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Astrocyty u neurodegenerativních onemocnění

Astrocytes in neurodegenerative disorders

Bakalářská práce

Školitelka: Ing. Miroslava Anděrová, CSc.

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 7.5.2019

Podpis

Poďakovanie:

Chcela by som sa poďakovať predovšetkým svojej školiteľke Ing. Miroslave Anděrovej, CSc. za jej cenné rady, odborné vedenie a trpezlivosť. Osobitná vďaka patrí aj mojej rodine a priateľom, ktorí pre mňa boli veľkou podporou pri písaní tejto práce.

Abstract

Astrocytes are the most abundant glial cells in a mammalian brain. They play an important role not only under physiological conditions, but also during pathological changes. They are involved in miscellaneous functions in a healthy tissue, such as: structural and developmental function, homeostasis maintenance, metabolic support for neurons, or reduction of oxidative stress. In a damaged brain, however, their activity is altered. The most common astrocytic changes in a diseased or injured brain/spinal cord are known as reactive astrogliosis and glial scar formation. Other alterations like cellular atrophy, membrane transporters impairments, or over-expression of certain astrocytic proteins may occur as well. These morphological and physiological changes often lead to an increased excitotoxicity which is one of the factors involved in neurodegeneration. This thesis discusses the astrocytic changes during selected neurodegenerative diseases, namely: Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's disease and Parkinson's disease.

Key words: brain, astrocytes, reactive astrogliosis, homeostasis, neurodegeneration, Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis

Abstrakt

Astrocyty jsou nejpočetnějšími gliovými buňkami v savčím mozku. Hrají důležitou roli nejen za fyziologických podmínek, ale také při patologických změnách. Jsou zapojeni do různých funkcí ve zdravé tkáni, jako jsou: strukturní a vývojová funkce, udržování homeostázy, metabolická podpora neuronů nebo redukce oxidačního stresu. V poškozeném mozku se však jejich aktivita mění. Nejčastější astrocytické změny v nemocném nebo poraněném mozku/míše jsou známé jako reaktivní astroglióza a tvorba gliálních jizev. Mohou se vyskytnout i jiné změny, jako je buněčná atrofie, poruchy membránových transportérů nebo nadměrná exprese určitých astrocytických proteinů. Tyto morfologické a fyziologické změny často vedou ke zvýšené excitotoxicitě, která je jedním z faktorů zapojených do neurodegenerace. Tato práce pojednává o astrocytických změnách během vybraných neurodegenerativních onemocnění, a to: Alzheimerovy choroby, amyotrofické laterální sklerózy, Huntingtonovy choroby a Parkinsonovy choroby.

Klíčová slova: mozek, astrocyty, reaktivní astroglióza, homeostáza, neurodegenerace, Alzheimerova choroba, Parkinsonova choroba, Huntingtonova choroba, amyotrofická laterální skleróza

List of abbreviations

Aβ	- β-amyloid
AD	- Alzheimer's disease
ALDH1L1	- aldehyde dehydrogenase 1 family
ALS	- amyotrophic lateral sclerosis
ANLSH	- astrocyte neuronal lactate shuttle hypothesis
AQP4	- aquaporin 4
ATP	- adenosine triphosphate
CA	- carbonic anhydrase
CNS	- central nervous system
COX-2	- cyclooxygenase
Cx30	- connexin 30
Cx43	- connexin 43
EAAT	- astrocytic excitatory amino acid transporter
ECS	- extracellular space
fALS	-familial amyotrophic lateral sclerosis
FDH	- 10-formyltetrahydrofolate dehydrogenase
GABA	- gamma-aminobutyric acid
GFAP	- glial fibrillary acidic protein
GLAST	- glutamate-aspartate transporter
GLUT1	- glucose transporter
GS	- glutamine synthetase
GSH	- glutathione
γGT	- γ-glutamyl transpeptidase
HD	- Huntington's disease
IL-1β	- interleukin 1 beta
iNOS	- inducible nitric oxide synthase

Kir	- inwardly rectifying potassium channels
LDHA	- lactate dehydrogenase isoenzyme A
LDHB	- lactate dehydrogenase B
LPS	- lipopolysaccharide
MCT-1/2/4	- monocarboxylate transporters 1/2/4
NBC	- Na+-HCO3- cotransporter
NFT	- neurofibrillary tangles
NG2 cells	- polydendrocytes
NHE	- sodium-hydrogen exchange
NO	- nitric oxide
PD	- Parkinson’s disease
PSP	- progressive supranuclear palsy
ROS	- reactive oxygen species
S100B	- calcium and zinc binding protein, astrocytic marker
sALS	- sporadic amyotrophic lateral sclerosis
SN	- substantia nigra
SOD1	- copper-zinc superoxide dismutase
TNF-α	- tumor necrosis factor α
UTP	- uridine-5'-triphosphate

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Introduction

A couple of decades ago the neurobiological research focused almost exclusively on neurones. Only recently were the neurocentric concepts compromised by novel views on this matter, promoting the importance of glial cells in both physiological conditions and during brain pathologies.

Glia, previously considered only as components with passive, mostly structural functions, are now believed to be substantially involved in all sorts of mechanisms accounting for healthy functioning of central nervous system (CNS). Astrocytes, in particular, represent cells playing a crucial role in the maintenance of brain homeostasis. Furthermore, they are involved in neuronal metabolic support, antioxidant protection against oxidative stress, and provide for many other CNS functions. Based on this knowledge we may assume that astrocytes are essentially contributing to all sorts of pathological events in the CNS, since all neurological defects are basically disruptions of neuronal tissue homeostasis (Verkhratsky et al., 2013).

Neurodegeneration is a process of progressive neuronal loss leading to extreme cognitive defects. This study is focusing on the role of astrocytes during onset and progression of several neurodegenerative disorders, namely Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and Huntington's disease. The precise mechanisms underlying the astrocytic behaviour in the course of neurodegeneration are only yet to be resolved. Nevertheless, there is a wide range of evidence suggesting that the astrocytic morphology and physiology may be altered already at the early stages of the neurodegenerative process (Verkhratsky et al., 2013).

The aim of this work is to highlight the importance of astrocytes and to outline their principal roles in both physiological and pathophysiological conditions. The second part is dedicated to introduction of the major characteristics of selected neurodegenerative diseases and description of astrocytic changes accompanying the process of neurodegeneration. At the conclusion a comparison of the similarities in astrocytic responses to a previously mentioned neurodegenerative disorders is given.

Astrocytes

Neuroglia

Glial cells are regarded as the most numerous cells of the CNS. This broad cell group consists of microglia and macroglia, the latter comprising astrocytes, oligodendrocytes, NG2 cells and ependymal cells. They all contribute to maintaining the homeostasis of the CNS and have a major role in supporting the neurons (Simard & Nedergaard, 2004; Pekny & Pekna, 2018).

The term Neuroglia (Nervenkitt) was first introduced in 1856 by a German pathologist Rudolf Virchow. However, his idea of glia was somehow different from what we consider it to be nowadays. He ascribed Nervenkitt to a sort of connective tissue functioning only as a substance holding the brain cells together. During the next few decades new staining techniques have been developed, bringing more light to categorisation of the glial cells and understanding the importance of their roles in nervous system (Somjen, 1988; Parpura, 2012a).

Defining an astrocyte

It was in the 1890's when Michael von Lenhossek came with the name „Astrocytes" to refer to the glial cells in the higher vertebrates. By changing the nomenclature from the previous "glia" to astrocytes he pointed out the function of these cells and their equality with the nerve cells, since the original term (glia) indicated the role of these cells to be only passive, glue-like (Matyash & Kettenmann, 2009).

