Role of Actin-mediated Motility of Peripheral Astrocytic Processes in Synaptic Function

Dmitry Molotkov

Neuroscience Center
and
Division of Physiology
Department of Biosciences
Faculty of Biological and Environmental Sciences
and
Finnish Graduate School in Neuroscience

Academic Dissertation

To be presented for public examination with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki in Telkänpönttö (lecture hall 2402), Biocenter 3, Viikinkaari 1, Helsinki, on Thursday the 12th June 2014, at 12 o'clock noon.

Helsinki 2014

Supervised by:

Docent Leonard Khirug, PhD
Neuroscience Center
University of Helsinki, Finland
and
Professor Eero Castren, MD, PhD
Neuroscience Center
University of Helsinki, Finland

Reviewed by:

Docent Sarah Coleman, PhD
Department of Biological and Environmental Sciences
University of Helsinki, Finland
and
Nathalie Rouach, PhD
Center for Interdisciplinary Research in Biology
College de France, Paris, France

Opponent:

Professor Dmitri Rusakov, PhD
Institute of Neurology
University College London, United Kingdom

Custos:

Professor Juha Voipio, PhD
Department of Biosciences
University of Helsinki, Finland

ISBN 978-952-10-9963-2 (paperback)
ISBN 978-952-10-9964-9 (PDF)
Unigrafia Oy, Helsinki 2014

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I. **Molotkov D***., Zobova S*., Arcas JM., Khiroug L. (2013) Calcium-induced outgrowth of astrocytic peripheral processes requires actin binding by Profilin-1. *Cell Calcium 53: 338-348.*

The candidate substantially contributed to the experimental design, designed and performed molecular biology manipulations, performed microscopic experiments together with SZ, designed analysis approaches, analyzed the data together with SZ and wrote the manuscript together with SZ and LK.

II. **Molotkov D**., Yukin A., Afzalov R., Khiroug L. (2010) Gene delivery to postnatal rat brain by non-ventricular plasmid injection and electroporation. *J of Vis Exp 43.*

The candidate designed experiments together with AY, performed experiments and analyzed the data, prepared the video together with RA, LK and AY, wrote the manuscript together with LK.

III. **Molotkov D**., Kislin M., Zobova S., Toptunov D., Castren E., Khiroug L. (2014) Suppression of astrocytic morphological changes does not affect BOLD signal during visual processing in anesthetized mice. *Manuscript.*

The candidate substantially contributed to the experimental design, planed and performed molecular biology and viral work, performed BOLD signal acquisition experiments, participated in *in vivo* microscopy experiments, designed data analysis approaches together with DT and MK, analyzed the data and wrote the manuscript.

*Equal contribution

ABSTRACT

Among other glial cell types such as microglia, oligodendrocytes and radial glia, astrocytes are known to be involved in brain function; metabolically supporting neurons, regulating blood flow dynamics, participating in the development of pathological states, sensing and modulating synaptic activity. At the same time the complex astrocytic morphology, with a number of highly ramified peripheral processes located near the synaptic terminals, suggests them as a possible source for morpho-functional plasticity in the brain. This thesis summarizes the work on the *in vitro* development and further *in vivo* implementation, using a gene delivery system, of a tool for suppressing activity-dependent astrocytic motility. Calcium-induced astrocyte process outgrowth and its dependence on Profilin-1, novel *in vivo* gene delivery approaches, a demonstration of astrocytic motility *in vivo* and the independence of visual processing from astrocytic motility rates are the main findings of the project. The results described in this work increase our understanding of the interactions occurring between astrocytes and neurons as well as the consequences for brain function.

ABBREVIATIONS

AAV –adeno-associated virus
AD – Alzheimer disease
AMPA – α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
Ang1 – angiopoietin 1
AQP – aquaporin (channel)
ATP – adenosine triphosphate
BBB – blood-brain barrier
BOLD – blood oxygenation level-dependent
$[Ca^{2+}]_i$ – intracellular calcium concentration
CAG promoter – chicken beta actin promoter with cytomegalovirus enhancer
cAMP – cyclic adenosine monophosphate
CAR – coxsackie and adenoviral receptor
CMV promoter – cytomegalovirus early genes promoter
CNS – central nervous system
CSPG – chondroitin sulfate proteoglycan
DHK – dihydrokainic acid
EAAT – excitatory amino acid transporter
ECM – extracellular matrix
EGFP – enhanced green fluorescent protein
F-actin – filamentous actin
FGF – fibroblasts growth factor
GABA – gamma-aminobutyric acid
GAT – gamma-aminobutyric acid transporter
GDNF – glial cell-derived neurotropic factor
GFAP – glial fibrillary acidic protein
GLAST – glutamate-aspartate transporter
GLT – glutamate transporter
GLUT – glucose transporter
GlyT – glycine transporter
GPCR – G-protein coupled receptor
HIV – human immunodeficiency virus
InsP3 – inositol-3-phosphate
IP3 – inositol-3-phosphate
Kir – potassium inward rectifying (channel)
LCMV – lymphocytic choriomeningitis virus
LDH – lactate dehydrogenase

LTD – long-term depression
LTP – long-term potentiation
mGluR – metabotropic glutamate receptor
miRNA – micro ribonucleic acid (molecule)
MMP – matrix metalloproteinase
NKCC – neuronal potassium-chloride cotransporter
NMDA – N-methyl-D-aspartic acid
NMDAR – N-methyl-D-aspartic acid receptor
OAPs – orthogonal arrays of particles
P2X – purinoreceptor
PAPs – peripheral astrocytic processes
PKC – protein kinase C
PNS – peripheral nervous system
shRNA – short hairpin ribonucleic acid (molecule)
SON – supraoptic nucleus
SR101 – sulforhodamine 101
SVZ – subventricular zone
TBOA – DL-threo-beta-benzyloxyaspartate
TCA – tricarboxylic acid
TGF – tumor growth factor
TPEM – two-photon excitation microscopy
TRP – transient receptor potential (channel)
VSVG – vesicular stomatitis virus glycoprotein

INTRODUCTION

The general assumption that brain functionality relies exclusively on wired neurons is far from the truth. Among the neurons there are several other cell types that play essential structural and functional roles in the brain. Indeed, astroglia, microglia, oligodendrocytes and radial glia cells are involved in brain development, maintenance, re-wiring, synaptic turnover and modulation of synaptic properties (Nicholls et al., 2001, pp. 133-46; Volterra et al., 2002). Modulation of basal synaptic transmission (Navarrete and Araque, 2011), facilitation of long-term synaptic potentiation (Henneberger et al., 2010), regulation of neuronal network activity (Pannasch and Rouach, 2013) and metabolic support of neurons (Magistretti et al., 2006) are the main points of astrocyte and neuron interactions. On the other hand astrocytes are known to display a set of unique morphological features: occupation of non-overlapping spatial domains (Bushong et al., 2002; Ogata and Kosaka, 2002), forming a complex 3D network of peripheral processes (Witcher et al., 2007; Shigetomi et al., 2013) and their strategic positioning near the synaptic terminals (reviewed by Reichenbach et al., 2010) that allow us to propose that astrocytes are the link between structural and functional changes occurring in the brain. In this study we posed the questions are astrocytes *in vivo* subjected to continuous morphological changes and will the suppression of these changes somehow affect neuronal activity in a restricted brain region?

REVIEW OF THE LITERATURE

1. Different glial cell types and their role in the mammalian brain

Glial cells were first described in 1840s by Rudolf Virchow (Virchow, 1846; Virchow, 1858), who coined a general name for them - “neuroglia”, i.e. “nerve glue” in 1856. Half a century later, Camillo Golgi (Golgi, 1903) in 1883 and Santiago Ramon y Cajal (Ramon y Cajal, 1995) in 1890s predicated that glial cells are more than “brain glue” and not merely metabolic suppliers for neurons. For many decades since, the doctrine that promotes neurons as central players of nervous system functionality was prevalent. Starting in the 1990’s, it became more and more evident that some aspects of nervous system physiology as well as pathophysiology could not be explained in light of exclusive neuronal doctrine, and the idea emerged that some glial cells might be also involved in information

processing within the nervous system (Kettenmann and Ransom, 1995). Glia is a general term for a diverse population of different non-neuronal cell types in a brain, including microglial cells that act as immune cells in central nervous system (CNS), oligodendrocytes and Schwann cells – myelinating cells, radial glia playing important role in brain development, NG2 and Bergman glial cells (Ransom, 1991), and astrocytes that metabolically support neurons and are involved in synaptic transmission and development (Ransom, 1991; Kettenmann and Ransom, 1995; Araque et al., 1999; Haydon, 2001) (Fig. 1).

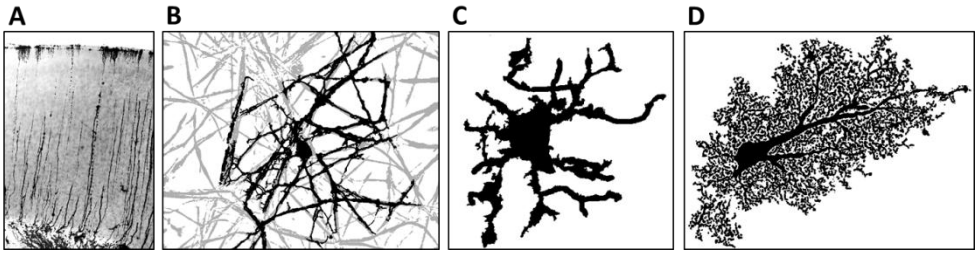

Figure 1. Different types of glial cells perform a variety of functions in the brain. Radial glia cells (black) in neonatal rat cortex (grays) extending their processes from lateral ventricle towards cortical surface and guiding neuronal precursors and other cells to their final destination during brain development (A). Myelinating oligodendrocyte (black) enwraps axons (grey), supports their integrity and facilitate conductance of action potentials (B). Highly mobile microglial cell with a number of processes is continuously probeing the surrounding area (C). Astroglial cell with highly ramified peripheral process associated with synapses can influence synaptic transmission (D). (Images modofied from Fields and Petryniak (NIH), Bouscein et al., 2003 and Shigetomi et al., 2013).

1.1 Radial glia and glial progenitor cells

Radial glia play an important role in guiding neuronal progenitor cells from sub ventricular zone to their place of final differentiation and action. These glial cells have their cell bodies located in the ventricular zone and exhibit bipolar processes extending to reach the pial and ventricular surfaces (Fig. 1A). These type of glial cells are transient and present only at embryonic and early postnatal stages of brain development (Rowitch and Kriegstein, 2010). Radial glia cells are formed from neural stem cells at ventricular and sub ventricular zones and can later differentiate as neuronal progenitors to almost any neural cell type including ependymal cells, subventricular zone astrocytes, oligodendrocytes and neurons (Malatesta et al., 2003). Notably, apart from the peculiar shape, in many species no markers are known that allow discrimination of radial glia from astrocytes. This is the case, for instance, in primates, where the immunoreactivity for GFAP is shown by both cell types (Choi and Kim, 1985; Choi, 1986). These cells clearly fulfill a multi-purpose role, contributing to the generation, migration and probably even the specification and/or differentiation of distinct neuronal subtypes.

1.2 Oligodendrocytes and Schwann cells

Both Oligodendrocytes and Schwann cells are very specialized glial cells types that have reached their final differentiation step. They are formed at the latest stages of the nervous system development. Oligodendrocytes are presented in a CNS where they myelinate several axons simultaneously (Fig. 1B) whereas Schwann cells were found in peripheral nervous system (PNS) where they can be associated with single axon or neuro-muscular junctions. One of the most important functions of oligodendrocytes and Schwann cells is to form a myelin sheath around axons (Nave, 2010). This kind of axon insulation enables rapid action potential propagation within thin and long mammalian axons that relies as a key concept of neurophysiology that providing possibility for complex and at the same time compact nervous system. In addition to their insulation role these cells can also support axonal integrity and provide functions that may be independent of myelin itself (Griffiths et al., 1998). Additionally myelinating glia are involved in several demyelinating disorders, like multiple sclerosis, leukodystrophies and demyelinating neuropathies (Nave, 2010).

