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CALCIUM ACTIVITY IN ASTROCYTES

Bachelor Thesis

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ABSTRACT

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This Bachelor's Thesis is a literature review on the calcium signaling in astrocytes, concentrating on the molecule- and cell-level. The study briefly surveys neural communication, and more thoroughly goes through the morphological astrocyte types, the major calcium pumps and receptors and the calcium signaling of the various astrocyte types. According to multiple studies, there are many morphological and functional astrocyte types with a characteristic location in the human brain. The types differ in their morphology and calcium activity patterns. The main contents of this study are the calcium signals, which are small local changes in the intracellular calcium concentration. It is still under debate on which pathways and mechanisms the calcium waves derive from, and which are the properties of the calcium signals in each astrocyte type. A further research is still needed to get a better understanding on the boundaries and properties of the different astrocyte types, as well as calcium signaling mechanisms of them.

TIIVISTELMÄ

JOHANNA LAPPALAINEN: Astrosyyttien kalsiumaktiivisuus
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Tämä kandidaatintyö on laadittu taustoittamaan kirjallisuuden avulla astrosyyttien kalsiumsignaalien syntyperää solutasolla. Työssä käydään läpi lyhyesti neuronien kommunikaatiota, määritetään astrosyyttien morfologiset tyypit, perehdytään tärkeimpiin solutason kalsiumpumppuihin ja –reseptoreihin ja selvitetään astrosyyttityyppien signalointia kalsiumin kannalta. Tutkimukset viittaavat moniin morfologisiin ja toiminallisiin astrosyyttityyppeihin, joista kukin sijaitsee tietyillä aivojen alueilla. Ne ovat anatomialtaan erilaisia ja saattavat erota myös kalsiumsignaaleiltaan. Työn pääosassa ovat kalsiumsignaalit, jotka ovat pieniä paikallisia kalsiumkonsentraation muutoksia solussa. Väittelyn alla on, mistä kalsiumin konsentraationmuutokset kulloisessakin astrosyyttityypissä johtuvat ja millaisia ominaisuuksia syntyneillä kalsiumpiikeillä on. Jatkossa tarvitaan lisää tutkimusta astrosyyttityyppien ominaisuuksista ja tyyppirajoista, kuten myös niiden kalsiumsignaloinnista, jotta aivojen toiminnasta saataisiin tarpeellista lisätietoa.

PREFACE

This Bachelor's Thesis is a part of the major Biomedical Engineering in the Master's Degree program in Electrical Engineering. The study sorts out the biological nature of the degree and justifies the fact that it is important to understand the biological processes when creating new technologies.

I would like to thank the examiners of the work, professor Jari Hyttinen, and the researcher Kerstin Lenk for their valuable support and patience during this study. I would also like to thank Olli Erälaukko for encouraging and believing in me along the way.

Helsinki, 24.02.2018

Johanna Lappalainen

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ABBREVIATIONS

ATP	adenosine triphosphate, the chemical energy source within cells
Aβ	amyloid-β, protein accumulating in the plaques of Alzheimer's disease
Ca^{2+}	calcium ion
CERCA pump	sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase pump
CNS	central nervous system
ER	endoplasmic reticulum
GABA	gamma-aminobutyric acid
GFAP	glial fibrillary acidic protein
GPCRs	G protein-coupled receptors
HDML	hippocampal dentate molecular layer
ICWs	intercellular calcium waves
in situ	viewing structures as they appear in healthy body
in vitro	studies performed outside their normal biological surroundings
in vivo	studies performed inside their normal biological surroundings
IP3	inositol triphosphate, an internal messenger molecule transmitting messages from the metabolic glutamate receptors to endoplasmic reticulum
IP3R2	inositol triphosphate type 2 receptor
mGluR	metabolic glutamate receptor
NG2-positive glia	nerve/glial antigen 2 –type of the glia cells
NMDA	N-methyl-D-aspartic acid
PLC	phospholipase C
P2Y	purine receptor
TRP channel	transient receptor potential channel
TRPA1	transient receptor potential A1
2PLSM	two-photon laser scanning microscope

1. INTRODUCTION

This Bachelor's Thesis work is a literature review about the calcium activity in astrocyte cells. The aim of the work is to study the different astrocyte cell types, both in morphological and functional state, and their differing calcium signaling. To understand the complex mechanisms of the calcium activity in astrocytes, the main view of the study is on the cellular and molecular level. Therefore, the brain as surroundings is not represented as widely.

During the last decade, researchers have revealed a more active role of astrocytes in the central nervous system. Astrocytes are star-shaped supportive cells with functions of for example maintaining blood-brain barrier, regulating blood flow and mediating neuronal uptake and providing nutritional and metabolic support to neurons. [34] We are just beginning to understand the heterogeneity of astrocytes. The calcium activity of astrocytes in the different regions of the brain have been studied for years now, but the information is still quite contradictory and scattered.

Calcium imaging *in vivo* have confirmed that astrocytes can generate various patterns of activity, and the excitability of astrocytes appears as elevations in intracellular calcium levels. Local intracellular calcium concentration transients can be generated spontaneously or by different stimuli, such as mechanical stimulation or release of transmitters. [17] The calcium concentration waves can also be transmitted to the connected cells, being called intercellular waves in that case, and affecting the interactions between cells. [11] The complexity of the fundamental understanding of calcium signals lies in the multiple mechanisms they are derived from, as well as in the differences between the astrocyte types.

The first section of the work concentrates on the brain networks, viewing shortly the structure of the brain as well as signaling of neurons and the interaction between them and astrocytes. The anatomy of the brain is represented quite lightly, and only on the cellular level. The second section studies astrocytes and their calcium signaling pathways, including sub-sections on the morphological and functional types of astrocytes. Last sub-section concentrates on the comparison of the different types of astrocytes, comparing the calcium activity of each type.

2. BRAIN NETWORKS

During the last few decades a new concept of the physiology of synapses has been defined. Astrocytes were discovered to exchange more information than anticipated with the synaptic elements. The key element in this concept are calcium (Ca^{2+}) dependent mechanisms. [5] To understand the function of astrocytes in the brain networks, the basics of cell communication must be surveyed. Before getting deeper in the signaling of astrocytes, it is important to study the basic structure of the brain, signaling in the brain networks and the basic role of Ca^{2+} activity in it.

2.1 Structure of the brain

Neurons, so far well known as the specialized cells for the tasks of information processing, encoding and transfer, form the grey matter of the brain. Connected to each other, forming neuronal networks, neurons process and transmit information through electrical and chemical signals. [4] The central nervous system – the brain and spinal cord – has neurons as the core components. A neuron consists of a soma, dendrites and an axon. A synapse between axon and dendrite connects two neurons to each other.