The etymology of the term astrocyte indicates its stellate morphology hence astrocytes could be also translated as star-like cells (Parpura, 2012). They are the most abundant glial cells in the CNS and are characterised by their leaflet-like processes. Their endfeet forming processes can either give rise to a glial membrane on the CNS surface or a perivascular sheet by attachments to the blood vessel (Simard & Nedergaard, 2004).

For a long period of time the main attributes that helped distinguish the astrocytes from other glial cells were considered to be their stellate morphology and the presence of the astrocytic fibrils – consisting of glial fibrillary acidic protein (GFAP) (Kimelberg, 2004). As the tools used for identifying astrocytes improved the categorization of these cells became less straightforward (Wang, 2008).

Despite all the confusions around astrocyte definition there is one property unifying the cells described as the astrocytes. This characteristic resides in their function of maintaining the homeostasis of the nervous system (Verkhratsky, 2012). They play a key role in all sorts of processes including brain-blood barrier formation, ion and neurotransmitter regulation, mediation of trans-cellular communication via gap-junctions and other (Verkhratsky & Parpura, 2010).

Classification

Astrocytes are a very diverse and heterogeneous class of cells divided into several subclasses depending on various criteria. Based on their location and morphology, astrocytes have been divided in to two subtypes: fibrous and protoplasmic. This classification was first introduced back in 1890's by Kolliker and Andriezen (Verkhratsky & Parpura, 2010; Andriezen,1893) .

Protoplasmic astrocytes predominate in the grey matter. In terms of their morphology, they are characteristic for the "bushy" structure of their processes also referred to as spongiform. These abundantly branched processes facilitate intimate associations with synapses (Bushong, 2004; Raff, 1984). The white matter is where fibrous astrocytes can be found, contacting the nodes of Ranvier. The orientation of their processes is along the fiber tracts (Matyash & Kettenmann, 2009; Allen & Eroglu, 2017). There are gap junctions formed between distal processes of neighbouring astrocytes in both of these cell subtypes and they are also consistent in making extensive contacts with blood vessels (Sofroniew & Vinters, 2010).

There are other types of astrocytes as well. Radial glia, for example, function during the development of the brain. In the cerebellum these cells are called Bergmann glial cells or Golgi epithelial cells. Their function is related to the development of cerebellum while the retinal development is associated with Muller glial cells (Pinto & Go, 2007; Muller, Kettenmann 1995).

Molecular markers

The most common astrocytic marker is GFAP. It has been widely used ever since the development of a gold chloride-sublimate staining technique in early 20^{th} century (Parpura & Verkhratsky, 2012b). GFAP is an intermediate filament, 8-9 nm in diameter, expressed mainly in mature astrocytes. It is involved in structural stabilisation of the astrocytic processes resulting in cell motility and shape modulations of the astrocytes (Eng et al., 2000). However, not all the

cells morphologically identified as astrocytes express the same level of GFAP. Further studies revealed that some GFAP-positive cells evince the characteristics of neural progenitor or a stem cell (Wang, 2008) and on the contrary not all the cells possessing morphological features of astrocytes actually display GFAP presence (Kimelberg, 2004).

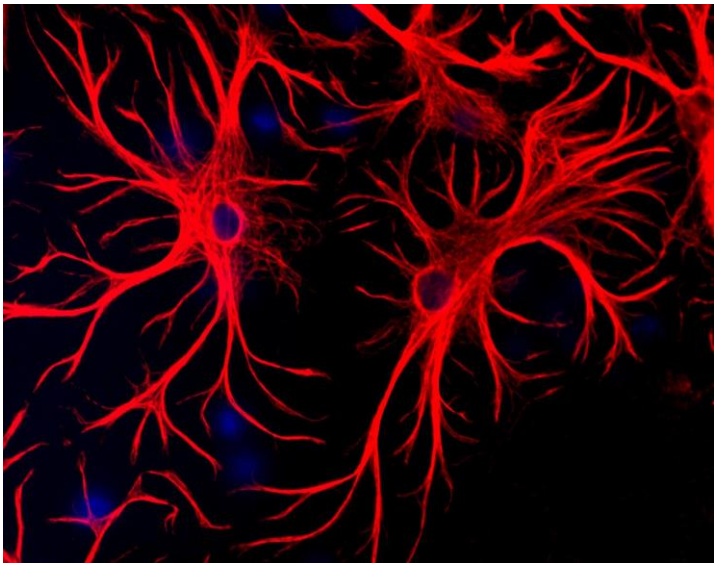

Figure 1: Mixed cultures of neurons and glia stained with Anti-GFAP Antibody (red) and DNA (blue). Astrocytes stain strongly and specifically in a clearly filamentous fashion with this antibody (from Antibodies.com).

Another molecule widely used as a marker for astrocytes is a calcium and zinc binding protein S100B (Ogata & Kosaka, 2002; Baudiers, 1986). Most of the GFAP-positive proliferating astrocytes are not immunostained for this protein, suggesting that it is mainly expressed in mature astrocytes. It has been proven that even multipotent astrocytic stem cells lack its expression (Deloulme et al., 2004).

Aldehyde dehydrogenase 1 family, member L1 (ALDH1L1) also known as 10-formyltetrahydrofolate dehydrogenase (FDH) is an astrocytic enzyme playing important role in cell division and growth. This folate enzyme has been proposed to be an astrocyte specific protein and is now used as a relatively new marker for astroglial visualisation (Yang et al., 2011; Kimelberg, 2010).

A number of studies have revealed the presence of glutamine synthetase (GS) in the astrocytes. However, this enzyme converting glutamate to glutamine has not been confirmed to be exclusively astrocytic protein however, it is considered to be one of their markers as well (Miyake & Kitamura, 1992).

Astrocytic functions

Developmental function

Astrocytes have been proven to be actively involved in neurogenesis regulation by releasing a number of growth factors, interleukins and other proteins with ability to control neuronal maturation and survival (Song, 2002, Wang, 2008, Parpura et al., 2012). Astrocytes also function as brain stem cells – they serve as a source for both neurons and glial cells. Moreover, they control synaptogenesis and provide a scaffold during neuronal migration (Verkhratsky & Parpura, 2010).

Structural function

The micro-architecture of the grey matter is divided into individual astrocytic domains. Each structural unit is established by separate protoplasmic astrocyte and is almost independent. Astrocytes provide for extensive connections with neurons, synapses and blood vessels within each astrocytic domain (Verkhratsky & Parpura, 2010).

There is only slightest overlap between these territories however, these areas are pivotal for further interconnections. The individual astrocytic domains are coupled through gap junctions and integrate into syncytia. The gap junctions are concentrated in the peripheral astrocytic processes and mediate transfer of the information enabling the inter-glial communication and broad signalling. Gap junctions are channels composed of two connexons, also called "hemichannels", localised in the plasma membrane of neighbouring cells. Connexins are the structural subunits, which are involved in connexon formation. The most prevalent connexins in astrocytes are Cx43 and Cx30.

Individual barrels comprised of astrocytic domains are formed at the next level of the organisation. There are only very poor connections between these anatomical units (Heneka et al., 2009; Parpura et al., 2012; Verkhratsky & Parpura, 2010).

Homeostatic function

Astrocytes are extensively involved in homeostasis maintenance in CNS. The properties of the astrocytic membrane play an important role in regulating the extracellular ion concentration, pH, brain water, and neurotransmitter homeostasis (Verkhratsky & Parpura, 2010).