1.3 Microglial cells

Microglia represents approximately 10% of total amount of cells in the mammalian CNS (reviewed by Kettenmann et al., 2011). They can appear as tree-like cells with many ramified processes (Fig. 1C) or as amoeboid cells with reduced morphology depending on developmental stage of the CNS and on their activation and/or migration status (Streit et al., 1988; Kettenmann et al., 2011). Common feature for all microglial cells is their high motility rates even in a resting state when changes occur within peripheral processes (Nimmerjahn et al., 2005). This continuous motility proposed to be linked with probing of perineuronal space (Kettenmann et al., 2013) or in case of activation could involve morphological remodeling of the whole cell. Both morphological changes and migration of microglial cells is thought to be caused by actin cytoskeleton remodeling regulated mainly via protein kinase C (PKC) and inositol-3-phosphate (InsP3) signaling pathways (Kettenmann et al., 2011). Migration of microglia in addition to their morphological changes in the adult CNS can be switched on by a variety of pathophysiological actions. For instance in laser induced brain micro lesion or thrombotic micro stroke microglial cells are known to extend their processes rapidly towards a lesion site or thrombotized blood vessels (Davalos et al., 2005).

Functions of microglial cells are very diverse and their portfolio has been extensively added to during last few years. The immune *status quo* of the CNS

caused by blood-brain barrier (BBB) has made it necessary to have a local separated immune competent system within CNS. Being macrophage-like cells with a potential for multiple cell divisions, their major role is to be immune cells in the brain and therefore act as pathology sensors at the CNS. They remove apoptotic brain cells and cell debris by phagocytosis, are involved in response to brain ischemia (Kettenmann et al., 2011) and are participating in developmental as well as in adult brain plasticity on the level of individual synapses (Wake et al., 2013).
Purinergic signaling mediated by ATP, which is suggested to be a major danger signal in the CNS, is one of the most studied and acknowledged pathways for triggering microglial activation, apoptosis and phagocytosis. However, there are many potential alternative pathways to be involved in a complex process of microglia interaction with other CNS cell types. Indeed, microglial cells express plenty of different channels on their surface including sodium channels, voltage-dependent Ca^{2+} channels, transient receptor potential (TRP)-generating channels, Ca^{2+} activated and G protein activated potassium channels, volume regulated chloride channels and also different types of aquaporins and connexons (Kettenmann et al., 2011). There are also some evidences that microglial cells might express glutamate receptors such as AMPA receptors with low Ca^{2+} permeability (Noda et al., 2000) and functional metabotropic glutamate receptors (mGluR) (Biber et al., 1999).
First described as immune cells in the CNS that can switch between resting and activated states microglial functions were extended first to developmental plasticity by synapse stripping (Kettenmann et al., 2013) and further to a variety of roles in such pathologies as Alzheimer disease and psychiatric disorders (Aguzzi et al., 2013). Without a doubt microglia have complex and important impact on the CNS development and function which is still enigmatic in the majority of its aspects.

1.4 Astroglial cells (a.k.a. astrocytes)

The name “astrocyte” stems from the stellar, or star-like, shape of the most numerous and best studied glial cell type in mammalian brain (Fig. 1D). There are probably many sub-types of astrocytes, but scientists have yet to agree on the classification principles that would allow distinguishing between various taxonomic groups within an astrocytic population. For example, anatomical evidence suggests that fibrous and protoplasmic astrocytes are two distinct groups (Penfield et al., 1932); it is not clear, however, whether the same astrocyte can make a transition between these two anatomical states. Furthermore, the level of expression of glial fibrillary acidic protein (GFAP), which is a classical astrocytic marker, varies in a large range between individual cells as well as between

different patho-physiological conditions (Chiu and Goldman, 1985; Baba et al., 1997; Gomes et al., 1999; Messing and Brenner, 2003; Middeldorp and Hol, 2011). Finally, brain region-dependent specialization of astrocytes is likely to occur, and in some regions astrocytes even have been assigned a separate name (e.g., Bergmann glia in cerebellum (Bergmann, 1857)).

Functionally, astrocytes may also be divided in a number of subtypes (although again, a consensus in the field is still lacking). Undoubtedly, though, functions of astrocytes are numerous and vary widely from vital metabolic support of neurons and formation of blood-brain barrier to enabling synaptogenesis and synaptic plasticity all the way to elimination of synapses. Some of these classical and more recently discovered functions will be discussed in detail in following chapters. It seems important to note that, although astrocytes have been studied for decades (anatomically since 1850s and functionally since 1990s), their role in the CNS is still somewhat enigmatic as more questions arise from each new study.

2. Astroglia functions in the brain

2.1 Metabolic support of neurons by astrocytes

Neurons are supposed to be the main consumers of energetic substrates over the mammalian body. The energy is utilized for maintenance of membrane potential in resting condition as well as for action potential generation during neuronal network electrical activity (reviewed by Magistretti, 2006; Allaman et al., 2011) and long term memory formation (Suzuki et al., 2011). Regional blood flow changes, energy and as a consequence oxygen and glucose consumption by neural tissue are related to neuronal activity and are used as a basis for different brain functional imaging techniques (Magistretti and Pellerin, 1999; Raichle and Mintun, 2006; Hyder, 2009; Bandettini, 2012). It is a well-established fact also that, in comparison to other cell types, glucose is not the main energetic substrate for neurons and that glycolysis is much more ineffective in neurons as compared to the tricarboxylic acid (TCA) cycle that utilizes lactate for energy production (Pellerin et al., 2007; Magistretti, 2006). On the other hand astrocyte metabolism demonstrates remarkably more active glycolysis and provides a source of lactate for neurons. This metabolic complementarity is not likely to be a fortuity but a result of metabolic coupling between astrocytes and neurons (Magistretti, 2006). This neuro-glial metabolic coupling includes active transport of glucose by astrocytes from a blood flow, glycolysis in astrocytes and active lactate transfer from astrocytes to neurons in an activity-dependent manner (Volterra et al., 2002; Kasischke et al., 2004).

Glucose from blood flow is transported into astrocytes via specific glucose GLUT-1 type transporters that are expressed in endfeet surrounding blood vessels (Morgello et al., 1995; Yu and Ding, 1998). This glucose is used in classical glycolysis that results in a anaerobic production of pyruvate and further to lactate in astrocytes with a muscle form of lactate dehydrogenase 5 (LDH) (Bittar et al., 1996). Increased glucose utilization in response to glutamate uptake (Pellerin and Magistretti, 1994; Takahashi et al., 1995) as well as lactate production following sensory stimulation of neuronal activity (Fellows et al., 1993) are key signs for coupling between astrocytic metabolism which acts as a glutamate source and neuronal activity. Astrocytes can actively evacuate glutamate from active synaptic sites by specific excitatory amino acid transporters (EAAT) 1 and 2 (reviewed in Volterra et al., 2002; McKenna, 2007). The glutamate transport is accompanied by 3 Na^+ ions transfer for each glutamate molecule that cause remarkable sodium current in astrocytes involved in glutamate scavenging. Both maintenance of sodium homeostasis and glutamate conversion to glutamine are ATP-dependent processes and thereby stimulate glycolysis and lactate production in astrocytes.

It seems that the role of astrocytes in activity-dependent neuro-glial metabolic coupling might be even more extensive and includes such aspects as regulation of vasoconstriction (Mulligan and MacVicar, 2004; Gordon et al., 2011) in response to Ca^{2+} transients in soma and endfeet as well rapid vasodilation mediated by local Ca^{2+} increase in astrocytic endfeet (Takano et al., 2006; Gordon et al., 2011). Astrocytic regulation of local blood flow and oxygenation level of brain tissue is not restricted to their influence on vessel diameter but also includes more generalized mechanisms of pH-dependent control of breathing via ATP signaling pathway (Gourine et al., 2010). Metabotropic glutamate receptor mediated Ca^{2+} transients in astrocytes caused by stimulation of neuronal networks activity (Wang et al., 2006), might also be involved in fine tuning of astrocytic homeostasis and metabolism.

It is also worth mentioning that astrocytes form an extensive metabolic network in the brain. Their coupling through connexin 43 and 30 channels allows trafficking of energetic substrates such as glucose (reviewed in Giaume et al., 1997) and ATP (Kang et al., 2008) between neighboring astrocytes and influences excitatory glutamatergic synaptic transmission within hippocampal neuronal networks (Rouach et al., 2008; Pannasch et al., 2011). Astrocytic K^+ homeostasis is mediated by Na^+,K^+-ATPase activity in astrocytic plasma membrane and thus is dependent on ATP level and glucose utilization via glycolytic pathway in astrocytes. Ca^{2+} dependent uptake of extracellular K^+ by astrocytes can modulate neural network activity by transient local decrease of K^+ ions leading to neuronal hyperpolarization and synaptic suppression (Wang et al., 2012). Thus, not only are Ca^{2+} and Na^+ ions

connected with maintenance of neuro-glial metabolic coupling but K^+ changes might also be involved in regulation of brain metabolism in an astrocyte-dependent manner.

Astrocytes provide not just metabolic support for neuronal cells by lactate supply for TCA cycle but are involved in a metabolic crosstalk with neuronal cells that could even include such features as suppression of neuronal activity. They are also actively participating in regulation of cerebral blood flow, breathing control and can act as a metabolic network connected by gap junctions. Astro-neuronal metabolic coupling and the role of astrocytes in brain metabolism is strongly related to the fundamental role that astrocytes play as a component of multipartite synapses.

2.2 Blood-brain barrier (BBB) formation and regulation by astrocytes

The modern concept of BBB was summarized by Hugh Davson in 1976 (Davson, 1976) and includes such features as barrier function *per se*, active transport and facilitated transport options across the barrier, leading role for endothelial cells in barrier formation and support, maintenance of brain homeostasis and ontogenetic developmental changes in BBB as well as significant role for astrocytes in BBB transport and homeostasis. To further develop this concept it is now an accepted fact that even though BBB is a relatively stable structure it continuously changes under different modulating factors among which astrocytes play not the least role (Abbott, 2005).

BBB starts to form at embryonic developmental stages with the help of pericytes at the time when astrocytes have not yet appeared (Daneman et al., 2010). At later stages of BBB formation astrocytes participate in its establishment by direct contacts with pericytes and endothelial cells by their endfeet. Astrocytes are also supposed to play a role in BBB maturation and tight junctions formation by means of secretion of such angiogenic compounds such as Ang1, TGFβ, GDNF and FGF2 (Quaegebeur et al., 2011). Particularly it was shown that factors derived from astrocytes can induce BBB-like phenotypes of endothelial cells and formation of tight junctions *in vitro* (Lee et al., 2003) suggesting an important role for astrocytes in BBB formation *in vivo* as well.

Besides participation in BBB formation during ontogenesis, astrocytic components are involved in transport across the barrier. While small lipophilic molecules (less than 400 Da) can cross BBB by lipid-mediated diffusion (Pardridge, 2007), other compounds need to be transported actively or by mean of special channels and transporters. Strategic location of astrocytes between neurons and blood vessels makes them major players in glucose transport through GLUT1 transporters, water

through AQP4 channels (Abbott, 2006) and also ions, glutamate and other amino acids (Ohtsuki and Terasaki, 2007) across the barrier. For instance, spatial K^+ buffering by astrocytes provided by Kir4.1 channels located on perivascular astrocytic endfeet (Kofuji and Newman, 2004) as well as by other transporters such as Na^+,K^+-ATPase and NKCC1 (Abbott et al., 2006) were proposed to be one of the major mechanisms for maintenance of K^+ homeostasis in the brain and for regulation of neuronal firing.

By cryo-electron microscopy studies it was shown that astrocytic endfeet form orthogonal arrays of particles (OAPs) containing specialized sets of proteins located at contact sites with endothelial cells (Fallier-Becker et al., 2011; Nico and Ribatti, 2012). These polarized structural protein expressing micro domains are involved in maintenance of water and K^+ homeostasis since they express aquaporin 4 (AQP4) channels and Kir4.1 K^+ channels segregated by agrin and α1-syntrophin (Abbott et al., 2006). Using dystrophin deficient mice it was shown that actin organization in astrocytic endfeet is crucial for AQP4 distribution and function (Nico et al., 2003) suggesting the role astrocytic actin cytoskeleton for clustering of OAPs and as a consequence its importance in regulating BBB properties.

There is more and more evidence that most CNS pathologies involve some aspects of BBB disruption at least at some stages. Thus diabetes, alcohol, Ischemic conditions, HIV-1 infection are factors for BBB leakage (Zlokovic, 2008; Eugenin et al., 2011); inflammation processes can also cause opening of BBB (Huber et al., 2001). Some of these pathological states involve astrocytes, thus astrocytic activation due to Alzheimer disease (AD) pathology and amyotrophic lateral sclerosis disease plays a role in BBB disruption in severe AD (Zlokovic, 2008). One of the possible mechanisms of astrocytic regulation of BBB permeability is based on ATP-mediated astrocytic and endothelium Ca^{2+} signaling. In this situation astrocytes act as a network connected by gap junctions and thus can propagate Ca^{2+} and ATP mediated signals to neighboring cells. At the same time intracellular Ca^{2+} changes in endothelial cells may act as a trigger for phosphorylation of cytoskeleton proteins and tight junctions opening (Abbott et al., 2006) increasing BBB permeability.