However, the major of the brain consists of glia cells. Glia or “glue” cells give the brain structural, chemical and immunological support. [4] In the nervous system, four major groups of glia cells exist: Schwann cells and oligodendrocytes, microglia, nerve/glial antigen 2 and astrocytes. Schwann cells and oligodendrocytes form the myelin layers covering axons. Microglia is an immune cell type of the nervous system and NG2-positive glia are located in the mature brain. [8]

Astrocytes are connected with blood vessels, other glia cells and neurons. As neurons display electrical excitability, astrocytes exhibit calcium excitability. [11] Astrocytes have multiple ways to regulate and optimize the surroundings of neuronal activity. They maintain pH homeostasis, provide metabolic substrates, clear neuronal waste and deliver glucose. [4; 7; 8] Astrocytes can form bridge structures from neuronal to vascular level within different brain structures enabling networks that otherwise are disconnected from each other. This most common glia cell type generates various regulatory signals and functions as local communication element of the brain, playing an active role in information processing within neuronal circuits. [7; 15]

The number of astrocytes, whether presented as a proportion of total cell number of the brain or as a ratio to the number of neurons, is higher in more complex brains and with increasing phylogeny. For every neuron there are 1.4 astrocytes in the human cortex (for review see [4]). Recent evidence supports the fact that the greater quantity of astrocytes enables the function of complex networks. More dense and sophisticated brain networks require more modulation and control locally. [4]

2.2 Signaling of neurons

The basis of signal processing in the brain are based on synaptic transmission between neurons that construct networks together. This transmission includes processes within the pre- and post-synaptic parts, but also several extrasynaptic signaling pathways can have an effect in it. Transmission over synapses is chemical but the information transmission in neurons is based on membrane voltage changes called action potentials. [2; 3]

The voltage gradient across the membrane is maintained by metabolically driven ion pumps combined with ion channels. Action potentials are formed when the membrane of the area called axon hillock depolarizes and generates the opening of voltage gated sodium channels. Also, other ions like calcium, chloride and potassium are involved in constructing the concentration differences. The depolarization of the membrane spreads away from the soma of the neuron and towards the synapse between the sending (presynaptic) and receiving (postsynaptic) cell. As the action potential moves along the axon, the channels behind the forwarding edge are closing and causing the neuron a refractory period, when it cannot transmit another signal. [2]

The action potential arrives to the end of the axon and causes the messenger molecules to activate the ion channels in the synapse, inducing it to release neurotransmitters into the synaptic cleft. Instead of voltage gated sodium channels, there is voltage gated calcium channels in the presynaptic terminal. Calcium enters these channels binding to SNARE-proteins and forming a molecule that merges with a vesicle separated from the membrane with the neurotransmitters in it. This merging causes the neurotransmitter release. The transmitters are detected by the receptors of the postsynaptic neuron and a new action potential is occurred as the postsynaptic membrane is hyperpolarized. [2] Calcium signals in neurons are characterized by properties like large amplitude, long duration of tens of seconds, and regular but infrequent appearence of 0,5-5 minutes. [15]

2.3 Neuron-Astrocyte interaction: tripartite synapse

There is a lot of new evidence about active communication of astrocytes to neurons from recent experimental studies. Astrocytes and the Ca^{2+}-dependent microscopic mechanisms are still quite unknown, and the comprehensive understanding of these actions could lead to a better understanding of the whole nervous system. [5; 6] The discovery of astrocytes being important in the function of the synapse in addition to the pre- and postsynaptic components was introduced as a concept of a tripartite synapse. [3]

Tripartite synapses consist of two neurons and an astrocyte. One astrocyte can control multiple synapses, but there is not more than one astrocyte connected to one synapse. [3; 8] Some of the neurotransmitter molecules released from the presynaptic terminals into the synaptic cleft can bind to metabotropic glutamate receptors (mGluRs) on the astrocytic processes. The binding activates G-protein mediated signaling cascades resulting an increase in IP3 production and phospholipase C (PLC) to activate. The IP3 in the intracellular stores binds to IP3-receptors in astrocytes, triggering calcium ions to be released into the cytoplasm from endoplasmic reticulum (ER). Increased intracellular Ca^{2+}-concentration triggers the release of gliotransmitters into the extracellular space. This can have an effect on both the pre- and postsynaptic parts of the neuron. [3; 12]

Astrocytes can chemically both receive and send signals to neurons. They can react to multiple neurotransmitters and factors and also release glutamate, D-serine, ATP, gamma-aminobutyric acid (GABA), prostaglandins and neuropeptides. This process is called gliotransmission. [7; 12] In addition to evoking neuronal signals, astrocytes can also inhibit them. For example, an increased frequency of inhibitory postsynaptic currents is proposed to depend on activation of GABAergic interneurons. The activation is probably resulting from astrocytic glutamate activity. [14]

Low synaptic activity leads to local intracellular calcium concentration elevations, meaning that the elevations stay only in the activated astrocyte processes, in short-distance. High synaptic activity leads to global intracellular calcium elevations, as the IP3 binds to the neighboring cells and calcium is released in those adjacent astrocytes. [12]

3. ASTROCYTES

The outdated consideration about astrocytes is that they are ancillary maintenance and support cells for neurons, because they do not have sodium channels and are electrically nonexcitable [4; 10]. Later this idea about astrocytes being quite inactive has been revealed to be wrong, as the interaction with the vasculature to form a gliovascular network was found. It is true that the network organizes the structural construction of the brain, but also the communication pathways, activation, thresholds and plasticity of it. [4; 35] Calcium imaging *in vivo* and in acute brain slices have confirmed that astrocytes can generate various patterns of activity [14; 17]. The excitability of astrocytes appears as transient or prolonged elevations in intracellular calcium levels. [11; 17] Local intracellular calcium concentration transients can be generated spontaneously or by different stimuli, such as mechanical stimulation or release of transmitters such as glutamate, GABA and ATP. [17; 31; 35]

3.1 Astrocytes in general

There is no such marker labeling all astrocyte types but not the other cell types existing in the brain [29]. A general shape of an astrocyte cell bears a likeness to a star – a bush with processes radiating out from a cell body. As seen in the Figure 1A, an individual astrocyte tends to occupy distinct, nonoverlapping domains. An astrocyte has an approximate soma diameter of 7-9 micrometers and a volume of 66,000 μm^3 [8]. The Figure 1B shows an example of an astrocyte extending its processes to a neuronal dendrite. The processes of a single astrocyte can surround approximately 140 000 synapses in human brain. At the same time, the different regions of that single astrocyte can interact autonomously with certain synapses, while other parts of the cell interact with other cellular elements. [8; 17; 29]

Examples of astrocytes controlling non-neuronal brain cells can be the processes of astrocytes sending rapid coordination signals to blood vessel cells promoting neurovascular coupling and attracting cells to their territories by releasing chemokines. Chemokines are agents activating receptors on other cells. By these actions, astrocytes can coordinate the spatial positioning of oligodendrocytes during development, provoke microglia and lymphocytes in inflammation, and might attract reparative neural stem cells to lesion cites. [15] The other functions of astrocytes are for example the regulation of extracellular concentrations of potassium ion and neurotransmitters, promoting synapse

formation and neuronal growth, regulating blood-brain-barrier and producing neurons. [30; 34; 35]

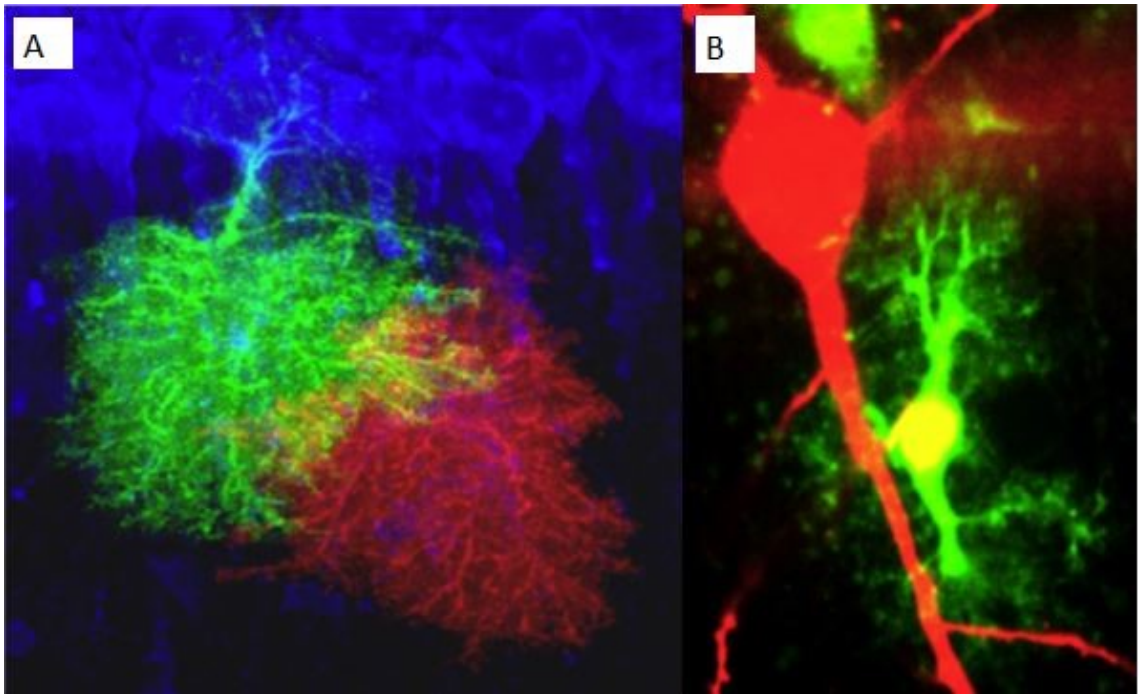

Figure 1. *A) Two neighboring astrocytes labeled with different colored fluorescent dyes. B) An astrocyte (dyed in green color) reaching its processes in close proximity to a neuronal dendrite (dyed as red), modified from source [8].*