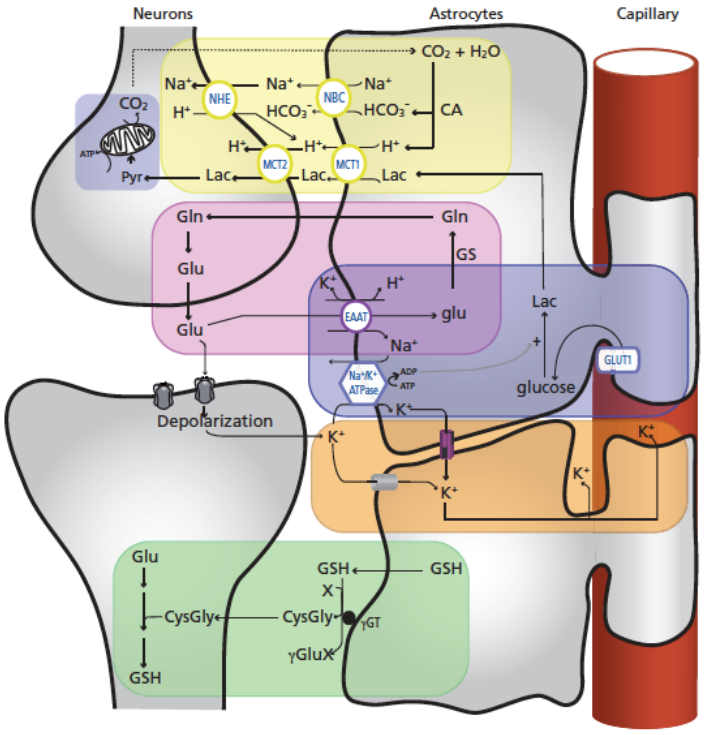

Figure 2: Simplified representation of the main roles of astrocytes in brain homeostasis. Pink box: glutamate-glutamine cycle. Astrocytic excitatory amino acid transporters (EAATs) are responsible for the uptake of a large fraction of glutamate at the synapse. Glutamate is converted into glutamine by glutamine synthetase (GS) and shuttled back to neurons for glutamate resynthesis. Blue boxes: Lactate shuttle. Glutamate uptake by astrocytes is accompanied by Na+ entry which is counteracted by the action of the Na+/K+ ATPase. The resulting increase in ADP/ATP ratio triggers anaerobic glucose utilization in astrocytes and glucose uptake from the circulation through the glucose transporter GLUT1. The lactate produced is shuttled to neurons through monocarboxylate transporters (mainly MCT-1 in astrocytes and MCT-2 in neurons), where it can be used as an energy substrate after its conversion to pyruvate. Yellow box: pH buffering. Abundant carbonic anhydrase (CA) in astrocytes converts CO2 into H+ and HCO3-. Two HCO3- are transported into the extracellular space along with one Na+ via the Na+-HCO3- cotransporter (NBC), thereby increasing the extracellular buffering power. Protons left in the glial compartment may drive the transport of lactate outside of astrocytes and into neurons through MCTs. Excess H+ in neurons is extruded via sodium-hydrogen exchange (NHE). Orange box: K+ buffering. Astrocytes buffer excess K+ released into the extracellular space as a result of neuronal activity. Potassium ions travel through the astrocytic syncitium down their concentration gradient and are released in sites of lower concentration. Green box: Glutathione metabolism. Astrocytes release glutathione (GSH) in the extracellular space where it is cleaved by the astrocytic ectoenzyme γ-glutamyl transpeptidase (γGT). The resulting CysGly serves as a precursor for neuronal GSH synthesis. X represents an acceptor for the γ-glutamyl moiety in the reaction catalyzed by γGT (Bélanger, Magistretti, 2009).

Ion homeostasis

The concentrations of extracellular ions need to be controlled and maintained to preserve accurate neuronal excitability. Astrocytes are known to play an important role in this process owing to their unique membrane properties. Even though astrocytic channels and receptors do not differ a lot from those expressed in neurons, their proportion and density varies between these cell types (Seifert et al., 2006; Heneka et al., 2009).

Astrocytes control a level of extracellular K^+, which is important for repolarization phase of action potential (Heneka et al., 2009). In a process known as potassium spatial buffering astrocytes disperse local extracellular K^+ from areas with increased concentrations to sites with low [K^+]. This mechanism of K^+ redistribution through astrocytic syncytium is preceded by K^+ uptake mediated by a number of ion channels and transporters. Inwardly rectifying K^+ channels (Kir), with their ability to favor an influx of K^+ to astrocytes, play an important role in spatial buffering. The influx of K^+ needs to be accompanied by either efflux of other cations or anion influx. Thus, presence of Na^+/K^+ ATPase pumps and $(Na^+)/K^+/Cl^-$ cotransporters is not insignificant and they are considered to be one of the key players in K^+ uptake. Astrocytes also express calcium and voltage-dependent K^+ channels, which are, however, mostly in a closed conformation under the conditions of hyperpolarized membrane potential (Kofuji & Connors, 2003; Kofuji & Newman, 2004).

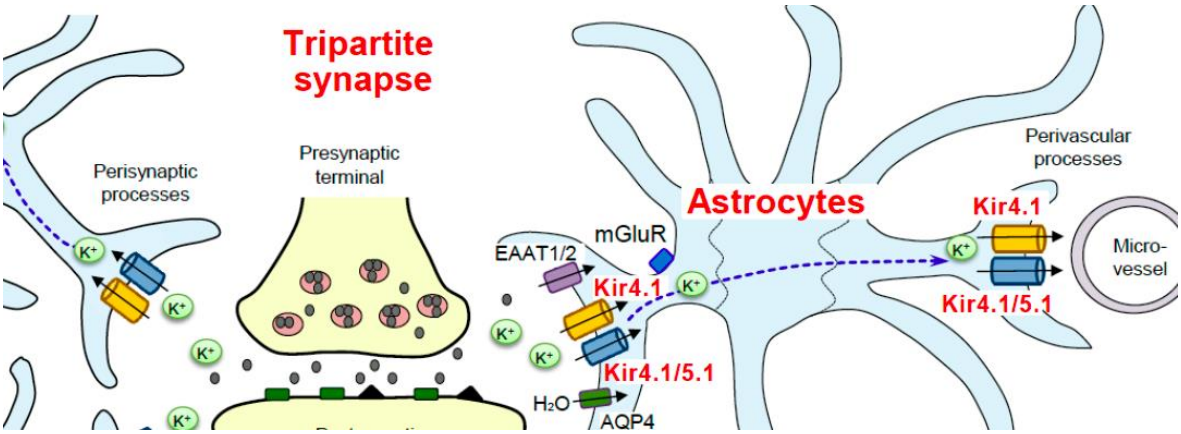

Figure 3: Kir4.1 channels mediate the spatial K+ buffering action of astrocytes. The spatial K+ buffering action of astrocytes is essential for controlling extracellular K+ concentrations at tripartite synapses. Kir4.1 and Kir4.1/5.1 channels located in perisynaptic and perivascular processes conduct the K+ buffering currents in astrocytes. Kir4.1 channels facilitate glutamate and water uptake into astrocytes via coupling to excitatory amino acid transporters (EAATs) and aquaporin 4 (AQP4) (Ohno et al., 2018).