Although BBB is mainly formed by endothelial cells and pericytes, astrocytes form numerous connections with both endothelial cells and pericytes and, as recently discovered, can regulate their functionality at least in terms of cerebral blood flow adjustment (Attwell et al., 2010). It is also unclear to what extent astrocytes can influence formation and integrity of tight junctions – one of most critical component of BBB. Although *in vitro* studies show astrocytic roles in almost every function of BBB (reviewed by Abbot et al., 2006), their role in BBB formation, maintenance and pathophysiology *in vivo* seems to be under studied. Focusing on

distal BBB components, like astrocytic endfeet, in the natural environment of the intact brain may shed light on novel therapeutic strategies based on selective BBB permeability, and open the way for new diagnostic approaches for CNS pathologies implicating unidirectional trans-BBB transport of diagnostic markers from CNS to the blood flow.

2.3 Astrocytic function in maintenance and regulation of extracellular matrix (ECM)

Regarding ECM we intend to take into account not just scaffold and cell-adhesion molecules but also soluble macromolecular factors derived from astrocytes, neurons and other cell types in the CNS. Roles of ECM can be divided in two main categories: mechanical that includes its implications in general and local diffusion properties of brain tissue (Sykova, 2004; Frischknecht et al., 2009; Zamecnik et al., 2012) and as a consequence influencing synaptic as well as extrasynaptic transmission and cell migration in physical aspects; and biochemically active components of ECM affecting CNS properties via cell adhesion molecules, secretion of soluble proteins in extracellular space and protein-protein interactions in ECM (Dityatev and Schachner, 2003; Kochlamazashvili et al., 2010; Dityatev and Rusakov, 2011). Molecular scaffolds, cell adhesion, and soluble proteins that are synthetized and secreted by astrocytes play complex and important role in physiological (Ehlers, 2005; Faissner et al. 2010; Wiese et al., 2012; Hawkins et al., 2013) and pathological states of CNS (Pantazopoulos et al., 2010; Giordano et al., 2011; Beretta, 2012).

Early *in vitro* co-culturing studies discovered the role of astrocytes in synaptogenesis and showed that astrocytes are able to produce some soluble factors that promote synaptogenesis even without direct astro-neuronal contacts (Pfrieger and Barres, 1997; Nägler et al., 2001; Ullian et al., 2001). Later it was shown that astrocyte-derived cholesterol (Mauch et al., 2001) and thrombospondins 1 and 2 stimulate formation of functional synapses at retinal ganglion cells (Christopherson et al., 2005). These findings suggested that astrocytes can be involved in synaptogenesis via secretion of ECM molecules. Additionally, it was demonstrated later that synaptogenic action of astroglia-derived thrombospondins is facilitated via gapapentin receptor, the $\alpha 2\delta 1$ auxiliary subunit of voltage gated Ca^{2+} channel in neurons (Eroglu et al., 2009).

Recent studies have demonstrated that astrocytes *in vitro* secrete a number of proteins including procollagen, enolase, protein disulfide isomerase and Ser/Cys protease inhibitor (Schubert et al., 2009). It was also shown earlier that astrocytes can form a fibrillar collagen matrix when cultivated *in vitro* (Heck et al., 2003) and

in case of brain injury repair (Hirano et al., 2004). Taking into account that astrocyte-derived glioma cells also have epithelial-like phenotype (Lin et al., 2002), it is tempting to speculate that activated astrocytes have some fibroblast features, particularly in their ability to form primary extracellular scaffolds containing collagen, laminin and fibronectin for cellular adhesion.

In addition to general scaffold ECM proteins astrocytes are known to express different glycoproteins, such as chondroitin sulfate proteoglycans (CSPG) brevican, aggrecan, versican and phosphacan that are involved in CNS synaptogenesis regulation and can also influence Schwann cell migration (Pyka et al., 2011; Afshari et al., 2010). ECM glycoprotein tenascin C that plays a role in developing and mature CNS functionality including regulation of neuro-glial interactions in synapses (Theodosis et al., 2004; Faissner et al., 2010, Geissler et al., 2013), particularly it is able to regulate patterning genes in astrocytes during development of spinal cord (Karus et al., 2011) and modulate activity of membrane Ca^{2+} channels (Evers et al., 2002). Such glycoprotein as dystroglycan was shown to regulate general astrocytic endfeet organization and AQP4 distribution by linking ECM protein agrin with the cytoskeleton (Noell et al., 2011). In turn, heparan sulfate proteoglycan agrin, whose main function is related to astrocytic maintenance of BBB (reviewed by Abbott et al., 2006), is also known for its role in formation and maintenance of neuromuscular junctions by Schwann cells (Burden, 1998). The story of astrocytic adhesion molecules would not been completed without mentioning ephrine-A3 ligand that is exposed on astrocytic surface and mediates signaling between neurons and astrocytes contributing to dendritic spines development (Murai et al., 2003).

Integrin family proteins are also important for regulation of cell migration and proliferation mediated by astrocytes. Thus expression of α6β4 Integrin in astrocytes is related to their activation due to hypoxic conditions and act as a promoting factor for proliferation of endothelial cells (Li et al., 2010). At the same time another astrocyte-derived integrin αvβ8 is involved in sprouting of blood vessels during retinal development (Hirota et al., 2011).

Astrocytes are known to express several types of matrix metalloproteinases (MMP) that are involved in cell migration by selective degradation of ECM molecules. The most studied MMP-9 whose activity is dependent on inflammation factors and on astrocyte activation (Hantamalala et al., 2012), has a pivotal role in a formation of glial scar after spinal cord injury (Hsu et al., 2008) and is involved in proteolysis of many ECM molecules that can promote neuronal death by degradation of vital laminin matrix or, on the other hand, can facilitate neurite outgrowth by degrading CSPG inhibiting components of surrounding ECM. It is known that such pro inflammatory agents that cause astrocyte activation as bradykinin can also induce

MMP-9 expression in astrocytes (Hsieh et al., 2008). Much less is known about MMP-2, but since it utilizes different distribution and trafficking compare to MMP-9 (Sbai et al., 2010) it is possible to assume that it might have different function or at least different regulation mechanism. A little aside from mentioned MMP-9 and MMP-2 there is another astrocyte-derived MMP-13 that can enhance permeability of brain endothelium under hypoxic conditions (Lu et al., 2009). Interestingly that astrocytes can regulate their MMPs on site by secreting specific inhibitors (Moore et al., 2011) providing local feed-back control of their activity.

It is interesting to trace the role of astrocyte-derived ECM in different pathological states of the CNS as well even though some pathological aspects of ECM remain controversial. For instance, astrocytes are responsible for fibronectin production which can form aggregates on sites of multiple sclerosis lesions that proposed to be one of the factors that prevent remyelination and increase disease severity (Stoffels et al., 2013). Similarly different strategies for treatment consequences of CNS injuries are based on facilitation of neuronal process regrowth by prevention of glial scar formation and/or decreasing the level of CSPGs that thought to suppress neuronal regeneration (Gris et al., 2007; Cua et al., 2013). Effectiveness of such strategies is not univocal yet.

Generally, active ECM deposition by astrocytes requires their activation by injury or internal inflammation process. Thus, pro inflammatory cytokine interleukin 1β is required for astrocyte activation followed by ECM production, regulation of cell adhesion and morphological changes (Summers et al., 2010). Interestingly, that some abnormalities in ECM, particularly related to reelin and CSPGs, were found in case of clearly psychiatric disorders, like schizophrenia, with no signs of severe brain damage and astrocyte activation (Pantazopoulos et al., 2010; Beretta, 2012). It is worth to mention in this context that some other CNS pathologies, like epilepsy, are related to changes in ECM and diffusion properties of the brain (Sykova, 2001) that is likely involve astrocytes.

Despite there are comprehensive data about ECM molecules expressed by primary astrocytes *in vitro* (Dow and Wang, 1998; Heck et al., 2003; Schubert et al., 2009), unfortunately there is no detailed proteomic analysis for astrocytes *in vivo* under different conditions. Since astrocytic expression profile can change dramatically upon activation, we can only speculate what extracellular molecules derived from astrocytes are related specifically to pathological states where inflammation and astrocyte activation take place, how subset of proteins excreted by astrocytes changes over developmental stages and what characteristically for astrocytes and neurons in normal mature CNS.

2.4 Astrocytic coupling through gap junctions

Being a unique feature of astroglia, coupling through gap junctions is of special interest. Connexins can act as adhesion molecule that provide formation and maintenance of astrocyte-astrocyte contacts as well as contacts between astrocytes and other cell types (Lin et al., 2002; Elias et al., 2007) or regulate functions of membrane receptors (Scemes and Giaume, 2006). Such connections through connexin 43 and 30 channels facilitate transmission of metabolic substrates and signaling molecules such as glucose, lactate and ATP (Tabernero et al., 2006; Kang et al., 2008; Rouach et al., 2008), ion permeability providing Ca^{2+}, K^{+} and Na^{+} waves (Harris, 2007; Rouach et al., 2008; Bernardinelli et al., 2004), and even the possibility for translational regulation by shRNA and miRNA transport (Valiunas et al, 2005; Katakowski et al., 2010).

The first evidence for glial communication via gap junctions was obtained in sixties (Kuffler et al., 1966). There are at least 20 different connexin and 3 pannexin genes that are expressed in mammals (Willecke et al., 2002; Bennett et al., 2012), but just two of them, connexins 43 and 30, are shown to be involved in formation of gap junctions connecting astrocytes (Wallraff et al., 2006; Rouach et al., 2008). Experiments with dyes and labeled molecules, such as Lucifer yellow and propidium, transferring between astrocytes showed that the connexin channel with a pore diameter of 1.0-1.5 nm is permeable for ions and small metabolites (with a cut-off of approximately 0.5 to 1.0 kDa) with almost no charge selectivity (Harris, 2001).

Asking questions about the complexity of astrocytic network we should consider several facts and concepts. Thus, the exact limits (if they exist) for the number of individual astrocytes connected by the continuous gap junction network is not known, giving rise to the "pan glial syncytium" concept (Theis et al., 2005). The network might be even more tangled if reflexive gap junctions that connect different processes of the same cell (Giaume, 2010; Wolff et al., 1998), are taken into account. Diverse regulation of astrocytic network based on selectivity, gating properties, regulation and combinatorics of different gap junction channels also gave rise to the connexin "language" concept (Bruzzone and Giaume, 1999).

Coupling through gap junctions mediates metabolic support functions performed by astrocytes via Ca^{2+} signaling. It was shown, for instance, that astrocytes can locally regulate vasoconstriction and vasodilation in a Ca^{2+} dependent manner (Koehler et al., 2009) and at the same time astrocytic coupling by gap junction is known to contribute to Ca^{2+} wave propagation (Hoogland et al., 2009). Thus, it is possible to conclude that astrocytic coupling via gap junctions can regulate direction (constriction or dilation), strength and determine the zone of blood flow

changes. Interestingly, Ca^{2+} waves could propagate both by connexins hemichannels-dependent mechanism, when Ca^{2+} increase generated *de novo* in each neighboring cell, utilizing ATP as a mediator (Kang et al., 2008; Hoogland et al., 2009), as well as by hemichannels-independent mechanism, when Ca^{2+} spreads directly through gap junctions in adherent astrocytes (Bennett et al., 2003). These two mechanisms presumably should differ by kinetics of Ca^{2+} signaling as well as by localization and distance for which Ca^{2+} signals are propagating throughout the astrocytic network.

Often astrocytes are considered as separate cells that can individually influence neuronal functionality by different ways. Here we want to ask two major questions about involvement of astrocyte-to-astrocyte communication in regulation of neuronal network activity and *vice versa*. Does the astrocytic network regulate neuronal activity and does neuronal activity have an impact on the properties of the astrocytic network?