Intracellular calcium

In the early 1970s the investigators found out that glia cells like astrocytes exhibited G protein-coupled receptors (GPCRs) in culture. These receptors are linked to a diverse group of intracellular signaling cascades. In 1997 the GPCRs were shown to be expressed by astrocytes also *in vivo*. [8] GPCRs stimulation evokes several different cell responses, among which the elevation of intracellular calcium can be considered as the most important one. [8; 10] The studies have shown that astrocytes can generate signals, bridge pathways and networks between structures that are not functionally connected, in addition to the well-known patterns of modulating the signals arising from neurons. They can send feedback and feedforward output responses to their neighboring cells. [15] Transient Ca^{2+} content rises independently of synaptic activity occurs particularly during development and Ca^{2+} fluctuations in consequence of transient receptor potential A1 (TRPA1) channel activity. The activity contributes to resting calcium ion levels. [18]

Also, the speed of the intracellular calcium concentration rise depends on the mechanism by which it is raised. [31] IP3-mediated intracellular calcium waves propagate

rapidly and they have a short delay between cells at most. The amplitude of the intracellular calcium transient decreases significantly from cell to cell, thus the waves only propagate for a few cells. The delay time between astrocytes is longer in ATP-mediated signals, leading to slower intracellular wave propagation and decay of intracellular calcium waves. [10]

Other factors contributing to the intracellular Ca^{2+} turnover are Ca^{2+}-dependent ryanodine receptor channels and sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase pumps (CERCA pumps). [24] Local endoplasmic reticulum structures are important in the intracellular generation of calcium waves, as the process depends on the activation properties of intracellular Ca^{2+} store receptors. These properties are exceedingly nonlinear. The smallest possible size of the interconnected ER structures is 50-100 *nm* and the volumetric ER-to-local cytoplasm ratio is 2-10%, so that the smooth ER can fit to small neuronal structures. This ratio is important for controlling the cytosolic calcium. This neuronal ER-versus-cytosol volumetric ratio is not possible in ultrathin protrusions of astrocytes, being 50-200 *nm* wide. The ER and mitochondria are typically located more than 0,5 μm from synapses in the thicker branches of the cells, so the regenerative calcium wave formation associated with the ER stores takes place mainly in the thicker processes of astrocytes. Passive diffusion of Ca^{2+} therefore mostly happens within and between the ultrathin processes. [24]

It has also been observed, that restricting the intracellular Ca^{2+} concentration in astrocytes can prevent the release of transmitters at neighboring synapses by inhibiting the modulation of neurotransmission. Therefore, another model states that presynaptic neuron releases transmitters, activating the astrocyte's and postsynaptic neuron's receptors. The endoplasmic reticulum of the astrocyte then liberates Ca^{2+} ions, causing the release of transmitter molecules that control neurotransmission. [26]

Intercellular calcium waves

Together the astrocytes form a network called synctum. The cells are connected by connexin proteins called gap junctions, forming tunnel like structures through the outer membrane of the cells. These channels allow the concentration between astrocytes to get steady fast and effectively by letting through inositol-1,4,5-triphosphate (IP3) and therefore inducing so called calcium waves or glissandi by releasing Ca^{2+} ions from stores in the endoplasmic reticulum. [8; 24] There are also other pathways for calcium waves to spread, like the release of adenosine triphosphate (ATP) or glutamate into the extracellular space of an astrocyte cell. The release of these particles results to the activation of the respective receptors on the adjacent astrocytes. [11] The astrocyte subtype affects on the selection of the pathway used in the signaling, leading to a great diversity in interactions with adjacent cells. [10]

These waves, also known as intercellular calcium waves (ICWs), consist of localized increase in the concentration of cytosolic calcium following a series of similar signals in wave-like form. ICWs can either be transmitted to the cells connected (intercellular), or restricted to one cell (intracellular). [11; 13; 16; 17] The waves decay in time and in space when propagating. The maximal propagation range is around 200-350 μm and a maximal velocity 15-27 $\mu m^2/s$. [10] The passage of a wave alters some calcium dependent mechanisms in astrocytes, and sometimes the appeared change persists and modifies the responses of the cells. [11; 16]

Astrocytes that are lacking physical contact with each other have still been observed to be engaged in Ca^{2+} waves. In the physical contact of astrocytes, gap junction proteins, composed of the two apposed hemichannels of nearby cells, have an inner pore freely permeable to large anions like ATP. Based on the lacking contact of some cells, a model whereby astrocytes could also potentiate ICW propagation without the gap junctions was suggested. One way to the ICW propagation without those pathways is by enhancing ATP release. Bioluminescence imaging of extracellular ATP reveals that ATP is released by a single point source of a single cell causing the propagating wave front to reflect the diffusion of ATP following a burst event. Under this scheme, the adjusting astrocytes increase intracellular calcium levels in response to the activation of purine receptor P2Y, but they cannot amplify the propagation more by releasing some extra ATP. This model applies to the observation that ICWs of astrocytes do not expand further than 300-400 μm from their origin very often. [4]

It is certain that in cell-cell signaling in the astrocyte networks, both indirect coupling via extracellular messenger ATP and direct coupling via intracellular messenger IP3 are involved. [10] There still are some questions about the importance of each method in general and in different astrocyte types. It has been demonstrated that different messengers induce intracellular calcium waves with distinct characteristics. These characteristics are propagation speed, propagation distance, delay between cells and intracellular calcium transient profiles. [10]

However, according to Kirchhoff [26], “there are concerns about whether such gliotransmission is merely an experimental artifact”. There are multiple researches with different results concerning this model, but differences in experimental setups or method-intrinsic artifacts may be responsible for the divergence. Kirchhoff then suggests, that other molecules affecting Ca^{2+} waves should be taken into account in the studies, such as voltage-gated Ca^{2+} channels or transient receptor potential channels (TRP). TRP channels are Ca^{2+} -permeable nonselective cation channels. They can be gated by stimuli like oxidants, phospholipids, acidity, osmolarity or cell volume changes. In astrocytes, it is possible to prepare transmitter-containing vesicles which can provide a fast release of particles without the need of a long process. Also, the requirements for calcium ions in releasing gliotransmitters may differ in different subcellular regions of an astrocyte.