The presence of functional ion channels is not the only factor influencing the ion homeostasis in the CNS. The transfer of ions is also interconnected with aquaporin channel-mediated brain water transport. The predominant channel enabling an influx of water in astrocytes is aquaporin 4 (AQP4), localised in the astrocytic perivascular endfeet and perisynaptic processes, usually in a close proximity to potassium channels Kir4.1(Dossi et al., 2018).

Neurotransmitter homeostasis

Astrocytic membrane is enriched with numerous transporters taking part in transfer of various neurotransmitters like glutamate, GABA, and glycine from the synapses. After the uptake by astrocytes, these neurotransmitters are enzymatically converted into precursors, which are subsequently recycled to synapses for their reconversion back into active neurotransmitters (Sofroniew & Vinters, 2010).

The principal amino acid engaged in neuronal excitability is L-glutamate. The role of astrocytes is to maintain low extracellular concentrations of the glutamate via its uptake. The process is mediated by two main glutamate transporters: excitatory amino acid transporter 1 (EAAT1), known as glutamate-aspartate transporter (GLAST) in rodents; and excitatory amino acid transporter 2 (EAAT2) also referred to as glutamate transporter 1 (GLT1). Both transporters are predominantly localized in astrocytic processes. The transport of glutamate is an electrogenic process and requires energy of transmembrane Na+ gradient. The glutamate transporters exchange one glutamate and three Na+ (or two Na+ and one H+) for one K+ and OH- (Seifert et al., 2006; Simard & Nedergaard, 2004).

Glutamate uptake is followed by a conversion of glutamate into non-toxic glutamine, which is catalysed by an astrocytic enzyme glutamine synthetase. Afterwards, glutamine is transported back to presynaptic terminal of a neuron via extracellular space. The final step in a glutamate – glutamine shuttle, necessary for glutamatergic neurotransmission, is a recycling of glutamine back to glutamate (Figure 3) (Simard & Nedergaard, 2004; Heneka et al., 2009).

pH regulation

A number of studies have proven a contribution of astrocytes to pH maintenance in the CNS. A constant pH within a brain is one of the essential requirements for a proper

neurotransmission and excitability of the neurons (Obara et al., 2008). Various astrocytic transport mechanisms take part in a proton transfer including, but not limited to bicarbonate transporters, Na+/H+ exchanger, proton ATPase, and monocarboxylic transporters (Sofroniew & Vinters, 2010). The reaction of carbonic anhydrase, an enzyme reversibly converting carbon dioxide and water into to a proton and bicarbonate, is a major process preserving the acid-base homeostasis (Obara et al., 2008).

Antioxidant function

The CNS is notably vulnerable to damages caused by oxidative stress. An imbalance between reactive oxygen species (ROS) production and antioxidant processes frequently appears under neuropathological conditions. Both neurons and astrocytes carry a neuroprotective force against oxidative injuries in a form of antioxidant production. A number of different antioxidants like glutathione, ascorbate, and vitamin E are present in astrocytes, together with a variety of ROS-detoxifying enzymes. The most significant antioxidant molecule in the CNS is glutathione (GSH). Astrocyte-neurone GSH shuttling provides precursors for the neuronal synthesis of GSH (Figure2, green box). Astrocytic ectoenzyme γ-glutamyl transpeptidase (γGT) converts a GSH, extracellularly released from astrocytes to extracellular space, into CysGly, which is then either directly transferred to neurons or at first cleaved into glycine and cysteine and only then transferred (Bélanger, Magistretti, 2009).

Metabolic function

Glycogen functions as a major energy source for the CNS cells. The distribution of brain glycogen is uneven throughout the brain, with astrocytes being the primarily sites for its storage. Active neurons are provided with energy substrate by astrocytes, which have a substantial location in the CNS organization, with processes contacting both blood vessels and neurons (Brown & Ransom, 2007).

Under the physiological conditions blood glucose is taken up by the astrocytes via glucose transporters (GLUT1) and is then converted into pyruvate in a process of aerobic glycolysis. Lactate is a product (pyruvate being the substrate) of reaction catalysed by lactate dehydrogenase isoenzyme A (LDHA) expressed in astrocytes and is transported into neurons, where it serves as a fuel for neuronal processes (Figure 4). This mechanism has been introduced as astrocyte neuronal lactate shuttle hypothesis (ANLSH). This process can be stimulated either

by glutamate, which increases the uptake of glucose from capillaries or by glycogen breakdown (Kimelberg, 2010; Brown & Ransom, 2007).

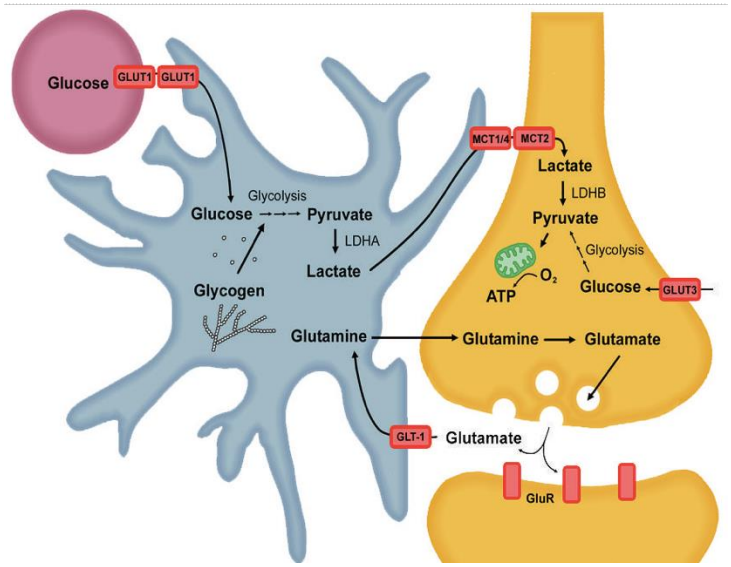

Figure 4: The astrocyte-neuron lactate shuttle hypothesis. The activation of nerve cells leads to the release of the neurotransmitter glutamate. Glutamate is actively taken up into astrocytes by glutamate transporters (GLT-1) and is converted into glutamine. The uptake of glutamate into astrocytes stimulates both increased glucose uptake from surrounding capillaries via glucose transporters (GLUT1) and increased aerobic glycolysis. Aerobic glycolysis can also be stimulated by the breakdown of intracellular stores of glycogen. Pyruvate is converted to lactate by lactate dehydrogenase isoenzyme A (LDHA) and is exported out of the cell by the monocarboxylate transporter 1 or 4 (MCT1/4) and transported into nerve cells via MCT2. LDHB within nerve cells coverts lactate to pyruvate which is used to fuel oxidative phosphorylation within mitochondria. Glucose can also enter nerve cells via GLUT3 transporters (Newington et al., 2013).

Signalling function

Regarding the signalling function, astrocytes have the ability to regulate synaptic transmission. As it was mentioned previously, the concentration of neurotransmitters in the synapses can be controlled via uptake by specific astrocytic transporters. Another way of modulating synaptic transmission is by the release of gliotransmitters (Sofroniew & Vinters,

2010). Gliotransmitters are molecules produced by and/or stored in glia. They induce rapid responses in nearby cells and play an important role in both physiological and pathological processes. Several miscellaneous gliotransmitter release mechanisms have been described in astrocytes, such as: a release through volume-regulated anion channels, through unpaired connexons (hemichannels), ionotropic purinergic receptors, through cystine-glutamate antiporters, via Ca^{2+}-dependent exocytosis, etc. There are two principal categories of gliotransmitters expressed in astrocytes: amino acids and their derivates, comprising molecules like glutamate, aspartate, D-serine, GABA, taurine; and the second group consists of nucleotides and their derivates like ATP, UTP, UDP-glucose and others (Verkhratsky & Parpura, 2010).