There are several recent studies that address this question indirectly. For instance, ATP release (caused by Ca^{2+} mediated activation of astrocytes) from the glial network can promote distal synaptic suppression by adenosine (Serrano et al., 2006). Also astrocytic network is involved as a functional unit in buffering K^+ ions and thus, the modulation of general excitability of neurons (Wallraff et al., 2006). Although indirect evidence that astrocytic networks could modulate synaptic activity were known, a direct demonstration for this occurred only recently. It was shown that astrocytic coupling through connexins 43 and 30 is involved in regulation of neuronal excitability, release probability and insertion of new AMPA receptors in hippocampal synapses (Pannasch et al., 2011). These data suggest astrocytic coupling through gap junctions to be involved in such basic process as formation of new synapses and LTP induction. In addition there is interesting evidence for alignment between neuronal and astrocytic domain organizations in different brain areas (Houades et al., 2008; Roux et al., 2011). Thus, within somatosensory barrel cortex astrocytic coupling through gap junctions resembles the columnar organization of neurons, indeed, astrocytes within a single column have very strong coupling with much less intra columnar coupling (Houades et al., 2008) suggesting the role of astrocytic communication in functional intracolumnar neuronal organization.

One of the first studies that address directly this question was investigation of coupling between neuronal activity and increase in intra glial permeability in frogs (Marrero and Orkand, 1996). Later it was shown that neurons itself and neuronal activity upregulate connexin 43 expression in astrocytes and their coupling through gap junctions in mixed astrocyte-neuronal cultures (Rouach et al., 2000). The mechanism for tuning of astrocytic networks by neuronal activity is possibly

underlined by K^+ dependent astrocyte depolarization (Enkvist and McCarthy, 1994).

In addition to maintenance of normal CNS homeostasis in some cases astrocytic coupling by gap junctions can disservice brain function and aggravate neuropathology. Thus, in case of HIV infection there is just small fraction of astrocytes of around of 8 % that are taking up the virus but coupling of infected astrocytes with healthy ones via gap junctions provides severe breach in BBB integrity (Eugenin et al., 2011). It was also shown recently that astrocytic coupling by connexin 43 but not by connexin 30 is involved in generation of neuropathic pain followed by spinal cord injury and consequent inflammatory reaction (Chen et al., 2012). In addition it has been reported that deletion of connexins 43 and 30 leads to demyelination phenotype due to oligodendrocytes dysregulation (Lutz et al., 2009). All in all, reactive gliosis that is characterized by astrocytic morphological changes, increase in GFAP expression level and other signs of astrocytes activation, accompanying commonly different pathological states of the CNS and is mediated by signaling via gap junctions (Kuchibhotla et al., 2009; Koulakoff et al., 2012; Chen et al., 2012).

Now it is accepted that gap junctional coupling of astrocytes is a subject for modulation by extra- and intracellular signals. Astrocytic syncytium not just summarizing properties of individual cells but acts as a new functional unit in the brain, influencing neuronal network properties (Giaume et al., 2010; Pannasch et al., 2011), and representing quantity to quality transition in some instances.

2.5 Ion channels, transporter and receptors expressed in astrocytic plasma membrane

Interaction with the environment as well as reaction to different stimuli coming from outside and inside the cell is mediated by receptors, channels and a variety of different transporters which facilitate information transfer between different cell types by means of signaling. Astrocytes that play an active role in communication with other cell type in the CNS, including neurons and microglia, express a range of transmembrane receptors, transporters and channels that provide permeability for different ions, transport of metabolic substrates, neurotransmitters and water.

2.5.1. Glutamate transporters

One of the major functions of astrocytes is glutamate uptake from a synaptic cleft that regulates spillover of glutamate, reduces the time for its action on postsynaptic receptors and maintains presynaptic pool of neurotransmitter by glutamate-glutamine cycle (Fig. 2). Astrocytes express mainly two types of glutamate

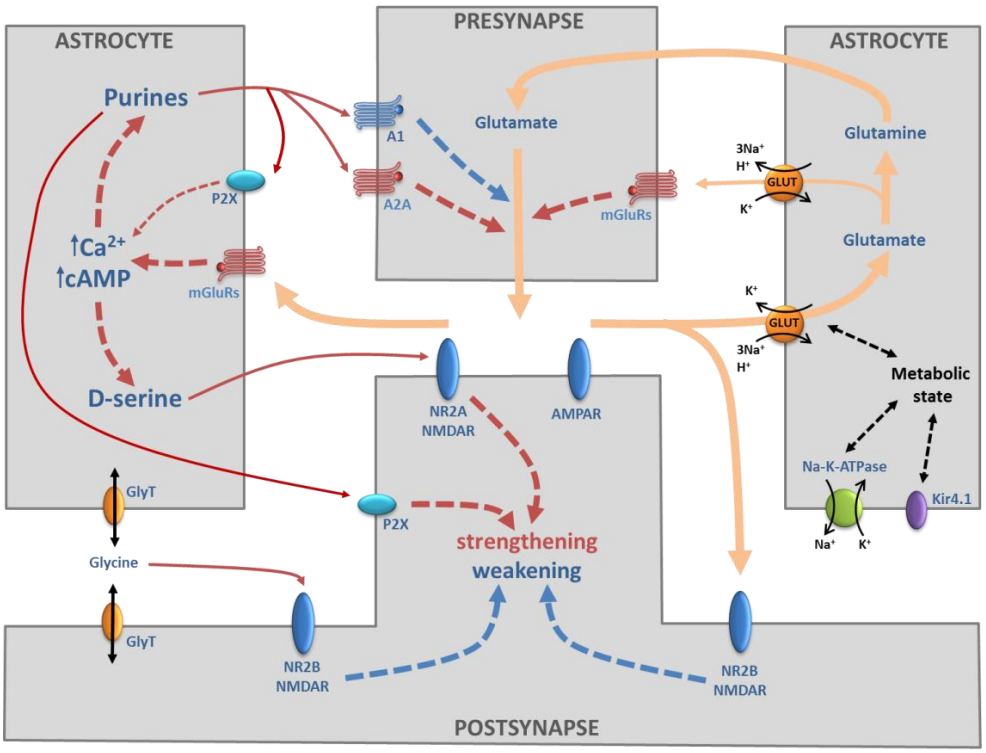

Figure 2. Neuron-astrocyte signaling in the excitatory multipartite synapse. Glutamate-glutamine cycle shown in orange has several key steps: after release from a presynaptic terminal glutamate can activate postsynaptic receptors mGluRs at the astrocytic endfeet or extrasynaptic receptors. Glutamate scavenging kinetics by astrocytic GLUT as well as spatial positioning of PAPs determines both postsynaptic and astrocytic glutamate action. After been scavenged by astrocyte glutamate can be excreted by the reverse uptake or can be sent back to the presynaptic terminal in a form of glutamine. Glutamate metabolism due to its involvement in TCA cycle is tightly related to the metabolic state of the cell and maintenance of ion homeostasis via Na-K-ATPase and potassium channels. Signaling cascades involving cAMP and Ca^{2+} in the astrocyte can influence synaptic transmission in bidirectional fashion, causing presynaptic inhibition or activation via different types of metabotropic purinoreceptors or facilitate postsynaptic LTP via purines and D-serine release. Via releasing or scavenging glycine through the GlyT astrocytes are also involved in presynaptic glycine actions.

transporters: excitatory amino acid transporter 1 (EAAT1) also called as glutamate-aspartate transporter (GLAST) and EAAT2 that is also called glutamate transporter 1 (GLT-1) (Danbolt et al., 1992; reviewed in Danbolt, 2001). Most studied and most abundant astrocytic glutamate transporter EAAT2 facilitates transport of 1 glutamate molecule inside the cell accompanied with co-transfer of 3 Na^+, 1 H^+ and 400 H_2O molecules and pumping 1 K^+ ion outside the cell. In this process Na^+ ions are essential for substrate binding (Levy et al., 1998; MacAulay et al., 2001). Their activity could be chemically blocked in experiment with a range of selective

glutamate transporters antagonists such as dihydrokainik acid (DHK) (Johnston et al., 1979; Arriza et al., 1994) and DL-threo-beta-benzyloxyaspartate (TBOA).
Glutamate transporters distributed unevenly in astrocytic plasma membrane; they mainly located on astrocytic processes that are in close contacts with synapses and much less abundant in somatic areas (Chaundhry et al., 1995) suggesting their primary role in uptake of synaptically released glutamate. In agreement with it is an observation that transgenic mice that lack EAAT2 expression demonstrated increased mortality and behavioral abnormalities (Tanaka et al., 1997), at the same time mice that overexpress EAAT2 were resistant to induction of epileptiform activity and had problems with LTP induction (Martinowich et al., 2001).
In addition to uptake glutamate transporters can mediate release of glutamate via so called ”Reversed uptake” of glutamate (Rossi et al., 2000; Grewer et al., 2008), it could happened if cells loose energy source in case of ischemia or glucose deprivation (Nicholls and Attwell, 1990; Rossi et al., 2000) causing increase in spontaneous activity and excitotoxicity. It is worth to mention here that glutamate transporters are involved in coupling synaptic activity with astrocyte signaling (Volterra et al., 2002, pp. 46-56), thus glutamate to glutamine transformation cycle should be considered in a context of neuroglial metabolic coupling since glycolysis in astrocytes is dependent on glutamate uptake (see also part 2.1 for details).
Such characteristic features of astrocytic glutamate transporters like their high density in perisynaptic regions of astrocyte, their high affinity to glutamate and the fact that much of the transporter current might be produced by low concentration of glutamate (Volterra et al., 2002, pp. 62-75) allow to propose that glutamate spillover and uptake regulated not chemically but spatially and are correlated rather with synaptic geometry than with transporters permeability (Freche et al., 2011; Freche et al., 2012). This idea is bringing us to a conception where astrocytes can actively participate in synaptic events by mean of their structural rearrangement and is the first link for involvement of astrocytic morphological plasticity in modulation of synaptic function.

2.5.2. Connexin and pannexin hemichannels

Along with specialized glutamate transporters astrocytes also express different channels facilitating transfer of small molecules and ions. Connexins that are known to form gap junctions between astrocytes are also presented as hemichannels in astrocytic membranes where they can mediate astrocytic activation by ATP and Ca^{2+} signaling (Bennett et al., 2003; Bennett et al., 2012; Chen et al., 2012). In addition to connexins there is also pannexin-1 hemichannels expressed in astrocytes. They are permeable for ATP, arachidonic acid derivatives,

are involved in Ca^{2+} waves propagation and might play a role vascular regulation (Bennett et al., 2012; Suadicani et al., 2012; MacVicar and Thompson, 2010). It was proposed also that hemichannels in astrocytes might mediate glutamate release (Ye et al., 2003) and thus play a role in coupling astrocytes with synaptic activity.

2.5.3. Glutamate receptors

Presence of functional ionotropic glutamate receptors in astrocytic plasma membrane is mysterious. Even though there were evidences for expression of ionotropic glutamate receptors mRNA and even functional NMDA and AMPA receptors in cortical and hippocampal astrocytes (Porter and McCarthy, 1996; Gallo and Ghiani, 2000; Schipke et al., 2001), their functionality remains controversial (reviewed by Volterra et al., 2002, pp .34-46). In opposite metabotropic glutamate receptors in astrocytes are well characterized and were shown to be involved in regulation of synaptic function (Perea and Araque, 2007; Panatier et al., 2011). Astrocytes were demonstrated to express mRNA for several metabotropic glutamate receptors subtypes; mGluR3 coupled with adenylate cyclase signaling pathway and mGluR5 that are connected to IP_3 and Ca^{2+} signaling cascade (Schools and Kimelberg, 1999).

2.5.4. GABA receptors and transporters

Besides excitatory glutamate sensing and release astrocytes are also exhibit sensitivity to inhibitory neurotransmitter GABA. It was reported that there is a high affinity GABA membrane transporter GAT-1 expressed in astrocytes (Minelli et al., 1995). On the other hand astrocytes can indirectly affect GABA-mediated synaptic transmission by influencing glutamate-glutamine cycle in presynaptic GABAergic terminal (Liang et al., 2006). There are also some evidences regarding metabotropic $GABA_B$ receptors which can trigger glutamate or ATP release from astrocytes (Kang et al., 1998; Serrano et al., 2006) and ionotropic $GABA_A$ receptors expression (Steinhäuser et al., 1994). Latter are known to be developmentally regulated and disappears in mature astroglia (reviewed by Volterra et al., 2002, in pp. 34-46).