TRP or voltage-gated Ca^{2+} channels can mediate Ca^{2+} entry in the perisynaptic astrocyte processes thinner than 50 nm. [26]

Even though astrocytes are connected to each other, and even within a localized area of the brain where the cells appear identical morphologically and immunocytochemically, the expression and the response to the activation of GPCRs may vary. This kind of diversity is usually taken into account when considering neurons, but rarely noticed when interpreting data derived from astrocytes. [8]

3.2 Morphology types

The research and literature have contradictory information of the morphology types of astrocytes. Different methods suggest multiple results in defining the subpopulations of astrocytes and the boundaries of the different types. However, recent studies *in vivo* show that astrocytes can both respond to neural activity by calcium mechanisms and to perform spontaneous calcium increases. It is also likely that different levels of Ca^{2+} activity will be exhibited by different subpopulations of astrocytes or different microdomains in astrocytic processes [8; 34]. In this section, the morphology types of resting ("healthy") astrocytes and reactive (in response of some insult, "non-healthy") astrocytes are studied.

3.2.1 Morphology of resting astrocytes

Already in the 19th century the heterogeneity of astrocytes in different brain regions was noticed and two morphological subclasses, protoplasmic and fibrous astrocytes, were defined (for review see [29]). Later at least nine different morphological types have been distinguished by three different methods, but the function of all these cells is not yet compared in the required accuracy. [29] Therefore, only the types with further research are represented here.

The best-known method, within astrocytes can be divided into different types with distinct morphological features, is by immunostaining glial fibrillary acidic protein (GFAP) and using confocal imaging to follow and reconstruct cells. With this method, at least four major morphologic subclasses of astrocyte cells can be found in the temporal lobe of adult human. [22] A schematic of these cortical astrocyte types is presented in the Figure 2 below.

In the layer 1 of the human cortex, the majority of astrocytes is the type of densely packed interlaminar cells, with their millimeter long processes which terminate in the layers 2-4. Protoplasmic astrocytes populate the layers 2-6 and layers 5-6 are characterized by rare varicose projection astrocytes, which have long processes randomly extending around them. The white matter is populated by fibrous astrocytes. [22; 29] Several types can exist in different brain regions and the morphology is determined by the cyto-

architecture of the region, like in the white matter where the fibrous astrocytes are characterized by their processes being oriented along the fiber architecture of that brain region. [29]

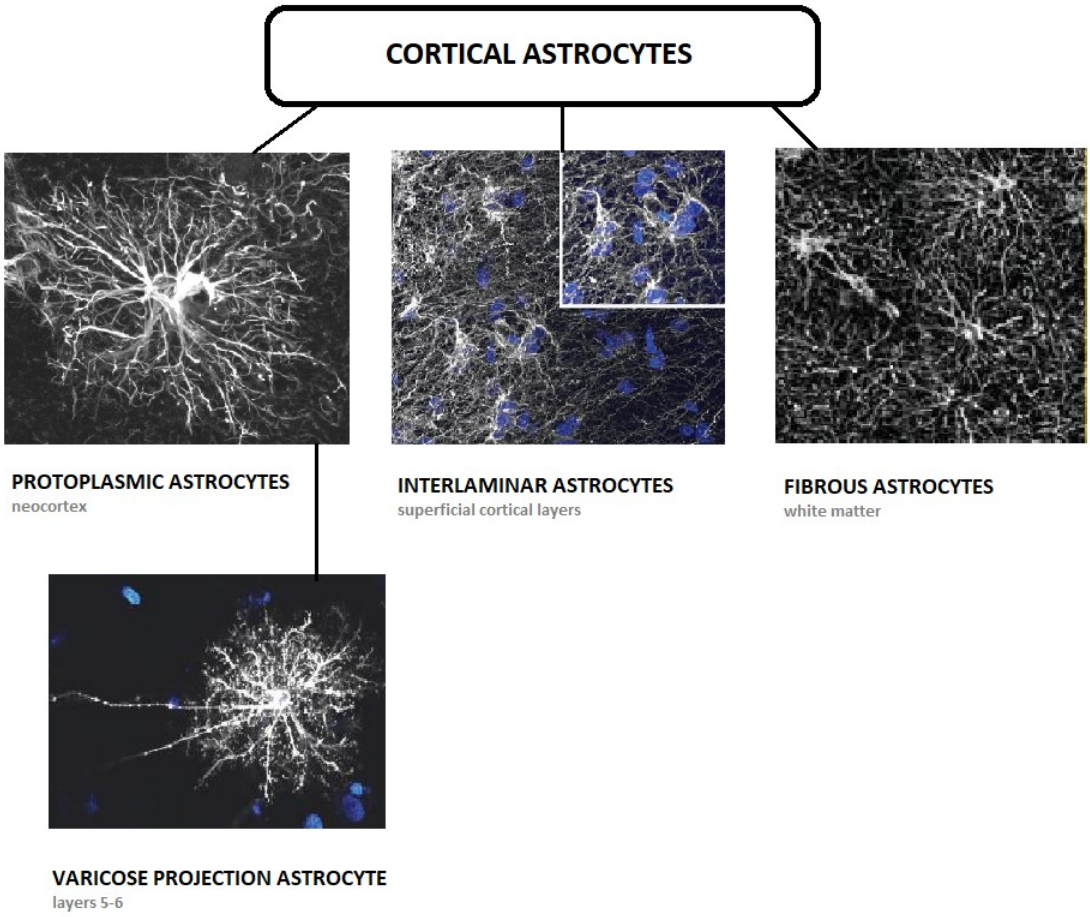

Figure 2. *Different morphology types and prevailing regions of human cortical astrocytes, modified from source [22].*

Protoplasmic astrocytes are the most common type of astrocytes in the human brain [8]. The primary processes of protoplasmic astrocytes are typically tortuous and highly branched processes [8; 15; 22]. These cells contact the most other types of cells in the brain and spinal cord [8], since their processes (diameter of 20 to 30 *nm*) are represented by thin, leaf-like structures that can penetrate the smallest extracellular gaps. [6] Human interlaminar astrocytes are round and have small cell bodies and their short processes extend in all directions and form a thick mesh of GFAP fibers. These fibers are not seen in other primates. Human interlaminar astrocytes also extend few processes from layer 1 to layers 2-4 of the cortex, and the processes wind back and forth as they travel. [22; 29]

Fibrous astrocytes are larger and do not have so fine bulbous processes than protoplasmic cells. This astrocyte type is specialized for structural support, having overlapping processes and cell bodies that are quite evenly located from each other. Fibrous astrocytes do not have synaptic contacts in the white matter. [22; 29] The amount of varicose projection astrocytes is quite limited in the brain, but it is one of the major features that separates humans and chimpanzee from other primates and species. The main $GFAP^+$ processes of this subpopulation are straighter than those of protoplasmic astrocytes, and they have varicosities that are spaced approximately every 10 μm, being typical only for this type of astrocytes. The processes are penetrating the process-delimited domains of neighboring astrocytes and some of them also extend a few millimeter long processes, terminating in the neuropil or on the vasculature. The function of these long processes is not known, but it is assumed that they help the cells in long distance communication across cortical layers, or they might even induce signals between gray and white matter. [22; 29]

Astrocytes in vitro

There are multiple options regarding *in vitro* studies of human astrocytes. The cells can initially be taken from fetuses or biopsies, or even generated from induced pluripotent stem cells. *In vitro* studies have few limitations, the main one being that astrocytes show signs of reactivity in a dish. Astrocytic reactivity is discussed thoroughly in the section 3.2.2, but as described shortly, the signs of reactivity means a flat, polygonal morphology of the cells *in vitro*. Co-culture with neurons triggers a bushy star-like morphology, as well as some new methods developed to reduce the astrocytic reactivity *in vitro*. These methods can be for example a 3D polymer matrix or an exposure to heparin-binding growth factor. [25]

At the submicroscopic level, astrocytes also have a substantial motility of cellular processes and an extraordinary ability to self-repair. This ability can be seen for example when putting a patch pipette through the astroglial projections and it not having any visible effect in the cell function like membrane currents or calcium signals. This is most likely a result of the high fusing and sealing capacity of the astrocytic membranes. Astrocytes tend to spread in a flat shape in monolayer cultures, unlike astrocytes *in situ*. This tendency might be explained by the relatively high membrane fluidity. [24]

3.2.2 Morphology of reactive astrocytes

In response to different brain insults, astrocytes can become reactive, meaning that they are characterized by morphological changes. The possible brain insults causing these changes can be for example trauma, infection, epilepsy and neurodegeneration. There is

also evidence that changes in gliotransmission, and therefore in astrocytic calcium signaling, contribute to the development of neurological disorders. [20; 25; 27]

When strong immunohistological staining for GFAP protein was discovered and noticed to have strong expression in reactive astrocytes, it became the hallmark of them. The clearest morphological change of reactive astrocytes is an enlarged cell body and processes (astrocytic hypertrophy). When visualized by GFAP, the thicker processes appear longer (as seen in the Figure 4), since they can be followed over greater distances. Their arborization is also reorganized: the number of primary processes changes or they might polarize toward the injury site or amyloid plaques in the case of Alzheimer's disease. The changes in perisynaptic processes of astrocytes that are in contact with synapses are not so well-known. [20; 25; 27] Alzheimer's disease and epilepsy are used as examples of reactive astrocytes and thus described here more specifically.