Ca^{2+} waves

Astrocyte signalling across the syncytium is mediated by gap junctions (see "Structural function") and this mechanism is a means of Ca^{2+} waves spreading. Ca^{2+} waves are generated either spontaneously or as a result of neuronal activity and have an effect on several pathways, including those engaged in modulation of synaptic transmission and plasticity (Parpura et al., 2012).

Vascular function

Blood brain barrier

The blood brain barrier is a selective physical barrier composed of microvessel endothelial cells enclosed by basal lamina and astrocytic endfeet (Abbott et al., 2006). Endfeet are astrocytic structures adapted for the direct interaction with the vessels (Gordon et al., 2007). Tight junctions between endothelial cells play an important role in the permeability of the blood brain barrier. Only small lipophilic molecules and gases like oxygen, CO_2, and ethanol are able to penetrate a lipid bilayer of the endothelium. All other components (small polar solutes, large molecules,…) need a specific carrier to be transported into or out of the CNS. Astrocytes have been suggested to promote the tight junction formation and tightness (Abbott, 2002; Abbott & Romero, 1996).

Blood flow regulation

As previously mentioned, astrocytes are connected with both neuronal synapses and brain vessels which is an excellent position for neurovascular coupling or in other words a blood

flow regulation in response to neuronal changes. Astrocytes are able to control the physiology of blood vessels leading to either vasoconstriction or vasodilation. In which direction a vessel diameter changes depends on astrocytic molecular mediators to which an arachidonic acid is converted. This process is also regulated by NO levels (G. R. J. Gordon et al., 2007).

Astrocytes under pathological conditions

Glial cells, and astrocytes in particular, are significant for their irreplaceable role in the maintenance of CNS homeostasis, thus the pathological conditions brought about by brain injuries, diseases or other impairments have a noticeable impact on astrocytic physiology and morphology. Despite all the differences between the individual CNS disorders, there are some common features accompanying these diseases, for instance: inflammation, ROS accumulation, excitotoxicity, and metabolic failure. Majority of these processes are under astrocytic control in a healthy brain (Bélanger, Magistretti, 2009).

The most common astrocytic response to the physiological changes in a diseased or injured brain is undoubtedly the one known as reactive astrogliosis. A wide range of different astrocytic changes and reactions has been observed in a process of astrogliosis, some of them being contradictory or only poorly explained. However, Sofroniew suggested a model in his review on Molecular dissection of reactive astrogliosis and glial scar formation which comprises general characteristics of the process in these four points:

1. Reactive astrogliosis includes a range of astrocytic changes occurring during CNS injury or disease of any form and severity.
2. The changes in astrocytes depend on nature and severity of the damage and vary along a gradated continuum of progressive alterations in molecular expression, progressive cellular hypertrophy and proliferation and scar formation which is the most severe.
3. The changes accompanying the astrogliosis are regulated by precise signalling pathways with the ability to customize their nature and degree, in a context-specific manner.
4. Astrocytic activity during reactive astrogliosis is altered through gain and loss of functions that have the ability to affect the surrounding cells both positively and negatively (Sofroniew, 2009).

There is a vast array of factors in astrocytic microenvironment, such as: cytokines, adhesion molecules, growth factors, and various signals from glial, neuronal and endothelial cells, all contributing to a modulation of astrocyte reaction (Markiewicz & Lukomska, 2006).

Even though astrogliosis represents a gradated continuum of changes it has been categorized into three stages: mild, moderate, and severe. All three present with altered gene expression resulting in GFAP up-regulation and cellular hypertrophy. Mild to moderate reactive astrogliosis usually appears during some forms of trauma and in bacterial or viral infections, further from focal CNS lesions. However, severe reactive astrogliosis is accompanied by astrocyte proliferation resulting in tissue reorganization and astrocyte domain loss. These structural changes result in a so called glial scar formation, isolating the area of tissue damage, necrosis, infection, or inflammation by compact border formation around it. Glial scar formation is known to be induced by CNS impairments like invasive infections, chronic neurodegenerative changes, neoplasm, trauma and others. Glial scar separates defective brain areas from healthy tissues, prevents axon regeneration, and hinders inflammation from spreading. This barrier persists for a long period of time and it requires astrocytes with proliferative potential for its formation (Verkhratsky et al., 2012; Sofroniew, 2009).

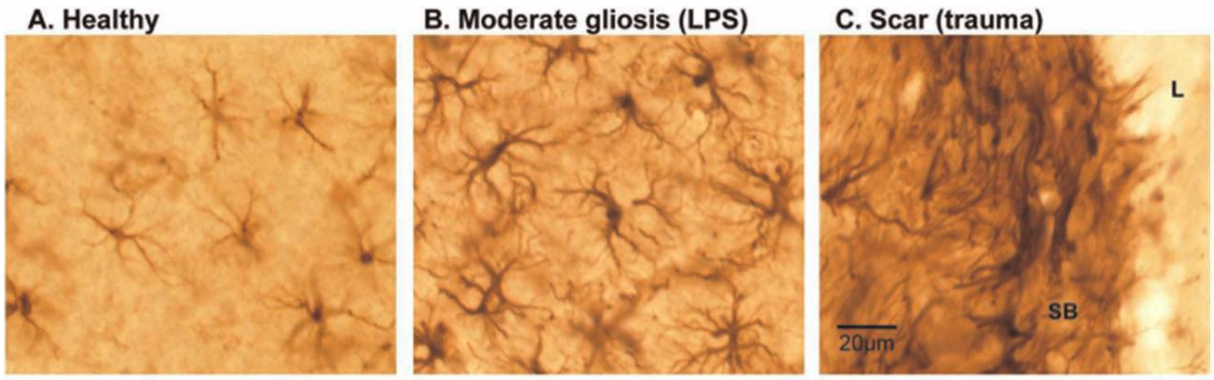

Figure 5: Appearance of astrocytes and different types of reactive astrocytes in mouse cerebral cortex. Images show immunohistochemistry for the intermediate filament protein, GFAP, which visualizes the cell cytoskeleton. (A) In healthy cortex, some, but not all astrocytes express detectable levels of GFAP; cell bodies are small and the cytoskeleton is thin and restricted largely to the proximal portions of cell processes. (B) In response to the bacterial antigen, lipopolysaccharide (LPS) injected into the lateral cerebral ventricle, cortical astrocytes become moderately reactive, with up-regulation of GFAP expression such that it is now detectable in all astrocytes. In addition, there is substantial hypertrophy of the astrocyte cell bodies as well as hypertrophy of stem processes and associated cytoskeleton. However, there is no astrocyte proliferation and individual cells continue to respect their individual, non-overlapping domains. (C) In response to a severe traumatic injury that creates a lesion (L) with tissue necrosis and invasion of inflammatory cells, astrocytes not only become reactive but also proliferate in the immediate vicinity of the lesion and form a scar with a dense scar border (SB) that comprises many newly generated astrocytes that do not exhibit individual domains and instead have many overlapping and intermingling processes. All images are at the same magnification. Scale bar520 mm (Photos courtesy of the Sofroniew laboratory) (Sofroniew, 2009).