2.5.5. Channels and transporters involved in D-serine release from astrocytes

Within a special interest astrocytic involvement in recently described D-serine actions (Panatier et al., 2006; Henneberger et al., 2010; Papouin et al., 2012). Astrocytes are proposed to express selective transporters for NMDA receptors co-agonists such as D-serine transporter and glycine transporter 1 (GlyT1) (Papouin et

al., 2012). These transporters are thought to mediate glutamate stimulated release of D-serine and control of synaptic function through neuronal NMDA receptors co-activation (Schell et al., 1995; Panatier et al., 2006). D-serine can also be secreted by astrocytes by less specific release mechanisms. It could be mediated by exocytotic release as well as by non-exocytotic release via $P2X_7$ channels or volume regulated anion channels in case of astrocyte swelling (reviewed in Hamilton and Attwell, 2010). Despite it was clearly shown that D-serine released from astrocytes is a key regulator for synaptic plasticity and transmission (Yang et al., 2003; Panatier et al., 2006; Henneberger et al., 2010; Papouin et al., 2012), actual mechanism for D-serine release from astrocytes remains mostly elusive.

2.5.6. Water and potassium channels

Astrocytes intermediate exchange between neurons and blood vessels and thus are the main players in water homeostasis in the CNS. Their ability to regulate water balance is based on dense expression of aquaporin channels arrays. Aquaporins are the family of trans-membrane water channels. Whereas these channels are presented in different cell types, astrocytes express only AQP4 channel that at the same time is the most abundant water channel in the brain (reviewed by Papadopoulos and Verkman, 2013). AQP4 channels are mainly located on astrocytic endfeet surrounding blood capillaries as well on astrocytic processes that enwrap synaptic terminals (Nagelhus et al., 2004; Papadopoulos and Verkman, 2013). Interestingly that AQP4 channels are coexpressed with Kir4.1 K^+ channels both at astrocytic endfeet (Nagelhus et al., 2004; Abbott et al., 2006) and at perisynaptic astrocytic processes (Nagelhus et al., 2004) suggesting their synergetic role in maintenance of water and K^+ homeostasis. Notably that astrocytic AQP4 also plays a critical role in epilepsy (Binder et al., 2012) and during formation of traumatic brain edema (Nase et al., 2007).

2.5.7. Purinoreceptors

Purinergic signaling underlies many signaling events in astrocytes. Purine receptors family $P2X_{1-7}$ mediating exchange of K^+ to Ca^{2+} and N^+ in a response to extracellular ATP, thus providing Ca^{2+} and Na^+ influx to astrocytes. Astrocytes express at least two types of purinoreceptors $P2X_1$ and $P2X_7$ that differ by their affinity to agonist (reviewed by Illes et al., 2012) and are involved in different signaling cascades. As a part of ATP signaling system in astroglia it is worth to mention exocytotic mechanisms for ATP release in a response to intracellular Ca^{2+} elevations (Bal-Price et al., 2002; Coco et al., 2003; Pangrsic et al., 2007; Pryazhnikov and Khiroug, 2008), suggesting exocytosis as one of the major

mechanism for bioactive substances release from astrocytes (reviewed in Hamilton and Attwell, 2010).

2.5.8. Ephrin mediated reception

In order to make the story about astrocytic receptors complete we should consider that signaling might be underlined not just by soluble factors that diffuse from one cell to another but also by integral membrane molecules that are working while cells have direct physical contacts. Commonly known ephrin-A3 ligands expressed on astrocytic surface can interact with their neuronal partners ephrin-A4 receptors located on dendrite. These interactions promote regulation of glutamate transporters in astrocytes and also involved in AMPA receptor endocytosis and degradation in dendritic spines as well as in Rac-mediated spine stabilization (reviewed in Murai and Pascuale, 2011).

2.6 Chemical excitability and calcium signaling in astrocytes

Astrocytes were long thought to be non-excitable cells when studied using electrophysiological tools applicable for neurons (reviewed in Verkhratsky et al., 1998; Agulhon et al., 2008; Kirischuk et al., 2012). But as many other cell types they have signaling system underlying information transfer within the cell, astrocyte to astrocyte interactions as well as astrocyte interaction with other cell types like neurons, microglia and endothelial cells. Along with general signaling mechanisms astrocytes exhibit some unique signaling features that are originated from their gap junctional coupling and their intercalating position relative to neurons, synapses and blood vessels.

Ca^{2+} is the most popular second messenger in different mammalian and non-mammalian cell types (reviewed in Akerman, 1982; Gardner, 1989; Webb and Miller, 2003). It logically led to a general concept of Ca^{2+} excitability of astrocytes (Bowman and Kimelberg, 1984; Kettenmann et al., 1984; Jensen and Chiu, 1990; Verkhratsky and Kettenmann, 1996). Indeed, it was demonstrated that astrocytes can exhibit Ca^{2+} waves induced by glutamate application (Cornell-Bell et al., 1990, Charles et al., 1991) emerging the idea of glutamate dependent neuro-glial signaling and supporting the concept of astrocytic Ca^{2+} excitability.

Even though astrocytes express channels that are permeable for Ca^{2+} ions, mainly changes in $[Ca^{2+}]_i$ are mediated by Ca^{2+} mediated Ca^{2+} entry either from endoplasmic reticulum or mitochondria (reviewed by Verkhratsky et al., 1998). These astrocytic Ca^{2+} spikes are regulated by signaling cascades that involve G-protein coupled receptors (GPCR) particularly mGluR5 (Panatier et al., 2011), protein kinase C phosphorylation and IP3 mediated signaling (reviewed by

Verkhratsky et al., 1998). It is worth to mention also that during brain maturation mGluR5 are replaced by mGluR3 that cause prevalence of adenylate cyclase mediated pathway and shifting mature astroglia signaling from developing one (Sun et al., 2013). On the other hand astroglial Ca^{2+} spikes mediate a range of different processes including exocytosis of ATP (Bal-Price et al., 2002; Pangrsic et al., 2007), D-serine (Panatier et al., 2006; Henneberger et al., 2010) and glutamate release (Liu et al., 2011). Unique astrocytic feature – their coupling through gap junctions and expression of hemichannels (Giaume, 2010; Bennett et al., 2003) provides additional options for Ca^{2+} signaling along ensembles of astroglial cells and can cause remarkable Ca^{2+} waves and synchronous astrocytic activity under physiological (Scemes and Giaume, 2006; Hoogland et al., 2009) and pathological conditions (Kuchibholta et al., 2009).

It is interesting that if the modulation of Ca^{2+} signaling in astrocytes via GPCR activation caused by synaptic activity is doubtless (Dani et al., 1992; Porter and McCarthy, 1996; Wang et al., 2006; Perea and Araque, 2007; Gordon et al., 2009; Panatier et al., 2011; Min and Nevian, 2012) the feedback loop that includes modulation of synaptic function by astrocytic Ca^{2+} transients and transmitters release from astrocytes is under debate (Fiacco et al., 2007; Petravicz et al., 2008; Agulhon et al., 2010). There are several explanations for such ambiguous role of astrocytic Ca^{2+} in glia-neuronal communication. Most of them are relied on the idea of inadequate tools used for astrocytic stimulation (Agulhon et al., 2010; Nedergaard and Verkhratsky, 2012), possible involvement of another universal second messenger replacing Ca^{2+}, for instance, Na^{+} ions (reviewed by Kirischuk et al., 2012; Bhattacharjee and Kaczmarek, 2005), or developmental shift in Ca^{2+} signaling occurring in astrocytes (Sun et al., 2013). It is worth to remember at this point that spatial localization of the signal, direction and rate of its propagation as well as dose dependence should always be considered when artificial stimulation approaches are used. For example ATP exocytosis from astrocytes triggered by Ca^{2+} transients has its own dose-dependent as well as temporal patterns (Pryazhnikov and Khiroug, 2008). Another elegant example where stimulus localization plays a critical role is provided by studies of local blood flow regulation by astrocytes. Exact location of Ca^{2+} elevations caused by local photolysis in soma or endfeet can cause opposite effects on blood vessels mediating either vasodilation or vasoconstriction (Takano et al., 2006; Gordon et al., 2011).

If stimulus amplitude and timing could be easily controlled in standard experimental paradigms, directionality, rate and precise localization should be refined. Possibly novel genetically encodable tools that can be delivered to a subset of astrocytes and affect defined and well predicted properties of the cell

(Armbruster et al., 2007; Airan et al., 2009; Molotkov et al., 2013) combined with proper delivery and localization system will shed the light on the ambiguous role of astrocytes in modulation of synaptic function.

Astrocyte excitability is accompanied by ion fluxes mediated by membrane channels in case of Na^+ and Ca^{2+} or by internal stores mainly in case of Ca^{2+} and is coupled to neuronal activity. Moreover Na^+ and Ca^{2+} mediated signaling might complement each other (Verkhratsky et al., 1998; Kirischuk et al., 2012). In fact Na^+ homeostasis that is strongly dependent on Na^+,K^+-ATPase activity and glucose utilization (Bernardinelli et al., 2004) might connect Ca^{2+} signaling to metabolic status of astroglia thus coupling indirectly internal astrocytic Ca^{2+} signaling with glycolysis and neuronal activity. In addition to it the role of K^+ ions also could be considered as possible indirect messengers for neuro-glial communication especially if astrocytic role in neuronal network activity regulation via K^+ buffering (reviewed by Simard and Nedergaard, 2004) would be taken into account. Hypothesizing further we can propose that water homeostasis in astrocytes is a candidate for playing a significant role both in maintenance and regulation of general and local ion mediated signaling, coupling metabolic activity of astrocytes with Ca^{2+} transients, glutamate uptake and possibly exocytosis events.

Despite application of advanced methodology the role astrocytic signaling inside the cell, among different astrocytes and also between astrocytes and neurons remains controversial with a prevalence of descriptive approaches to colligating ones. Diversity of methodological approaches might be one of the most critical factors that affect sensitive intracellular machinery. For instance, it was recently shown for awake mouse cortex that general anesthesia can disrupt Ca^{2+} signaling in astrocytes (Schummers et al., 2008; Thrane et al., 2012), but see also the work of Hirase and colleagues (Hirase et al., 2004) where astrocytic Ca^{2+} signaling was shown to be in a direct correlation with neuronal activity in anaesthetized rats. We can further suggest that signaling in primary astrocytic cell culture would be different from those in acute brain slice preparation that in turn would be different from whole brain *in vivo* situation.

2.7 Modulation of synaptic transmission by astrocytes: the concept of multipartite synapse

Complexity of chemical CNS synapse is often reduced to simple donor-acceptor model where presynaptic site releases neurotransmitter which is sensed by receptors on postsynaptic terminal. This view that pointing the synapse as one way neurotransmitter action site does not consider different important features of synapse such as geometry of synaptic cleft (Savtchenko and Rusakov, 2007), activation of extrasynaptic receptors (Kullmann and Asztely, 1998; Fellin and

Carmignoto, 2004), electrodiffusion of neurotransmitter caused by electrical fields generated near the synapse (Sylantyev et al., 2013), diffusion dynamics of neurotransmitter determined by ECM and surrounding cellular components (Sykova, 2004; Dityatev and Rusakov, 2011) and retrograde signaling that transform unidirectional synaptic transmission into crosstalk between pre and post synaptic terminals. This crosstalk which seemed to be a dialogue in a first approximation actually involves other active and passive participants besides neuronal components and generally synaptic events might be much more complex than proposed before.

It was known for quite a long time already that glutamate uptake by glial glutamate transporters is involved in synaptic transmission and neuronal excitability (Tanaka et al., 1997; Bergles and Jahr, 1998; Oliet et al., 2001) moreover efficacy of neurotransmitter clearance appeared to be affected by structural interplay between neuronal and glial compartments of synapse (Oliet et al., 2001) that raises a question about the role of structure-functional relationship between glia and synaptic structures. Glutamate uptake function of astrocytes is accompanied with a well-known fact that glial cells can produce and release neuroactive substances (Martin, 1992) and particularly glutamate in Ca^{2+} dependent manner (Bezzi et al., 2004; Marchaland et al., 2008; Santello and Volterra, 2009) and thus scale synaptic strength (Jourdain et al., 2007). It is also known that approximately 50% of hippocampal glutamate synapses are opposed to astrocytic processes and exhibit cooperative dynamics (Ventura and Harris, 1999; Witcher et al., 2007; Haber et al., 2006). These morphological changes also may underlie synaptic development and spine stabilization (Nishida and Okabe, 2007; Haber et al., 2006; Murai et al., 2003; Witcher et al., 2007). All mentioned above facts leaded to an idea of tripartite synapse (Araque et al., 1999; Perea et al., 2009) where astrocytes playing a role of active participants of synaptic events. Hypothesizing further it was proposed that ECM components represent a part of synaptic structure and involved in diffusion regulation, thus synapse representation could be upgraded to quadripartite structure (Sykova, 2004). Indeed, among non-neuronal cell types not just astroglia can actively participate in synapse functionality and structural changes. There are accumulating evidences that microglia could be an active synaptic partner as well (Bessis et al., 2007; Graeber, 2010; Tremblay and Majewska, 2011; Aguzzi et al., 2013; Kettenmann et al., 2013) allowing us to propose that the synapse represents a multipartite structure.