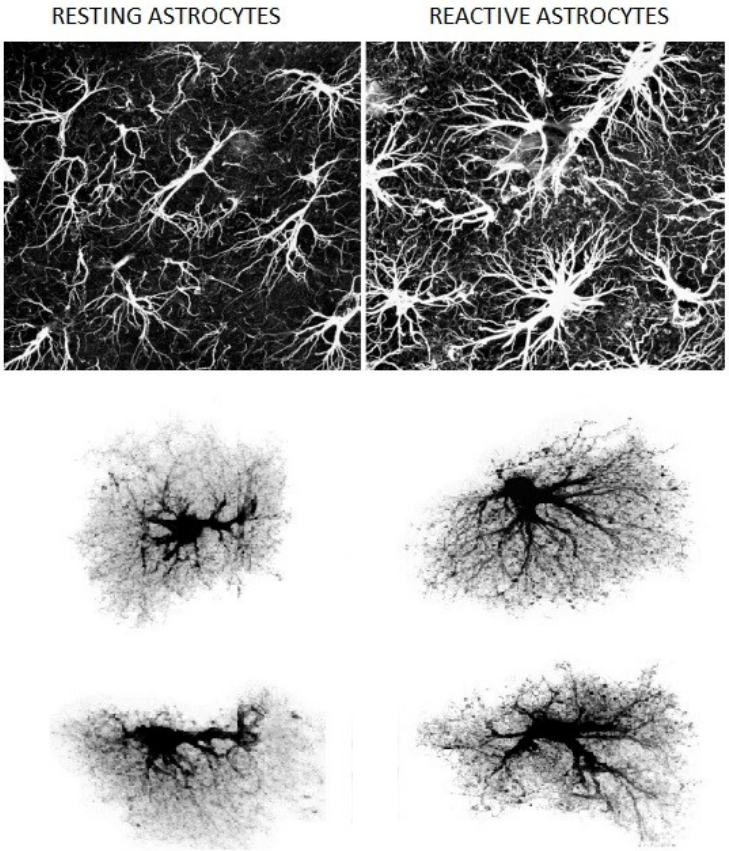

Figure 4. *Resting, "healthy" astrocytes on the left and reactive astrocytes on the right, modified from [27].*

Alzheimer's disease

Alzheimer's disease is a form of dementia, having characteristics of cognitive deficits like learning impairment and memory loss. The hypothesis in the mechanisms of Alzheimer's disease is that synapses fail and dendritic spines do not work properly in the plaque environment. The failure is caused by changes in synaptic drive, calcium overload and activation of calcium-dependent degenerative processes. [21; 25] Amyloid-β (Aβ) is a crucial protein in the formation of the disease, since it assembles in the plaques. Healthy astrocytes are important in the battle against the disease, as they specifically bind, internalize and degrade amyloid-β (Aβ). [25]

In this disease, the Aβ-generating enzyme β-secretase has an abnormal expression and together with a defect in the Aβ degradation, the role of astrocytes can be switched from the important part of the normal brain function to the promoters of the disease, as they start increasing the plaque formation. [15; 25] With imaging and proteomic techniques, the early phase of Alzheimer's disease can be detected in the brain before the symptoms start. The reactive cells are found in entorhinal cortex and hippocampus, with gradual progression to temporal, frontal and parietal lobes. [25]

Epilepsy

Normal brain function is disrupted in epilepsy, which is a neurological disorder caused by bursting activity of neuron groups. This is a result of long-lasting plastic changes in the brain that have effect on the expression of receptors and channels and reorganizing of synapses and reactive gliosis. [23] Long-term changes of astrocyte structure and function over weeks and months are a hallmark of mesial temporal lobe epilepsy. An outstanding finding in the later stages of the disease is hippocampal astrogliosis, which means a multiple changes of astrocyte density, morphology, biochemistry and physiology. [23; 36]

3.3 Functional types

According to Haim et al. [25], the next challenge in the field is that astrocytes show remarkable functional heterogeneity, as they display heterogeneity regarding their density, transcriptional profile, morphology and expression of transporters, receptors, channels and transcription factors. [25] Depending on brain region and age, the astrocyte networks can have notable differences. Using genetically encoded calcium indicators which are proteins reacting to an increase in Ca^{2+} levels by fluoresce, it has been possible to determine the differences in Ca^{2+} signaling dynamics between the regions of individual astrocytes. [29; 33; 34]

Until the recent years, most studies have reported slow calcium signals which are on a scale of seconds. It does not necessarily mean that the kinetics of intracellular Ca^{2+} concentration is slow, since these results might be a cause by a limited resolution and quite small signal-to-noise ratios in the measurements, not the fact that the signals were slow in the reality. [24] According to few studies, the astrocyte calcium wave in human astrocytes seems to propagate with a speed of 40 $\mu m/s$. [29] In the reality, there are probably multiple different functional types of astrocytes with differing propagation speeds in human brain, some of the types still unrevealed.

Ca^{2+} influx / Spotty Ca^{2+} signals

Spontaneously and randomly occurring microdomain calcium signals, "spotty Ca^{2+} signals", are mediated by calcium influx through TRPA1 channels in hippocampal astrocytes. Similar spotty Ca^{2+} signals in astrocytes are usually called microdomains, and they can be generated both in brain slices and *in vivo*. The molecular basis of these microdomains stay still unclear [33], but it seems that the transmembrane calcium fluxes are somehow involved in calcium waves in astrocytic processes. [19]

The detectable number of waves and microdomains in astrocytic processes, as well as basal calcium levels, are significantly contributed by the transmembrane calcium fluxes and also completely reversible. This means that calcium waves within processes are reproducible and stable over time. This activity is more frequent and dissociated from activity in the cell body, like they were functionally independent. [18; 19]

Multiple studies (for review see [33]) have revealed differences in spotty Ca^{2+} signals between subregions of the brain: astrocytes in different areas have calcium signals from different sources, of differing duration, and with different reliance on neuronal function. Evaluations done in hippocampal astrocytes show that around half of the spontaneous calcium signals in the processes are dependent on transmembrane fluxes. In the soma, almost none depend on that mechanism. The calcium ion concentration is locally elevated by around 300 nM with the spotty transmembrane fluxes, when with GPCRs it is globally elevated to at least 1 μM. [33]

Hippocampal astrocytes: focal and expanded events in astrocytic processes

Di Castro et al. [1] have done research on intracellular astrocytic calcium signaling analyzing the activity in the focal plane of the brain, taking into account the spatio-temporal characteristics, origin and underlying mechanisms of the signaling. The research considers if the astrocytic activity is spontaneous or driven by neuronal inputs. Di Castro et al. used high-resolution two-photon laser scanning microscopy (2PLSM) calcium imaging in the hippocampal dentate molecular layer (HDML) of the adult mouse. As a result, the research revealed two distinct types of calcium events, focal and expanded. [1]