Whether the impact of reactive astrocytes on CNS is rather positive or negative is a question that needs to be further investigated to be resolved properly. There is an evidence of both beneficial and deleterious astrocytic responses to CNS insults. A recent study on reactive astrocytes suggests an existence of two distinct types of astroglia, one characterised as damaging and the other one supportive, named "A1" and "A2" respectively. Neuroinflammation instigates an activation of A1 astrocytes which subsequently up-regulate the pathways involved in synapse destruction, unlike the ischemia-induced A2 astrocytes which implement their protective potential by stimulating neurotrophic factors production (Liddelow & Barres, 2017).

In principle, all CNS disorders could be characterised as homeostatic failures. Astrocytes may contribute to these imbalances in the homeostasis in various ways, often by altered expression or function of transporters and channels (Figure 6) (Verkhratsky et al., 2012).

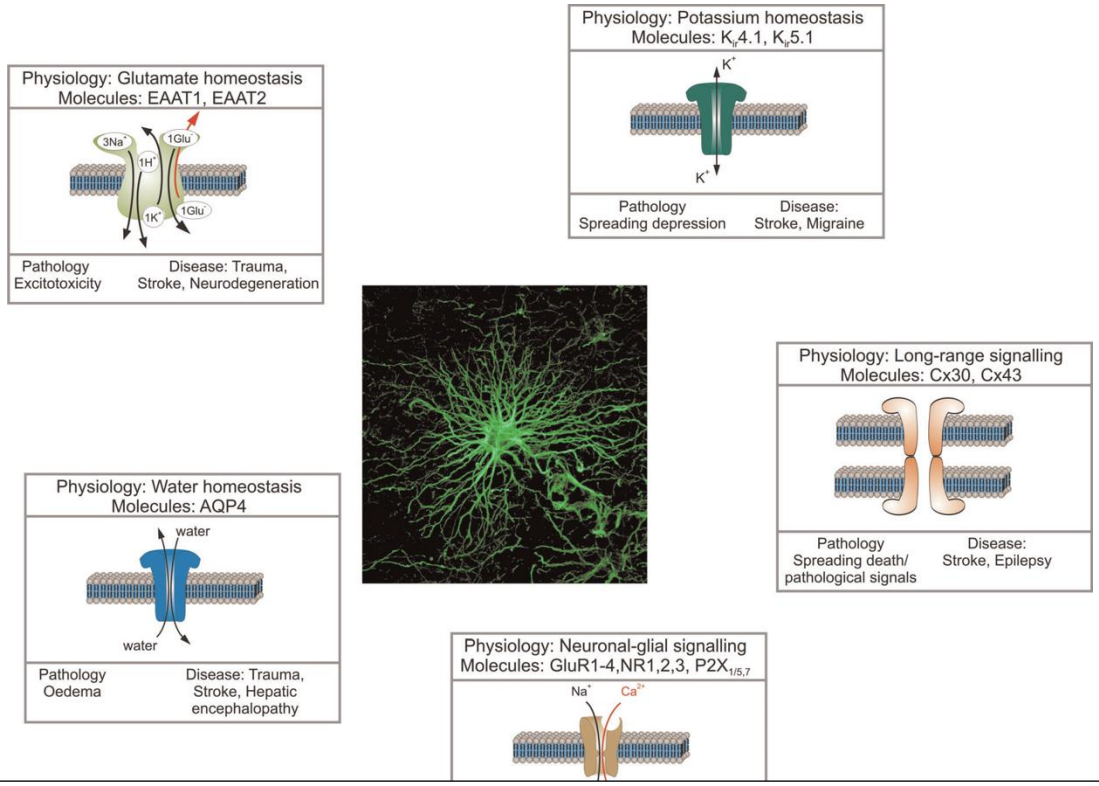

Figure 6: General pathophysiology of astroglia. The homoeostatic cascades expressed in astrocytes control various aspects of CNS homoeostasis including extracellular ion homoeostasis (K+ buffering via Kir channels, Na/K pump and K transporters), regulate movements and distribution of water (via aquaporins and connexins), control extracellular concentration of neurotransmitters (by dedicated transporters) and provide the main reactive oxygen species scavenging system. In pathological conditions, the very same systems may contribute to brain damage. Failure in water transport triggers brain oedema, reversal of neurotransmitter transporters contributes to glutamate excitotoxicity, inadequate K+ buffering promotes further overexcitation of neural cells and spreading depression, and connexins become a conduit for death signals (Verkhratsky et al., 2012).

One of the examples of such failures in ion homeostasis is represented by the alterations in potassium channel expression and location. Miscellaneous CNS pathologies, either chronic or acute, have the ability to trigger defects in K^+ channel functions, leading to an impaired K^+ spatial buffering. As a result of dysfunctional K^+ uptake a spreading depression may occur, mediated by extracellular K^+ accumulation. A disproportion of the ionic distribution leads to a depolarization of the astrocytes, which in turn inflicts the glutamate transport defects. Together with an affiliation of other proteins like aquaporins and connexins the whole process ends up with systemic collapse of homeostasis contributing to a neuronal damage (Verkhratsky et al., 2012; Anderova & Pivonkova, 2012).

Another abnormality observed in astrocyte behaviour under pathological conditions is a process called astrocyte swelling. An increased uptake of water via AQP4 on astrocytic endfeet impels the cells to extend their volume, thus contributing to a condition described as cytotoxic edema (Sofroniew & Vinters, 2010).

Neurodegeneration

Neurodegeneration is a chronic and progressive process of neuronal death, leading to brain atrophy and impairments of CNS connectivity. Onset and progression of neurodegenerative disorders can be triggered by miscellaneous factors from traumatic and infectious attacks to genetic susceptibility or sporadic errors accumulation. Astrocytes play a significant role in health of neuronal cells, thus their contribution to the development of neurodegeneration is critical (Heneka et al., 2009; Verkhratsky et al., 2013).

Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder manifesting itself by dementia and was first described by Alois Alzheimer in 1907 (Verkhratsky et al., 2013). From all types of dementia, Alzheimer's disease is the most frequent one among the elderly. The clinical symptoms of this neuropsychiatric disorder include progressive memory loss and dysfunction of higher cognitive domains. Histological features accompanying development of AD are senile/neuritic plaques and neurofibrillary tangles (NFT) formation. Neuronal degeneration and death are the consequences of these pathological changes. The senile plaques were shown to be formed by an extracellular aggregation and deposition of the β-amyloid peptide (Aβ) fibrils. Tau protein is the principal component engaged in NFT formation (Maccioni et al., 2001; Parpura et al., 2012).

AD occurs in two distinct forms: sporadic and familial. These two forms have strong phenotypical similarities and it is often difficult to distinguish between them. Even though the genetic factors may be involved in onset and progression of the disease, it seems that majority of AD cases are of non-familial origin (Maccioni et al., 2001; Selkoe, 2001).

There are three phases of the disease: pre-symptomatic phase, prodromal phase, and dementia. In the first one (pre-symptomatic phase) the cognitive functions are not affected, however, the patients may have AD pathological changes. The prodromal phase is also known as mild cognitive impairment. It presents itself with the earliest cognitive symptoms, such as episodic memory deficits. Dementia is the final and the most severe phase of AD and is characterised by extreme impairments in multiple domains causing loss of function (Jack et al., 2010).

Braak stageing is used to distinguish between six distinct pathological stages of AD. Changed intraneuronal neurofibrils in AD are found in three forms: neuritic plaques with

variable distribution, neurofibrillary tangles and neuropil threads. The latter two are distributed in a specific manner. The distribution patterns determine the stageing (Braak & Braak, 1991).