Although the combination of ability of astrocytes to perform bidirectional control of synaptic strength (Navarrete and Araque, 2011) and their highly organized territories with no spatial overlap and coverage of different dendritic trees (Bushong et al., 2002; Volterra and Meldolesi, 2005; Halassa et al., 2007) together with evidences for activation of astrocytic signaling pathways in a response to sensory stimulation *in vivo* (Wang et al., 2006; Winship et al., 2007; Petzold et al., 2008; Schummers et al., 2008; Nimmerjahn et al., 2009) allows to propose astrocytes as major CNS pacemakers. The astrocytic crosstalk with neurons is

controversial. Despite astroglia respond to neuronal activity by mean of metabolic and chemical activation, the influence of these events on synaptic events is under debate (Fiacco et al., 2007; Petravicz et al., 2008; Agulhon et al., 2010). In addition active glutamate conversion to glutamine in astrocytes by glutamine synthetase raises the question on sufficiency of glutamate concentration that is released by astrocytes (Barres, 2008). Even though astrocytes were shown to be able to generate Ca^{2+} responses fast enough (time to peak less than 500 ms) to intercalate with synaptic events (Marchaland et al., 2008; Santello et al., 2011; Di Castro et al., 2011; Winship et al., 2007). It is accompanied by the fact that vesicular glutamate transporters have not been detected in astrocytes with microarray expression studies (Lovatt et al., 2007; Cahoy et al., 2008) nor with microscopic investigations in cultured astrocytes and brain slices (Li et al., 2013) that puts a question mark on ability of astroglia to release glutamate in synaptic-like vesicles. We should consider there at least that neuro-glial communication differs across different brain areas and is a subject for developmental changes (Fiacco and McCarthy, 2006; Takata and Hirase, 2008; Nimmerjahn et al., 2009; Sun et al., 2013).

It is also need to be taken into account that astrocytic synaptic components are likely far away from being uniform; different parts of astrocyte can have different transporters and receptors profile they also can be on different distance from an active synaptic site, exhibit different morphological and functional responses. Actually if the release of neurotransmitter from presynaptic terminal is supposed to be all or nothing process its regulation by astroglia might be multimodal (Fig. 2).

2.7.1. Presynaptic mechanisms of astrocyte action

There are several ways how astroglial component of synapse can scale its activity via interaction with presynaptic neuronal terminal. These mechanisms involve astrocyte-neuron signaling through presynaptic glutamate GPCR and purinoreceptors. They are proposed to be involved in bidirectional control of synaptic strength both of basal synaptic transmition (Navarrete and Araque, 2011) (Fig. 2).

2.7.1.1. Presynaptic action of purines released from astrocytes

Astrocytes are known to release purines such as ATP and adenosine in a Ca^{2+} dependent manner (Haydon and Carmignoto, 2006). At the same time both adenosine and ATP are supposed to be one of the major molecules providing glia to neurons signaling in synaptic and non-synaptic regions within peripheral (Fields

and Stevens, 2000; Housley et al., 2009) and central nervous system (Kato et al., 2004; Haydon and Carmignoto, 2006; Rossi et al., 2007).
Adenosine action via presynaptically located metabotropic A1 receptors or ATP action mediated by ionotropic P2X receptors decrease release probability at presynaptic terminal and depress synaptic transmission by damping Ca^{2+} events (Koizumi et al., 2003; Kato et al., 2004; Pascual et al., 2005; Serrano et al., 2006). Interestingly also that ATP binding by P2X receptors located to the somatic region of GABAergic interneurons in hippocampus can facilitate neurotransmitter release from synaptic terminal and thus suppress pyramidal neurons activity (Bowser and Khakh, 2004; Kato et al., 2004). This example demonstrates dualistic role for ATP action in a respect of receptors localization and context.
The dualistic role for astrocyte derived adenosine was recently described as well (Panatier et al., 2011). Thus, it was shown that glutamate released from presynaptic terminal can activate astrocytic Ca^{2+} signaling via mGluR5 followed by exocytosis of ATP and adenosine that activate extrasynaptic A2A metabotropic adenosine receptors and increase release probability from the axonal terminal (Panatier et al., 2011). This mechanism provides possibility for bidirectional control of synaptic efficacy by astrocyte derived purines.

2.7.1.2. Modulation of synaptic events by presynaptic glutamate

Glutamate was known as a major neurotransmitter in CNS (Moore, 1993). Prolonged action of glutamate caused by inefficient uptake by astrocytes underlies many pathological states (Rothstein et al., 1996; Tanaka et al., 1997; Bergles and Jahr, 1998). Astrocytes are contributing to glutamate mediated events occurred in a presynaptic terminal in two ways. First, being responsible for glutamate clearance from synaptic cleft and for control of glutamate spillover astrocytes are modulators of extrasynaptic mGluRs and NMDA receptors activation by synaptically derived glutamate (Haydon and Carmignoto, 2006; Rossi et al., 2007). This implication is thought to be highly sensitive to morphological changes of astrocytic perisynaptic terminals. Second possible mechanism utilizes active Ca^{2+} dependent glutamate release from astrocytes that leads to activation of presynaptic neuronal mGluRs followed by Ca^{2+} increase and promoting finally additional release of glutamate from a presynaptic neuronal terminal (Schwartz and Alford, 2000; Fiacco and McCarthy, 2004; Perea and Araque, 2007, Navarrete and Araque, 2010). This principle is controversial since it is not clear if astrocytes are able to produce glutamate containing vesicles (Lovatt et al., 2007; Cahoy et al., 2008; Li et al., 2013) and if it is necessary for physiological synaptic functionality (Fiacco et al., 2007; Petravicz et al., 2008; Agulhon et al., 2010). In addition, there are some

evidences that activation of astrocytic mGluRs may reduce glutamate release from astrocytes (Ye et al., 1999) and potentially can scale up astrocytic glutamate uptake (Vermeiren et al., 2005). Potentially this kind of events should lead to down regulation of synaptic activity and are not entirely consistent with other studies.

2.7.2. Postsynaptic mechanisms of astrocyte action

Postsynaptic terminal is a primary site for neurotransmitter action. Astrocytes can affect synaptic transmission by modulating different aspects of presynaptic events, for instance, controlling neurotransmitter availability for neuronal receptors and duration of its action or secreting neuroactive substances that trigger activation of signaling pathways in dendritic spines and even play a role of glutamate source (Parri et al., 2001) (Fig. 2).

2.7.2.1. D-serine release from astrocytes

D-serine can bind at the glycine modulatory site of the NMDA receptor and promote its opening in a presence of glutamate thus acting as a co-activator (Wolosker, 2006). It was proposed quite a while ago that D-serine derived from astrocytes can play a role of synaptic modulator and that its action is tightly linked with glutamate-induced activation of non-NMDA receptors type on astrocytic surface (Schell et al., 1995). Lately it was also shown that D-serine release from astrocytes can be involved in LTP induction in hippocampal synapses and thus its exocytosis is linked to glutamate release from a presynaptic terminal (Yang et al., 2003). The conception that astrocytes can exclusively supply D-serine for NMDA co-activation during presynaptic stimulation and thus are a key component of LTP induction process was proofed in SON of hypothalamus (Panatier et al., 2006) and in classical hippocampal paradigm (Henneberger et al., 2010). Notably also that long term morphological changes of astrocytic synaptic component known for hypothalamus of lactating animals and Ca^{2+} clamping in astrocytes are also affect LTP induction by mean of availability of D-serine (Panatier et al., 2006, Henneberger et al., 2010; Fossat et al., 2012). There is also another link connecting D-serine release from astrocytic terminals with their structure-functional interplay with neuronal components of synapses. Indeed, it was shown recently that synthesis and exocytosis of D-serine from astrocytes is linked to Ephrin-mediated signaling that prerequisites direct contact between astrocytic and neuronal terminal (Zhuang et al., 2010). If D-serine deficit and lack of LTP induction in SON of lactating animals is caused by absence of direct astro-neuronal contact and Ephrin-mediated signaling is still an open question.

Although astrocytes a thought to be an exclusive source of D-serine in major brain structures (Schell et al., 1995; Panatier et al., 2006) there are accumulating evidences that neurons can produce and release D-serine that on a par with astrocytic one can act as a mediator for postsynaptic NMDA receptors (Kartvelishvily et al., 2006; Rosenberg et al., 2013).

2.7.2.2. Postsynaptic action of Glycine

Glycine is one of amino acids neurotransmitters in the brain mostly known for its inhibitory action. Its binding to postsynaptic NMDARs is known to promote their internalization, suppress synaptic activity and promotes LTD (Nong et al., 2003). On a par with neurons astrocytes are potential source for glycine (Holopainen and Kontro, 1989) that is most prominently facilitated by reverse action of glycine transporters (Henneberger et al., 2013). On the other hand astrocytes are scavengers for glycine mediated by GlyT1 and GlyT2 as well (Fedele and Foster, 1992; Verleysdonk et al., 1999; Zhang et al., 2008; Aroeira et al., 2013). Interestingly that glycine uptake by astrocytes as in many other cell types is mediated by protein kinase C signaling pathway (Morioka et al., 2008) and thus is a subject for Ca^{2+} dependent modulation. Recent studies also indicate that postsynaptic glycine action is mainly mediated by extrasynaptic NR2B containing NMDARs and that it may act synergistically with D-serine binding to synaptic NR2A containing NMDARs located at the synaptic site to promote LTD (Papouin et al., 2012).

2.7.2.3. Postsynaptic action of astrocyte derived ATP

Astrocyte derived ATP is known to modulate synaptic transmition by mean of postsynaptic action in addition to presynaptic one. Comparatively to diverse presynaptic purines action ATP interaction with postsynaptic P2X receptors increase synaptic strength likely by promoting insertion of novel AMPA receptors (Wang et al., 2004; Gordon et al., 2005) and underlie, for instance, astrocyte mediated norepinephrine action (Gordon et al., 2005). Both ATP release from astrocytes (Coco et al., 2003; Pangrsic et al., 2007) and ATP mediated changes in postsynaptic terminal (Wang et al., 2004; Gordon et al., 2005) are triggered by local intracellular Ca^{2+} mediated signaling. Contrarily to normal physiological conditions, under ischemia or hyper activation caused by epileptiform activity Na^{+} entry via postsynaptic P2X channels may trigger Ca^{2+} release from mitochondrial stores, reactive oxygen species (ROS) production and cause activation of proteases leading to spine collapse and even apoptosis (Rossi et al., 2007).

2.7.2.4. Neurotransmitter and ion clearance by astrocytic transporters and regulation of spillover

Involvement of astrocytes in glutamate-glutamine cycle that supplies neuronal demand in excitatory neurotransmitter and promote neuro-glial metabolic coupling also tightly linked to the ability of astrocytes to control glutamate clearance from a synaptic cleft via EAAT1, EAAT2 and maintain potassium homeostasis by K^+ syphoning through Kir4.1 channels and Na^+,K^+-ATPase. Glutamate clearance by astrocytes regulates duration of its action and also prevents or facilitates its extrasynaptic action. Indeed, glutamate spillover is an important factor involved in modulation of synaptic properties affecting both glutamatergic (Asztely et al., 1997; Kullmann and Asztely, 1998) and GABAergic transmission (Semyanov and Kullmann, 2000). Similarly activation of extrasynaptic NR2B containing NMDA receptors by astroglia-derived glutamate may lead to neuronal cell depolarization by slow inward current and even trigger generation of action potentials (Angulo et al., 2004; Fellin et al., 2004; Perea and Araque, 2005; Navarrete and Araque, 2008). In turn K^+ syphoning by astrocytes decrease neuronal depolarization and decrease neuronal network excitability (Pannasch et al., 2011).
Efficiency of glutamate uptake by astrocytes is thought to be dependent on structural interplay between neuronal and astroglial synaptic components and affected in supraoptic nucleus (SON) in hypothalamus of lactating animals (Oliet et al., 2001). Such morphological changes also involved in diffusion regulation (Piet et al., 2004) and might be the key to spillover and extrasynaptic transmission control (Rusakov, 2001; Scimemi et al., 2004; Papouin et al., 2012).