The brain circuits in hippocampus are proverbially involved in cognitive activity. Hippocampal astrocytes are thought to exert a range of effects in excitatory and inhibitory nerve cells and fibers through the action of gliotransmitters. Supposedly, the purpose of hippocampal astrocytes is to fine-tune the balance between excitation and inhibition. [15] Hippocampal astrocytes can also proliferate, since neural stem cells are located in the hippocampus. Most astrocytes in the adult brain do not proliferate. However, the electrophysical properties of these neural stem cells functioning astrocytes are similar to the other astrocyte types. [30]

Local calcium activity in the processes of mature hippocampal astrocytes is intense. This activity is confined to astrocytic processes, occur 10-30 times more frequently and lasts a shorter time than transients involving the astrocyte cell body. Hippocampal calcium signals can be identified into two groups with distinct properties, the first one and a majority (95,5%) of signals being called focal events and the second expanded events. Focal events are small intracellular calcium elevations which are usually restricted to a single subregion of one cell, whereas expanded events are larger intracellular elevations which appear almost simultaneously in multiple neighboring subregions of one cell. The distinct properties of these events identify them as separate phenomena which are most likely triggered by different stimuli. [1] The two types of calcium events can be seen in the Figure 5 below.

Focal events are the fastest and most spatially confined calcium signals in astrocytes with a rise time of 139 ± 12 *ms*, full-width half-maximum duration of 696 ± 57 *ms* and peak amplitude of 19,3 ± 1,6 %. Expanded events are called expanded, since they span several micrometers (12,85 ± 1,16 μm) of the analyzed process and the full extension is larger, as they invade adjacent processes at branch points. Expanded events do not usually involve the astrocyte cell body nor spread to the end of the process. They seem to be composed of intracellular calcium elevations occurring simultaneously (0-40 *ms*) or with delays (100-200 *ms*) in multiple sub regions. Their peak is often composed as a summation of smaller events with an average peak amplitude of three times higher than that of focal events. Also, their other average properties are distinct from those of focal events, with full-width half-maximum duration 4,5 times longer (3,18 ± 0,34 *s*) and a rise time of seven times longer (1,03 ± 0,12 *s*). [1]

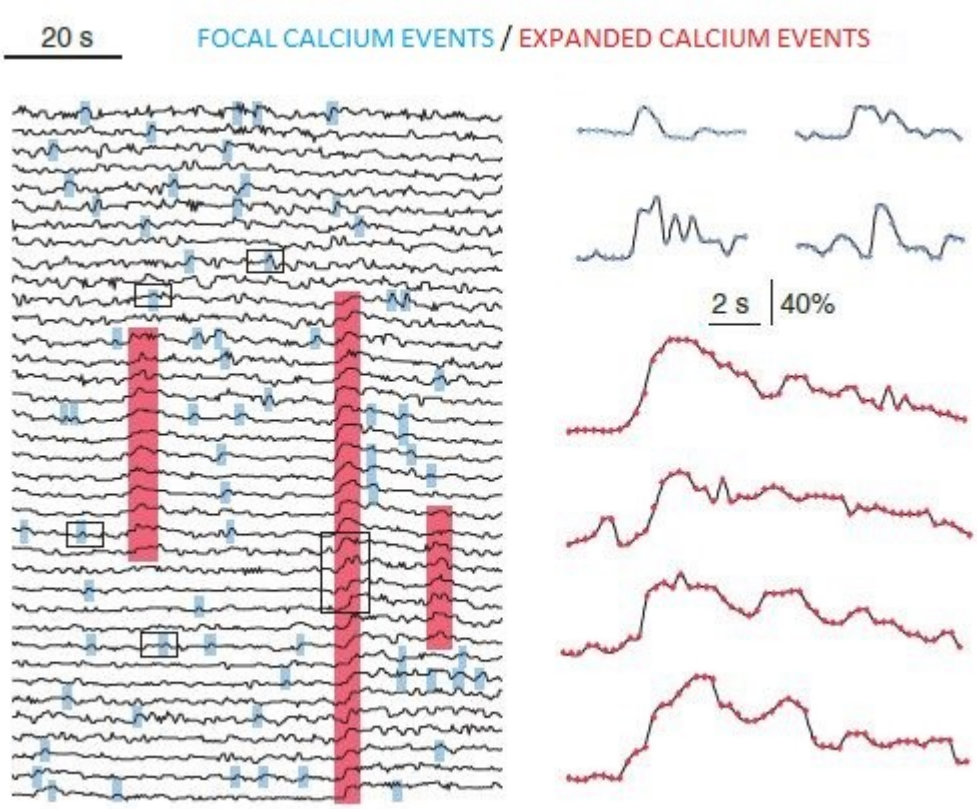

Figure 5. *Focal and expanded calcium events in astrocytic processes. Example of calcium activity imaged in frame scan mode for 80 seconds. On the left in the figure are traces showing occurrence of Ca^{2+} peaks in neighboring process sub regions. Expanded calcium events are shown in the red highlights, identified by relatively high-amplitude waves in several sub regions. Focal calcium events are shown in the blue highlights, identified by small waves often limited to one sub region and occurring randomly. On the right in the figure are magnified traces of the squared examples of focal and expanded calcium events. Modified from source [1]*

The study made by Di Castro et al. [1] suggests that individual action potential fired by an axon which lies beside the studied processes produces expanded calcium events. Occurrence of an action potential produces robust and long-lasting calcium rises involving micrometers long regions and their branches. However, expanded calcium events still differ from those larger, longer and more global astrocytic Ca^{2+} responses to intense firing activity of neurons. In contrary, focal calcium events are a result of transmitter release at adjacent synapses in spontaneous synaptic release which maintains dendritic spines and stabilizes their connections. Focal signals, and their continuous exchange between astrocytes and synapses, might be one crucial part in maintaining the tripartite connections in place. Based on multiple tests, it was also implied that calcium elevations in astrocytic processes have their part in the local transmitter release at excitatory synapses. [1]

Haustein et al. [32] made a following research on these calcium activities, and stated that astrocytic calcium signaling was tightly gated by glutamate clearance. This means that when the glutamate uptake was blocked, enhanced spontaneous calcium signals and elevated electrically evoked astrocyte responses were observed. Glutamate transporters do not regulate spontaneous calcium signals, but they gate the engagement of astrocytes and spontaneous calcium signals. [32]

Hippocampal astrocytes: intercellular Ca^{2+} waves

Using mechanical and chemical stimuli in hippocampal astrocyte cultures and in hippocampal slice cultures, researchers have been able to observe intercellular Ca^{2+} signaling as a response. The spatial propagation of these waves has been reported to be spiral shaped. [16] The main findings in the studies are that calcium waves evoked by mechanical stimuli are mediated by an intercellular mechanism and the waves rely on a diffusible messenger. The messenger used for calcium waves is ATP released from vesicles into the extracellular space, and acting via P2Y1 receptors. [11] However, there are contradictory results about the gliotransmitter messenger used in hippocampal astrocytes, since other studies state that calcium waves occur primarily through exocytosis of glutamate. [15] Astrocyte calcium waves can spread to around 200 μm from the origin point in hippocampal astrocytes. At further distances, the peak changes in intracellular calcium concentration for individual astrocytes are too short and small to enable the calcium wave to propagate. [11]

Hypothalamic astrocytes

In hypothalamic slices, the noradrenaline-dependent synaptic potentiation requires astrocytic release of ATP. In response to adrenergic input hypothalamic astrocytes release ATP onto nearby neurons. This leads to the activation of P2X7 receptors of these neurons, leading to the enhancement of AMPA receptor surface expression. Also the amplitude of miniature excitatory postsynaptic current reacts to these mechanisms, as an increase of it occurs. [20] Even if glutamate uptake is a characterized function of astrocytes, a group of hypothalamic astrocytes lacks the ability to take up extracellular glutamate. [30]