Astrocytes in AD

There are two major reactions of the astrocytes occurring in response to AD progression: reactive astrogliosis and astrocytic atrophy. Reactive astrogliosis is often recognized in post-mortem human tissues and was also observed in AD animal models. This reaction of astrocytes is typical for the late stages of the disease and presents with astrocytic hypertrophy together with upregulated expression of GFAP and S100β protein (Verkhratsky et al., 2013). A transformation of astrocytes into their reactive form is instigated by various factors like an exposure of astrocytes to extracellular Aβ deposition and signalling from damaged neighbouring cells (Verkhratsky & Parpura, 2010). Senile plaques represent the sites where hypertrophic astrocytes are often found. In transgenic AD animal models an aberrant Ca^{2+} signalling was observed in astrocytes present in senile plaques. Increased intracellular Ca^{2+} concentrations and Ca^{2+} waves propagation have been both observed in activated astrocytes (Heneka et al., 2009). Severity of astrogliosis seems to correlate with a cognitive decline (Verkhratsky et al., 2012).

The second astrocytic reaction described in early stages of AD presents with atrophy and degradation of the astrocytes. Naturally, the astrocytic decline is not negligible. It is the absence of synaptic coverage of astrocytes that most probably leads to a decrease in synaptic connectivity and subsequently an abnormal synaptic transmission (Verkhratsky et al., 2013).

There is a wide evidence of glutamate metabolism being altered in astrocytes in the progression of AD. An aberrant glutamate-glutamine shuttle, which seems to be the consequence of both decreased glutamine synthase expression and down-regulation of glutamate transporters, implicates the excitotoxicity and synaptic dysfunction leading to a loss of neurons (Yeh et al, 2013; Dossi et al., 2018).

One of the most controversial roles of astrocytes in AD, however, seems to be the one they play in the metabolism of amyloid β. Various studies have revealed opposing results on this matter. Activated astrocytes proved to be involved in degradation and clearance of Aβ and still in some cases they produce β-secretase, an enzyme functioning as an additional source of Aβ (Verkhratsky et al., 2013; Verkhratsky et al., 2012).

Inflammatory processes contribute to both production of the Aβ and impediment of its clearance. Amyloid precursor protein and β-secretase-1, which are both involved in Aβ production, show increased levels when influenced by inflammatory cytokines released by astrocytes. Moreover, inflammation-induced production of cytokines and chemokines leads to a neurodegeneration accompanied by axonal, dendritic and synaptic degradation.

Furthermore, astrocytes are involved in expressing the inducible nitric oxide synthase (iNOS). This enzyme is produced by neurons and microglial cells as well; astrocytes, however, proved to be the most important sites for its expression in human and mouse AD models. Released NO influences its close environment in various ways. It is involved in switching on signalling pathways leading to gene transcription and regulation, mitochondrial respiration impairment, and even cell death via apoptosis or necrosis. NO is also engaged in the process of nitration and Aβ protein is one of its targets. Nitrated form of Aβ protein is easily forming the Aβ plaques, unlike the non-nitrated one (Parpura et al., 2012).

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) also known as Lou Gehrig's disease is the third most common neurodegenerative disorder after AD and Parkinson's diseases. It is a fatal idiopathic motor neuron disease, which presents with upper and lower motor neuron degeneration and death, localised in the brain and spinal cord. ALS is more prevalent among elderly, men being at higher risk of developing the disease than women (Gordon, 2013; Verkhratsky et al., 2013) Only 5-10% of ALS cases are of genetic origin. Thus, a vast majority of the patient are diagnosed with a sporadic form of the disease (Kiernan et al., 2011).

Not only motor neurons supplying voluntary muscles are affected by ALS, but also lower medullar motor neurons and upper motor neurons in the cerebral cortex (Gordon, 2013). The most probable cause of death of amyotrophic lateral sclerosis patients is due to respiratory failure (Verkhratsky, 2013).

Many genes have been proved to be involved in both familial and sporadic ALS, however, approximately 20% of cases of fALS and 1% of sALS are related to a dominant mutations in the Copper-Zinc superoxide dismutase (SOD1) gene (Rossi & Volterra, 2009; Wijesekera & Leigh, 2009).

In about 70% of patients the first symptoms of the disease occur in the limbs (usually in the arms) and are focal and unilateral. They include foot drop, walking difficulties, hand dexterity loss, and weakness in the arms during movements. With the progression of the disease a complete loss of the ability to walk may occur. Older women usually present with bulbar-onset of the ALS, which means a poorer prognosis for the patients. Speech (dysarthria) and swallowing (dysphagia) difficulties are characteristic signs of the bulbar-onset ALS. The possible consequences of these conditions are excessive salivation, anarthria and even malnutrition. Furthermore, as a result of axial weakness, dropped head and kyphosis may also accompany the disease (Gordon, 2013; Kiernan et al., 2011).

Astrocytes in ALS

In ALS, similarly to AD, two distinct astrocytic changes may occur. The early stages of the disease are characterised by astrocytic atrophy and degeneration. These changes in the astrocytes occur even sooner than the first clinical symptoms appear (Heneka et al., 2009).

Astrogliosis is typical for the later stages of ALS and presents with an increased immunoreactivity for GFAP and S100β. Reactive astrocytes also contribute to an inflammation by releasing inflammatory markers like COX-2, iNOS and neuronal NOS (Verkhratsky et al., 2013; Markiewicz & Lukomska, 2006).

Reduced or dysfunctional EAAT2 astrocytic glutamate receptors are often present in ALS. Defective uptake then contributes to increased levels of extracellular glutamate, which have an excitotoxic effect and cause damage to motor neurons. The deposition of glutamate in the extracellular space may be supplied even by glutamate release from astrocytes, which can be enhanced by inflammatory processes (Barbeito et al., 2004).

Oxidative stress is another negative factor contributing to astrocyte activation. Nitric oxide and peroxynitrite are produced by both reactive astrocytes and damaged motor neurones and adversely affect a number of proteins and cellular processes, such as astrocytic gap junctional communication and glutamate uptake (Barbeito et al., 2004; Markiewicz & Lukomska, 2006).

Parkinson's disease

Parkinson's disease (PD) is a condition characterised by a progressive loss of dopaminergic neurons of pars-compacta - a part of midbrain-located substantia nigra (SN) - and region specific aggregation of α-synuclein. When the neurodegeneration hits over 50% of neurons (of SN), the disease appears clinically (Heneka et al., 2009; Lees et al., 2009).

Ageing and genetic predisposition are primary risk factors concerning PD. Other risk factors linked to a sporadic form of the disease have been also reported. They include rural living, middle-age obesity, injury of head, shortage of exercise, exposure to chemicals; and they are all only of a minor importance. Nicotine and caffeine are related to the disease as well, and they both contribute to a decreased risk of PD as they induce a dopamine release in striatum. Moreover, cigarette smoke has been suggested to inhibit monoamine oxidase, an enzyme producing reactive oxygen species, which account for increased risk of neuronal damage. Thus, it is not surprising that non-smokers and people with no or very low caffeine intake have higher susceptibility to develop the disease(Lees et al., 2009; Fowler et al., 1996).