3. Peripheral astrocytic process as a functional microdomain

Astrocytes are known to have complex 3D morphology *in vivo* and *in situ* (Ogata and Kosaka, 2002; Bushong et al., 2002; Witcher et al., 2007; Hirrlinger et al., 2004). Even though this complexity is reduced *in vitro* when individual astroglial cells are flattened on a culture dish bottom forming 2D structures astrocytes are able to form peripheral filopodia-like process containing actin (Molotkov et al., 2013), actin related proteins such as ezrin and also mGluRs (Derouiche et al., 2001; Lavialle et al., 2011). Peripheral astrocytic processes (PAPs) are supposed to be main astroglial compartment responsible for neuro-glial interaction at synaptic terminals. Indeed, it was shown that PAPs are located in a close proximity with a majority of synapses. Thus, 56% of synapses in rat neocortex and 64 to 90%, depending on spine type, of synapses in rat hippocampus have glial enwrapping component nearby (Wolff, 1970; Witcher et al., 2007).

One astrocyte can contact with more than 100,000 synapses in rodent and up to 1,000,000 synapses in the human brain (Oberheim et al., 2006). This huge integrating capacity is maintained by their highly ramified PAPs. At the same time astrocytes are known to occupy separate but partially overlapping territories (Ogata and Kosaka, 2002; Bushong et al., 2002; Derouiche et al., 2002; Livet et al., 2007). It is now accepted that each PAP can form separate functional microdomain with local Ca^{2+} signaling, localized receptors and channels (Grosche et al., 1999; Grosche et al., 2002; Reichenbach et al., 2010; Shigetomi et al., 2013(b)).
Interestingly that astrocytic processes have a preference to postsynaptic terminals *versus* presynaptic ones that additionally regulated during brain maturation (Lehre and Rusakov, 2002; Nishida and Okabe, 2007). At the same time PAPs are known to participate in synapse stabilization via ephrine signaling with spines (Murai et al., 2003; Haber et al., 2006) and can be active regulators of synaptogenesis in developing brain (Nishida and Okabe, 2007). It is also known that, for instance, tonic oxytocin action in hypothalamic SON during lactation in rats causes PAPs retraction from neurons and synapses causing changes in functioning of those synapses and providing as well a unique model for studying long-term plasticity between neuronal and astroglial component (Theodosis and Poulain, 1993; Oliet et al., 2001).

3.1 Ultrastructure of PAPs

The term peripheral astrocytic processes was introduced by Derouiche and colleagues just a bit more than ten years ago in order to describe very thin filopodia-like protrusion in cultured astrocytes (Derouiche and Frotscher, 2001; Derouiche et al., 2002). These protrusions were too thin to be visualized with transmitted light microscopy and remained undescribed prior ezrin immunolabelling and fluorescent tracing of astrocytic plasma membranes. Alternative term such as peripheral glial processes (PGP) for these kinds of protrusions but meaning their appearance in close proximity to synapses in the brain *in vivo* was introduced by Reichenbach and colleagues in 2010 (Reichenbach et al., 2010).
Thickness of astrocytic process can vary from 1 μm to 50-100 nm almost lacking the cytoplasm inside in this case; typically they are as narrow as 200-500 nm (Ventura and Harris, 1999; Witcher et al., 2007; Shigetomi et al., 2013). Although PAPs contain very small portion of astrocytic cytoplasm they represent a major fraction of cell surface of around 70-80% from total astrocytic membrane with extremely high surface to volume ratio that may reach 25 μm^{-1} (reviewed by Volterra et al., 2002, pp. 3-24; Grosche et al., 2002). Due to such high surface to

volume ratio visualization of PAPs could be effectively done using membrane bound fluorescent tracers *in situ* and *in vivo* (Benediktsson et al., 2005; Shigetomi et al., 2013(a); Molotkov et al.,*in preparation (study III)*). Interestingly there are evidences that the thinnest astrocytic process are also involved in neurotransmitter recycling and have all necessary machinery to participate in neurotransmitter exchange (Derouiche and Frotscher, 1991) at the same time it was shown using serial electron microscopy that thinnest parts of distal astrocytic process are mostly associated with synaptic structures (Witcher et al., 2007) suggesting their role in synaptic functioning.

The shape of PAPs is not uniform they typically do not taper towards the end but have thicker regions interconnected by more narrow ones (Peters et al., 1991; Witcher et al., 2007). Based on observations made with serial electron microscopy and two-photon microscopy with membrane-targeted fluorescent tracers, it was also proposed recently that PAPs may form net-like rather than tree-like structures (Shigetomi et al., 2013(a); Molotkov et al., *in preparation (study III)*). Usually PAPs contain just little amount of organelles (reviewed by Reichenbach et al., 2010) that differ them from dendrites but they might contain vesicles that thought to accumulate and release gliotransmitters such as D-serine, ATP and glutamate (Bezzi et al., 2004; Spacek and Harris, 2004; Jourdain et al., 2007; Witcher et al., 2007; Kang et al., 2013).

3.2 Receptors and transporters in PAPs

Even though astrocytic component of synapse represented by PAP has relatively small volume it demonstrates expression for broad spectrum of different transporters and receptors that underlie crosstalk between neuronal and glial components in the tripartite synapse as well as promote local signaling for structural PAP remodeling. Thus, it is known that PAPs express high levels of glutamate transporters of both types EAAT1 and EAAT2 which facilitate glutamate uptake by astrocytes from a synaptic cleft (Chaudhry et al., 1995; Zhang et al., 2004) and can also participate in regulation of neuronal mGluRs or excrete glutamate by reverse uptake mechanism (reviewed by Grewer et al., 2008). These transporters may also underlie glutamate induced swelling of astrocytes (Koyama et al., 1991; O'Connor and Kimelberg, 1993).

Besides glutamate uptake and secretion from and to synaptic terminals, PAPs are able to sense glutamate molecules via mGluRs (Lavialle et al., 2011; Panatier et al., 2011) particularly by mGluR5 and mGluR3 which translate glutamate binding to Ca^{2+} elevations or activation of cAMP signaling pathway depending on the brain developmental stage (Sun et al., 2013). As an alternative mechanism for internal

store-independent Ca^{2+} signaling in PAPs the Ca^{2+} entry into astrocytes through TRPA1 channels was proposed (Shigetomi et al., 2013(b)). This mechanism does not require activation of IP_3 signaling pathways and may operate in mGluRs independent way.

Another group of channels expressed in PAPs is tightly linked to morphological changes of astroglial cells due to regulation of chloride, potassium and water homeostasis. Thus, chloride channels presented in PAPs were shown to mediate glial reshaping and swelling (Ransom and Sontheimer, 2001). On the other hand one of the theories for astroglial reshaping is based on water entry through AQP4 which is enriched in PAPs where it is often colocalized with Kir4.1 potassium channels suggesting their mutual role in maintenance of water and K^+ homeostasis in PAPs (Nagelhus et al., 2004; Holen, 2011). Another trans membrane protein presented on PAPs surface Ephrin-A3 ligand is involved in contact glia-neuronal interactions and was shown to regulate dendritic spine development and stabilization in a bidirectional fashion (Murai et al., 2003, Haber et al., 2006; Nishida and Okabe, 2007). Additionally Eprin-A3 and A4 interactions are involved in expression regulation of glutamate transporters in perisynaptically localized PAPs and thus can influence synaptic functional properties via modulation of glutamate uptake and spillover (Filosa et al., 2009).

3.3 Morphological changes of PAPs: two different mechanisms proposed

3.3.1. Aquaporin mediated morphological changes

Water channel AQP4 is supposed to be potential regulator of cells shape (Nicchia et al., 2003; Nagelhus et al., 2004). Water uptake by astrocytes can cause swelling which, in turn, changes their contacting surface with neurons. Astrocytic swelling also affects activity of neuronal networks and is typical for several pathological states (Scharfman and Binder, 2013). Similarly rise of extracellular potassium might be one of keys for switching morphological changes of PAPs. Indeed, there is an evidence that increase of extracellular potassium ions can stimulate formation of new branches in cultured Muller cells (Reichelt et al., 1989).

3.3.2. Actin-dependent morphological changes

Actin cytoskeleton is the most common driver for different types of cellular motility including migration, rapid morphological changes of cell edge (Pollard and Borisy, 2003), lamellipodia and filopodia formation and extension (Faix and Rottner, 2006; Mattila and Lappalainen, 2008), exocytosis and endocytosis events (Muallem et al., 1995; Engqvist-Goldstein and Drubin, 2003). Generally actin

cytoskeleton is linking intracellular signaling to membrane dynamics and underlies as well receptor trafficking on the cell surface (Malinow and Malenka, 2002; Collingridge et al., 2004; Cingolani and Goda, 2008). Actin driving force is underlined by constant treadmilling cycle between monomeric actin pool and filamentous actin (F-actin) strands and can be regulated by many co-factors that acts as primers, exchangers and catalyzers for actin monomers (Pollard, 2003).

The evidence that PAPs motility can be reduced by cytochalasin treatment (Haber et al., 2006) suggesting the active role of actin cytoskeleton remodeling for morphological plasticity of PAPs. This observation is also confirmed by the fact that morphological changes of PAPs are diminished by overexpression of dominant-negative form of small GTPase Rac that is known to be involved in cytoskeleton regulation (Nishida and Okabe, 2007). Besides functional evidences for actin cytoskeleton based motility of PAPs there are several structural supports for this idea. It is based on the evidence for localization of actin-related proteins such as ezrin (Derouiche and Frotscher, 2001) and α-adducin (Seidel et al., 1995) inside PAPs. Indeed, direct demonstration for the presence of F-actin inside astrocytic filopodia in culture was recently shown as well (Molotkov et al., 2013).

PAPs outgrowth could be switched both by rise of $[Ca^{2+}]_i$ (Molotkov et al., 2013) or by application of extracellular glutamate (Cornell-Bell et al., 1990; Hirrlinger et al., 2004). In both cases they demonstrate relatively high outgrowth rates of around 1 $\mu m \cdot min^{-1}$ for brain slices with glutamate-induced outgrowth (Hirrlinger et al., 2004) and approximately 3 $\mu m \cdot min^{-1}$ for cultured astrocytes stimulated by photolysis of caged Ca^{2+} (Molotkov et al., 2013). Most likely PAPs outgrowth is triggered by similar mechanisms as filopodia outgrowth in other cell types, for instance, fibroblasts or keratinocytes. The role of plasma membrane-to-cytoskeleton linker Ezrin is within a special interest if focusing on reshaping of neuron glial connections. Regulated by phosphorylation/dephosphorylation it can potentially represent a key to rapid bidirectional activity-mediated morphological plasticity of PAPs (Gautreau et al., 2002; Lavialle et al., 2011). Other possible players are chloride channels that are known to have actin-dependent activity (Lascola et al., 1998; Ahmed et al., 2000) and bridging together "passive" astrocytic swelling and "active" actin-based astroglial motility.

Finally it is interesting to address the question whether mGluR3 mediated activation of adenylate cyclase pathway can promote rapid morphological changes in PAPs similar to those that could be obtained by local Ca^{2+} uncaging or local glutamate application in rat neonatal astrocytes that supposed to mimic store-operated Ca^{2+} entry mediated by mGluR5 receptors.

4. Methodological approaches to study neuro-glial interactions

4.1 Hypothalamus as a classical model of PAPs retraction

Morphology of the synapse is one of the critical aspects that underlies amount of postsynaptic receptors being activated and time course for neurotransmitter action as well as possible transactivation of neighboring synapses and extrasynaptic receptors (Freche et al., 2011; Allam et al., 2012).
It was known a while ago that oxytocin can induce morphological changes in SON of hypothalamus in lactating rodents (Theodosis et al., 1986). These changes represent evidence for mature brain structural plasticity and was a unique case until recent studies of antidepressant-induced plasticity (Maya Vetencourt et al., 2008; Karpova et al., 2011). Lactation and increased oxytocin level induced long term retraction of astrocytic process from synapses and neuronal somata facilitating direct contact between neighboring neurons and blood vessels (Theodosis and Poulain, 1989; Theodosis and Poulain, 1993). This also affected glutamatergic synaptic transmission (Oliet et al., 2001; Panatier et al., 2006). Morphological changes in SON could also be driven by dehydration and changes in vasopressin level which also caused astrocyte retraction (Hatton, 1997; Theodosis et al., 2008). Interestingly, the model of astrocyte retraction in SON in a response to oxytocin works *in vitro* as well. Thus, bath application of oxytocin on brain slices containing SON caused similar rapid (within hours) and reversible morphological changes in astro-neuronal contacts as observed *in vivo* in lactating animals (Langle et al., 2003). In addition dehydration and rehydration could be also used as a reverse switcher for GFAP level in astrocytes and may reversibly affect astrocytic morphology and coverage of neurons in SON (Hawrylak et al., 1998). If this model could be simplified for *in vitro* studies, by application of hyperosmotic solutions on an acute brain slice is questionable.