Striatal astrocytes

The astrocytes in the striatum have lower frequency in spontaneous calcium signaling and the extracellular entry for basal calcium levels is the dominant mechanism in the

homeostasis of them. It seems that striatal astrocyte territories are bigger than hippocampal territories, and they also have a contact with greater number of neuronal somas. However, hippocampal astrocyte territories contain more excitatory synapses, leading to some differences in the function of these cells. [28]

Striatal and hippocampal astrocytes seem to have significant differences in their gap-junctional coupling and spontaneous, electrically evoked GPCR-mediated calcium signaling. The differences between GPCR signaling seem to be reflected as differences in gene expression regulation, and the activation of some GPCR pathways in striatal astrocytes is more effective than in hippocampal ones. Chai et al. [28] also represented the first unbiased astrocyte subpopulation identifying protein, as they showed that μ-crystallin protein is specific for striatal astrocytes. [28] However, the study does not provide more specific information on the calcium signals of striatal astrocytes and leaves multiple questions to be answered later in the astrocytic research.

Astrocytes in vitro

The gap junctions are not the pathway in the coordination of the intracellular calcium concentration activity *in vitro*, but it seems that these signals require transmitter activation of N-methyl-D-aspartic acid (NMDA) or metabotropic glutamate receptors. The magnitude and extent of these effects depend on the preparation used. It can also involve some intracellular calcium concentration changes in the whole population or only a few adjacent cells, or not in any cells. [9]

3.3.1 Function in the morphology types of resting astrocytes

Independently of neuronal activity two forms of calcium signaling in astrocytes can occur: spontaneous calcium oscillations in individual cells and coordinated form of intercellular calcium wave. In the somatosensory cortex, layer-specific differences in spontaneous wave patterns of astrocytes can be detected in rats, leading to a conclusion that different morphology types have characteristic signaling. It can be seen, that in the cortical layer 1 calcium waves are asynchronous and have a higher frequency, when in the cortical layer 2/3 the calcium oscillations are infrequent and mostly synchronized between different cells. [29; 30]

Differences between intercellular calcium waves in white and grey matter can be seen in propagation of the waves. Functional gap junctions enable the propagation of the wave in the cortex, whereas in the corpus callosum the gap junctional coupling is low and the waves propagate through the release of ATP. [29] Gap junctional coupling, and therefore IP3-mediated intracellular calcium waves, seems to be important also in astrocytes

in the neocortex, where the propagation requires intact gap junctional conductance. Protoplasmic astrocytes are the most common subgroup in that brain region. [10; 29]

Protoplasmic astrocytes also have a low input resistance, voltage- and time-independent K^+ currents, very negative membrane potential, prominent glutamate uptake and extensive gap-junctional coupling. [15] However, Rusakov et al. [6] call protoplasmic astrocytes passive, stating that there is a poor understanding on the mechanisms on active communication between protoplasmic astrocytes and neurons. [6] Varicose projection astrocytes are so rare that it is hard to evaluate whether they perform any calcium signals, and by which pathways do they use if signaling occurs. [22]

3.3.2 Function in reactive astrocytes

Any changes in brain homeostasis can trigger astrocyte reactivity and the physiological alterations in reactive astrocytes are less understood than the morphological ones. [20; 25] Since there are multiple brain insults and diseases causing reactivity, there can be different physiological alterations as a result, few of being for example alterations in glutamate uptake, K^+ buffering and water homeostasis associated. Neurodegenerative disorders such as Alzheimer's disease, and epilepsy both seem to have alterations in astrocytic glutamate uptake and K^+ buffering. [20] Thus, some changes of calcium homeostasis in reactive astrocytes, especially in Alzheimer's disease, have been reported in multiple studies. [25]

The changes of astrocyte function when they become reactive are quite heterogeneous between brain regions. As astrocytes communicate with adjacent cells by releasing various molecules involved in signaling, the alteration of these mechanisms affects the astrocyte activity. Resulting from the reactivity changes in astrocytes, the gliotransmitter release is strongly altered, leading to the alteration of the calcium activity also. [25]

Alzheimer's disease

The function of the astrocytes in Alzheimer's disease show unique characteristics *in vitro* comparing to *in vivo* studies. The long-distance signaling properties seen *in vitro* cannot be seen elsewhere before a pathological trauma. In the Alzheimer's disease model, astrocytes show elevations in resting calcium and increased functional activity. The effects of focal amyloid deposition across a bigger cortical network are amplified by astrocytic network. This might lead to some changes in cortical function and therefore to the memory disorders of Alzheimer's disease. The amplification effect is believed to be a result to the propagation of intercellular calcium waves, which are observed to begin in the local plaque micro-environment. This suggests that plaques might induce these ICWs which travel across the cortex. [21]

The increased astrocyte activity is not a result of coupling mechanism with neuronal activity. There are few reasons to assume this, firstly because even if neurons show weakened calcium homeostasis near plaques, the resting calcium of astrocytes is elevated there. Secondly, astrocytes show increasing activity near and far from plaques, while neurons exhibit hyperactivity only near plaques. Thirdly, when the neuronal activity was blocked, it had no measurable effect on calcium waves of astrocytes. Thus, the Aβ deposits might catalyze intra- and intercellular signaling of astrocytes even if it induces local synapto- and neuro-toxicity. [21]

Sigetomi et al. [33] suggest mechanisms of increased astrocytic calcium signaling being produced because they display improved extracellular ATP-dependent P2Y1 receptor-mediated calcium signaling, meaning that increased calcium signaling in Alzheimer's may be caused by a combination of ATP release and/or increased P2Y1 receptor expression. [33]

More frequent spontaneous calcium waves can be distinguished in mouse models of Alzheimer's disease by two-photon live imaging with calcium dyes. Changes in calcium activity in reactive astrocytes may produce changes in calcium dependent mechanisms like intracellular signaling cascades, proteolysis and gliotransmitter release. [25]

Postmortem brains of patients have less aquaporin 4 channels in astrocytes and they also show reduction in astrocytic glutamate transporter expression and glutamate uptake by biochemical assays. In mouse models some synchronous calcium oscillations and exhibit higher resting levels of calcium can also be seen in astrocytes. [20] Another gliotransmitter involved in the function of reactive astrocytes is GABA, which is excessively released by reactive astrocytes. The inhibition of dentate gyrus granule cells in the hippocampus of Alzheimer mice can be a result of releasing too much GABA. Pharmacological blockage of GABA transporters brings back the synaptic plasticity and memory deficits in the mice. For review see [25].

