcium homeostasis. Cells affected by Aβ show increased levels of intracellular calcium concentrations, thus resulting in aberrant calcium signaling affecting neurons and other glial cells. This process supports AD progression by enhancing the glial inflammatory response and causing hyperactivity of neurons. Therefore, the calcium hypothesis of AD is suggested to bring together the amyloid cascade hypothesis and the inflammation cascade hypothesis creating a meaningful link. Above all, since there is no known cure for AD, treating the calcium homeostasis of glial cells by for example stabilizing their intracellular calcium concentrations might be a promising approach in therapy, thus more research in this area is necessary.

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