Astrocytes in PD

Substantia nigra, which is affected by the disease in the first place, is a brain region with a lower abundancy of astrocytes in comparison to the rest of the brain. This indicates a lack of astroglial support to the neurons, which might play a substantial role in the development of PD. Similarly to the previously mentioned neurodegenerative diseases, one of the possible astrocyte pathologies occurring during the early stages of PD development is the astrocytic atrophy, which gradually leads to a neuronal degeneration (Heneka et al., 2009). Concerning the reactive astrogliosis, which is the most common astrocytic response to the cerebral impairments, mostly mild and moderate forms have been observed in PD (Teismann & Schulz, 2004).

It has been proposed that neuronally released aggregates of α-synuclein protein are transferred into astrocytes, where they induce the inflammatory processes (Lee et al., 2010). Neuroinflammation is believed to promote the degeneration of dopaminergic neurons. A number of cytokines induces the process, part of them being produced by astrocytes. However, different cytokines can mediate diverse responses, for example TNF-α and IL-1β are characteristic with their neurotoxicity, other cytokines are engaged in astrocyte activation or contrarily, there are some with the ability to inhibit the activation of glia (Teismann & Schulz, 2004; Markiewicz & Lukomska, 2006).

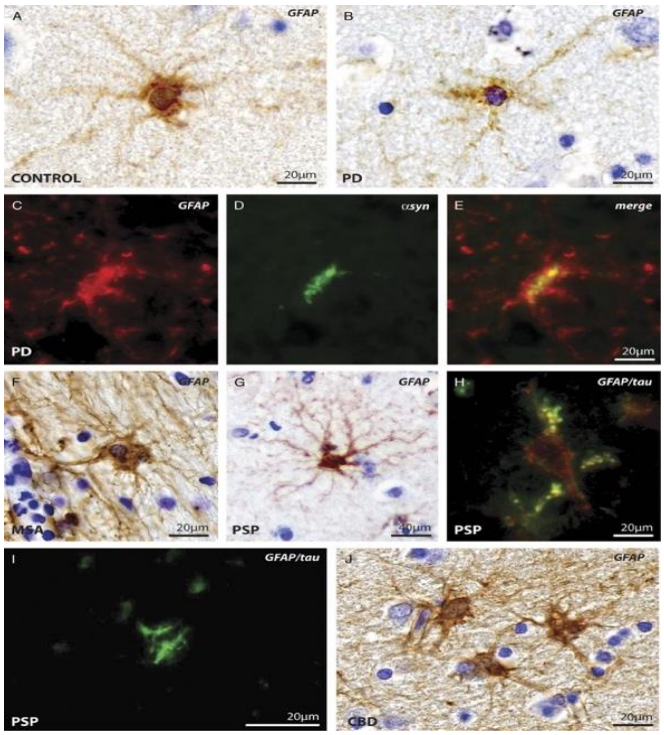

Figure 7: Pathologic changes in astrocytes. (A, B) Brightfield photomicrographs of similar typical stellate astrocytes with finely branching processes immunostained for glial fibrillary acidic protein (GFAP) in the putamen of a control (A) and a Parkinson disease (PD) case (B). (C-E) Immunofluorescent images of a typical stellate GFAP-immunoreactive (red Alexa 568) (C, E) astrocyte containing α-synuclein (green Alexa 488) (D, E) in the pons of a PD case. (F) Brightfield photomicrograph of an enlarged and distorted GFAP-immunoreactive astrocyte in a white matter bundle of a multiple system atrophy case. (G) Brightfield photomicrograph of an enlarged reactive GFAP-immunoreactive astrocyte in the putamen of a progressive supranuclear palsy (PSP) case. (H) Immunofluorescent merged image of a GFAP-immunopositive (red Alexa 568) reactive astrocyte containing phospho-tau (green Alexa 488) in the putamen of a PSP case. (I) Immunofluorescent merged image of a phospho-tau immunopositive (green Alexa 488), but GFAP-immunonegative (red Alexa 568) tufted astrocyte in the putamen of a PSP case. (J) A brightfield photomicrograph of enlarged reactive GFAP-positive astrocytes in the putamen of a corticobasal degeneration case. Brightfield photomicrographs are counterstained with cresyl violet (Song et al., 2009).

Interestingly, there is a vast amount of evidence that astrocytes have an appreciable neuroprotective effect on neurons. For instance, astrocytes are implicated in the clearance of the toxic substances produced by dying neurones; in addition they metabolize dopamine and thus contribute to a decreased oxidative stress. What's more, astrocytes have been suggested to

inhibit the negative neurotoxic effects of NO via glutathione dependent mechanism; and to control deleterious microglial reaction (Teismann & Schulz, 2004).

Huntington's disease

Huntington's disease (HD) is a progressive genetic disorder with neurodegenerative outcomes, emerging from expansion of triplet CAG repeats in a gene for huntingtin. The onset of HD depends on length of CAG repeats. The primary target of neurodegeneration in HD progression is a striatum. Clinically the disease presents itself with a variety of symptoms from cognitive decline and psychiatric breakdown to a progressive motor dysfunction. The patients diagnosed with HD typically struggle with involuntary movements (chorea), overall motoric slowdown (bradykinesia), incoordination, and rigidity often preceded by behavioural defects (Ross & Tabrizi, 2011).

Astrocytes in HD

An increased excitotoxicity which is known to contribute to a neurodegenerative processes has been observed in HD. One of the most probable reasons for excitotoxic neurodegeneration resides in the astrocytic glutamate transport alterations. A number of studies revealed a down-regulation of EAAT2 glutamate transporter in astrocytes and increased calcium-dependent glutamate release, which directly induce the excitotoxic glutamate accumulation in the extracellular space (ECS) (Verkhratsky et al., 2013; Estrada-Sánchez & Rebec, 2013). There is still a lot of data missing concerning the astroglial behaviour in HD and a further research would be required to complete a whole picture of the matter. Even though, there is an evidence of various astrocytic alterations, which might play a relevant role in HD progression, such as: increased gap junctional coupling, mitochondrial impairments, neuroinflammation mediators' release, Kir 4.1 channels down-regulation, or abnormal cholesterol production (Phatnani & Maniatis, 2015).

Conclusion

Astrocytes are a subtype of glial cells and their most important function in CNS is to maintain brain homeostasis. Homeostatic disruption occurs during neurodegeneration, suggesting that astrocytes can play a substantial role in the onset and progression of neurodegenerative diseases, or even contribute to their formation. Even though astrocytes are receiving more and more attention from researchers, their role in neurodegeneration is still not fully understood.

Over the past few years, there has been a lot of evidence of their positive but also negative effects on neurons and their environment. The most typical astrocytic response observed in most neurodegenerative diseases is reactive astrogliosis. It is often accompanied by a change in the expression of various genes. And such changes were demonstrated in AD, PD but also in ALS. However, during the early stages of these diseases there is a sudden loss of astrocytes, which in turn leads to an increased excitotoxicity and neuronal death. Neurodegeneration often results in decreased or altered expression of glutamate transporters, thereby leading to the accumulation of glutamate in the extracellular space, causing a neuronal damage. Down-regulated astrocytic EAAT2 transporters is another typical feature observed in AD, ALS and HD damaged brains. Furthermore, neuroinflammation also occurs in most of these diseases. The function of astrocytes during inflammatory processes is controversial and depends primarily on the severity of the defect and the type of disease.

In order to obtain a better understanding of the role of astrocytes in neurodegenerative diseases, more detailed investigation and clarification of the processes accompanying reactive astrogliosis is required. At the same time, if we want to target the treatment of neurodegeneration to astrocytes, it is important to examine the pathophysiological processes in the neighbouring cells – both neuronal and glial - since the CNS is a dynamic structure in which intense communication occurs between its individual interacting components.

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