Epilepsy

According to Henneberger [36], a major part of the studies on epileptic activity of the brain have analyzed the mechanisms of astrocyte-neuron communication in reduced preparations like acute hippocampal slices. However, in a complex disease context like epilepsy, the brain network activity might increase the epileptic activity *in vivo* and we would need more studies taking this into consideration. Development of epilepsy may rely on normal mechanisms of astrocyte-neuron communication of the healthy brain, especially calcium dependent signaling is strongly implicated. The dominating effect is likely depending on the cell type (excitatory or inhibitory neurons and synapses), temporal characteristics and spatial extent. [36]

Several researches (for review see [23]) suggest that glutamate has a major impact on the pathogenesis of epilepsy. Excessive neuronal firing is triggered by local or systemic administration of glutamate agonists, and since astrocytes release glutamate via regulated calcium dependent mechanisms, it can be assumed it has a crucial role in synchronous firing of multiple neurons. Tian et al. [23] made some experimental observations involving astrocytes in initiation, maintenance or spread of seizure activity in epilepsy. Astrocytic glutamate release evokes prolonged episodes of neuronal depolarization and synchronized population spikes follow the seizure. It seems that astrocytes are the primary source in the extrasynaptic glutamate release, but other cells and pathways have a small effect too. [23]

The study does not suggest that astrocytes did not play a role in neuron originated seizure activity too. Astrocytes may amplify, maintain and expand neurogenic seizure activity, since neuronal firing induces changes in the extracellular ion proportions like an increase in K^+ and reduction of Ca^{2+}, which potently elicts astrocytic calcium signaling and glutamate release. Thus, a seizure focus of epilepsy might be partly a result of this kind of astrocytic activity. Spillover glutamate from excitatory synapses might also bind to mGluR, contributing to activation of astrocytic calcium signaling in that way. Astrocytes might be first activated by excessive neuronal activity, but maintaining and propagating abnormal electrical activity is probably carried on without the neuronal firing as a stimuli afterwards. [23]

The seizure focus may also expand locally because of similar mechanisms like lowering of the extracellular calcium. This process triggers the propagation of spreading astrocytic calcium waves, exciting neurons along their way by release of glutamate. In the other hand, this neuronal activity continues lowering the extracellular calcium and the distance from the seizure focus just keeps on growing. Thus, astrocytic calcium signaling is in a key role in the events causing normal brain tissue to start behaving in seizure-like manners in the further distance from the center of an epileptic focus. [23]

3.4 Comparison of the astrocyte types

The presented astrocyte types, morphological and functional, are gathered together into the following tables representing the substantial properties of the calcium activities of each type. The tables show the major pathways of calcium signals under the Pathway column, and under the column called Ca^{2+} waves are the distinct properties of each astrocyte type, if they happen to have some known patterns. The tables also gather together the locations of the types in the brain. In the table 1 below, the calcium activity of the four different morphological astrocyte types are seen.

Table 1. *A summary of the calcium activity in resting morphological astrocyte types representing the main pathways and properties of calcium signaling of them.*

Type	Location	Pathway	Ca^{2+} waves
Interlaminar astrocytes	Cortex, layer 1	Functional gap junctions	Asynchronous, high frequency of waves
Protoplasmic astrocytes	Cortex, layers 2-6	Functional gap junctions, glutamate uptake	IP3-mediated intracellular waves, infrequent, synchronized between cells
Varicose projection astrocytes	Cortex, layers 5-6	Functional gap junctions	-
Fibrous astrocytes	White matter	ATP release	-

The properties of the functional astrocyte types can be seen in the table 2. The location of the focal calcium events in the table explains these calcium waves being restricted into one subregion of hippocampal astrocytes, whereas the expanded events spreading to various subregions. For the two lowest rows of the table, the functional types located in striatum and hypothalamus were discussed in few studies, but did not have any specific name defined for them.

Table 2. *A summary of the calcium activity in resting functional astrocyte types representing the main pathways and properties of calcium signaling of them. Abbreviation [Ca^{2+}] means calcium ion concentration.*

Type	Location	Pathway	Ca^{2+} waves
Spotty Ca^{2+} signals / Ca^{2+} microdomains	Cortical astrocytes: Processes	Ca^{2+} release from intracellular stores	Random, spontaneous, reversible
	Hippocampal astrocytes: processes	Transmembrane Ca^{2+} fluxes	Local [Ca^{2+}] rise: 300 *nM* [33]
Focal Ca^{2+} events	Hippocampal astrocytes: one subregion in one process	Spontaneous transmitter release at adjacent synapses [1]	Random, rise time 139 ± 12 *ms*, amplitude 19,3 ± 1,6 % [1]
Expanded Ca^{2+} events	Hippocampal astrocytes: many subregions in one process	Adjoining action potential induces calcium rises in astrocytes	Simultaneous (0-40 *ms*) or with delays (100-200 *ms*), span rate 12,85 ± 1,16 *μm*, rise time 1,03 ± 0,12 *s*, amplitude 3 times higher than focal [1]
GPCR-mediated Ca^{2+} events	Striatal astrocytes	GPCR-mediated	lower frequency in spontaneous waves [28]
ATP-mediated Ca^{2+} events	Hypothalamic astrocytes	Release of ATP	-

The table 3 shows the properties of the reactive astrocytes presented in this study. The location of the reactive astrocytes in Alzheimer's disease depends on the state of the disease. The cells become reactive progressively in many regions of the brain when the disease proceeds.

Table 3. *A summary of calcium activity in reactive astrocytes representing the main pathways and properties of calcium signaling of the astrocytes in Alzheimer's disease and epilepsy.*

Type	Location	Pathway	Ca^{2+} waves
Astrocytes in Alzheimer's disease	Entorhinal cortex + hippocampus, progressing to temporal, parietal and frontal lobes	ATP-dependent P2Y1 receptor-mediated, GABA release	Synchronous, high calcium resting levels
Astrocytes in epilepsy	Mesial temporal lobe + hippocampus	Glutamate release	Spatial extent

As seen from the tables, there are many pathways and properties for the calcium signaling in the different regions of the brain. These mechanisms presented here are not the only possible ways for these astrocytes to perform their calcium activity, but they are the major and individual properties for the types.

4. DISCUSSION

Astrocytic calcium activity is a complex process which is not fully understood yet. As the morphological differences of different subpopulations of astrocytes are already recognized by many of the researchers, the functional differences are less-known. Also, there is no consensus about the principles of the division into the morphological astrocyte types either, nor there is unanimous supposition about the amount of the types existing in human brain. Thus, there is only a little bit of information on the calcium activity of these subtypes.

Different mechanisms and pathways in the rise of calcium signaling are already better-known, but the complexity lies in combining the morphological astrocyte types with certain mechanisms and pathways. Also, researches do not know if every mechanism is already found or not, and some of the information available is contradictory. For example, according to Di Castro et al. [1] the major part of calcium waves in hippocampal astrocytes are focal events, when Chai et al. [28] stated that when comparing global events (events encompassing the entire soma and some major branches) and non-global events, (events that include only a subregion) the major part of the signaling in hippocampal astrocytes is global events.

Thus, when discussing about the functional types of astrocytes, it is difficult to analyze the validity of studies when the differences in the results might be a consequence of multiple complex reasons. Things affecting the results are for example the methods the study is performed with, if the study is done *in vitro* or *in vivo*, where the neighboring cells might affect the signaling, if some proteins affecting the cells in some unknown way are used, or if the studies even consider the same mechanism or wave pattern, or just two different functional types in the same brain subregion.

Additionally, reactive astrocytes, which all seem to have some similar morphological properties, but differences in the function, is an another issue. For the future, it would be important to study the reactive astrocytes and their calcium signaling, to get more knowledge on the multiple diseases reactive astrocytes are involved in. This could lead to better treatments against these brain insults.

5. CONCLUSIONS

The heterogeneity of astrocytes in both morphology and function has been recognized already for years. Multiple methods must be used to distinguish the astrocyte types and trying to understand the differing calcium activity of them. It is certain that there are at least four morphological subtypes of astrocytes, interlaminar, protoplasmic, varicose projection and fibrous astrocytes, each located mainly in certain regions of the brain and having distinct properties in their calcium signaling patterns. The boundaries of the functional astrocyte types are not completely clear or restricted to certain morphological types or brain subregions.

Nevertheless, there are such distinct calcium activity patterns, that at least five different functional astrocyte types can generally be presumed to exist: spotty calcium signals, focal calcium events, expanded calcium events and the calcium wave types in striatal and hypothalamic astrocytes. The differences in calcium activity lie in the pathways the waves are formed as well as the features of the waves, like the span rate, amplitude and if the waves are synchronized or not. The morphology and calcium activity in reactive astrocytes in response to brain insults also differs comparing to resting, healthy astrocytes. To understand the astrocytic calcium activity properly, we must first understand the morphological types and the differences of these cells thoroughly.

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