4.2 Transgenic mouse models to study astrocytes

Genetic manipulation in mice is a powerful tool to understand CNS physiology and pathology. It allows the study of particular gene function by their ablation or overexpression in a subset of cells in the brain using cell-type specific promoters. There are a number of different genetic strategies to manipulate and study astrocytes in a rodent brain (reviewed by Pfrieger and Slezak, 2012). Some of these approaches are based on available transgenic mice models.
Glial fibrillary acidic protein (GFAP) is an intermediate filament component that is preferentially expressed in astrocytes and glial progenitors. Recombinant GFAP

promoter is the most studied and the most popular tool for transgene expression in astrocytes both *in vitro* and *in vivo* and is often used for generation of transgenic mouse lines.

GFAP promoter as a tool for transgene expression in astroglial and neuronal cells has been known for more than 20 years (Mucke et al., 1991; Su et al., 2004). Besides endogenous promoter elements, its expression efficiency and specificity also depends on protein coding sequence for the gene of interest, its genomic and temporal context, methylation and acetylation status and genetic background of the animal (Su et al., 2004; Bai et al., 2013). Although, it is known that the 2.2 kb human GFAP (hGFAP 2.2 kb) promoter facilitates astrocyte-specific expression (Brenner et al., 1994); the same promoter variant was also used in several studies to direct expression to astrocytes, Muller cells and Schwann cells in the peripheral nervous system in transgenic mice (Zhuo et al., 1997; Nolte et al., 2001). Particularly intriguing is that when the 1.7 kb mouse GFAP promoter is delivered to neonatal rat brain in the form of a bacterial plasmid by *in vivo* electroporation, it has an expression preference for neurons rather than astrocytes (Molotkov et al., 2011).

Novel variants of engineered transgenic mice provide more flexibility in exogenous gene expression in astrocytes and allow combining, for instance, specificity and efficiency of a well characterized promoter with flexibility of viral gene delivery approaches. Thus, transgenic mice expressing Cre recombinase under the control of hGFAP 2.2 kb facilitates homologous recombination and stable expression of the gene of interest in astrocytes (Zhuo et al., 2001; Hirrlinger et al., 2006). This system could have additional temporal control facilitated by inducible Cre (Hirrlinger et al., 2006). However, it was shown that some neurons also express Cre in this model (Zhuo et al., 2001), thus additional specificity filtering by addressed gene delivery done by viral vector has a good potential in this model, if an absolute specificity to astroglia is needed.

Time-lapse microscopy on thin structures such as PAPs was always a challenge due to poor signal to noise ratio and a size that is sometimes less than the diffraction limit for light microscopy. However, at least two transgenic mouse models expressing cytoplasmic EGFP were shown to be effective for studying PAPs dynamics. The first one is based on the mouse 2.6 kb GFAP promoter (Suzuki et al., 2003), and another one utilizes the 2.2 kb hGFAP promoter to direct EGFP expression to astroglia (Hirrlinger et al., 2004). Both models demonstrated that PAPs dynamics could be studied with cytoplasmic fluorescent reporters, and that the expression level of GFAP promoter is sufficient to get a good signal to noise level. Nevertheless both the resolution and expression efficiency in these studies were subject to future improvement.

The promoter is not a uniform sequence, it might contain parts responsible for transcription itself (core promoter), as well as parts that can modulate promoter efficiency in a bidirectional fashion. Thus, a large 2.2 kb segment of human GFAP promoter contains not only the elements that enhance transcription of a downstream gene, but also elements that can decrease promoter efficiency. Interestingly, the minimal 447 bp long gfa28 promoter directs expression only in a subset of astroglia and its expression efficiency is not altered by injury as classical 2.2 kb hGFAP promoter; moreover, it was shown that its restricted activity is dependent on chromatin remodeling by histone deacetylation during development (Lee et al., 2006). These finding led to an idea that some enhancer elements of GFAP promoter can increase transgene expression without affecting astrocyte specificity (Lee et al., 2006) and to the creation of improved astrocyte-specific promoter. The novel gfaABC1D promoter, which is only 681 bp in length, and which was obtained from the 2.2 hGFAP promoter, contains all necessary elements for strong and astrocyte-specific expression and lacks all the elements that suppress expression. It demonstrated higher efficiency and specificity when it was used for creation of transgenic mouse strain (Lee et al., 2008).
Another prospective strategy to improve expression specificity in transgenic mice utilizes the so called "Split-Cre" system that overcomes expression specificity problems, since simultaneous expression from two cell-type specific promoters is needed to activate recombination (Hirrlinger et al., 2009). This model has also demonstrated its usefulness for studying cell differentiation *in vivo* (Hirrlinger et al., 2009).

4.3 Acute brain slices and organotypic cultures to study neuro-glial interactions

The brain is a complex organ where structure and intracellular interactions are of the same importance as its individual components. Brain slices provide an easy-to-use and reliable alternative for *in vivo* approaches to study properties of individual cells as well as interactions between different cell types. This feature is the most valuable when such property of the brain as connectivity is investigated.
There are many examples of the use of acute brain slices obtained from different brain areas for studying neuro-glial and interglial interactions which demonstrate the potential of this model (Hirrlinger et al., 2004; Panatier et al., 2006; Perea and Araque, 2008; Rouach et al., 2008; Henneberger et al., 2010; Panatier et al., 2011).
Ease of accessibility to the slice has made it possible to perform advanced electrophysiological recordings and dye infusion via patch pipette (Perea and Araque, 2008; Rouach et al., 2008; Henneberger et al., 2010; Panatier et al., 2011;

Pannasch et al., 2012). Combination of slicing procedure with the use of transgenic mouse models or application of viral vectors expressing fluorescent reporters allows performing high resolution imaging of entire astrocytes and their peripheral processes (Hirrlinger et al., 2004; Shigetomi et al., 2013(a)). Despite their many advantages, acute brain slices were shown to be different when compared to an intact brain. Thus, it was shown that in acute hippocampal slices dendrites form additional synapses that are not characteristic for hippocampus *in situ* (Kirov et al., 1999). Another issue that should be addressed is the post-slicing recovery conditions and timing that significantly affect the morphology of glia and neurons in acute brain slices (Fiala et al., 2003; Kirov et al., 2004).

As an alternative to acute brain slices, the procedure for preparation of organotypic brain cultures has been developed (reviewed by Gähwiler et al., 1997; Gähwiler et al., 2001). Originally invented as a roller-tube culturing method (Gähwiler, 1981; Gähwiler, 1984), it results in obtaining a 1-2 cell layer of brain tissue that resembles the entire architecture of the brain region from which it was dissected (Gähwiler et al., 1997). The method was further simplified and improved to stationary interphase culturing method using porous membrane as a substrate for the slice (Stoppini et al., 1991). Cultivation results in 1-4 cell layer thick culture in which organotypic morphological and functional organization is relatively well preserved. The well-defined procedures for preparation of such slice cultures (De Simoni et al., 2006), their applicability for long term imaging experiments (up to 6-8 weeks) (Gogolla et al., 2006), good optical transparency and their convenience for different transfection procedures (Wirth and Wahle, 2003) combined with preserved cell morphology and connectivity, have made organotypic brain slices a popular tool to study astrocytic morphological changes and neuro-glial interactions (Benediktsson et al., 2005; Haber et al., 2006; Nishida and Okabe, 2007; Bernardinelli et al., 2011).

4.4 *In vivo* methods to probe astrocytic morphological and functional changes

4.4.1. Two-photon microscopy on living mouse brain

Since its invention more than twenty years ago (Denk et al., 1990), two-photon excitation microscopy (TPEM) became a powerful tool for understanding processes in the brain with cellular and subcellular resolution due to its good penetration depth and high resolution. TPEM is applicable for functional imaging in individual neuronal and glial cells as well as in their distal processes, using synthetic or genetically encoded Ca^{2+} indicators enabling longitudinal studies of

development of brain pathological states and tracing morphological changes underlying memory (reviewed by Bacskai et al., 2002; Helmchen and Denk, 2005; Svoboda and Yasuda, 2006). Recently, it became also possible to perform TPEM on awake rodents involved in virtual navigation tasks (Dombeck et al., 2007; Dombeck et al., 2010).Additionally, two-photon microscope is not just a tool for observation but also a tool for manipulation. Thus, it is possible to produce a local stroke by thrombotizing a single penetration arteriole (Nishimura et al., 2006; Shih et al., 2013), to use a laser beam for photolysis of caged compounds (Noguchi et al., 2011), photoactivate fluorescent markers (Piatkevich et al., 2013), optogenetic manipulation (Figueiredo et al., 2011; Prakash et al., 2012) or for direct activation of astrocytes (Choi et al., 2011).

In vivo microscopy on the brain requires an optically transparent skull. Currently, there are two distinct techniques available to prepare cranial optical window with their advances and limitations (Xu et al., 2007). The craniotomy method, in which a part of the bone is removed and replaced with glass (Holtmaat et al., 2009); has an advantage of higher transparency and as a consequence better penetration depth for light; on the other hand, post-surgical recovery period for such preparation is around 2-3 weeks. In addition, the glass transplant does not allow injecting any substances under the cranial window during experiment. As an alternative for craniotomy, there is a method for thinned polished skull preparation (Xu et al., 2007; Drew et al., 2010). This preparation induces much less inflammatory changes in the brain since it is almost non-invasive, but lacks perfect optical transparency and long-term stability featured by craniotomy.

Recent development of *in vivo* microscopic imaging techniques provides a good opportunity to manipulate and to observe neuro-glial interactions in the intact rodent brain and to study rapid changes in glial morphology as well as long term neuro-glial plasticity.

4.4.1.1. Probes and dyes to label astroglia in vivo for TPEM

In vivo patching techniques for unlabeled neuronal cells (Kitamura et al., 2008; Witte et al., 2011) are difficult to apply for astrocytes due to their small soma dimensions. This obstacle makes it problematic to load individual astroglial cells with tracers *in vivo*. The dye that can overcome these limitation should have intrinsic tropism to astroglial cells, thus being applied topically to the brain surface or injected to the brain parenchyma should and be able to enter specifically astrocytes. A synthetic water-soluble dye sulforodamine 101 (SR101) was shown to solve the problem of astrocyte-specific labeling via bulk loading in rodent neocortex *in vivo* and trace astrocytic morphology when used in combination with

two-photon excitation microscopy (Nimmerjahn et al., 2004). Most likely the dye enters cells via active uptake (Schnell et al., 2012). Later this dye was shown to be effective also for *in vivo* labeling of Bergman glia cells in the cerebellum of freely moving mice (Nimmerjahn et al., 2009) and for astrocyte loading in hippocampus in acute brain slices (Kafitz et al., 2008). Despite SR101 benefits, it has also some limitations (Nimmerjahn and Helmchen, 2012). Particularly it was shown that, when applied to hippocampus, SR101 can enhances activation of NMDARs, trigger spontaneous neuronal firing and may cause *in vivo* epileptic seizures (Kang et al., 2010) and, thus may interfere with results obtained from tracing astrocytic morphology. It was also shown that SR101 uptake to astrocytes changes during developmental stages (Kafitz et al., 2008).

In comparison to neurons, astrocytes are known to lack electrical activity but they can interact with each other by Ca^{2+} waves (van den Pol et al., 1992). Intracellular Ca^{2+} is also a key player for astro-neuronal signaling (Fiacco and McCarthy, 2004) and for induction of morphological changes in astrocytes (Molotkov et al., 2013). Monitoring Ca^{2+} events *in vivo* in astrocytes and their processes constitutes a special interest. Two prospective strategies are currently available for highly localized Ca^{2+} imaging. The first one utilizes newly developed fast and sensitive Ca^{2+} sensor GCaMP6 for detection of highly compartmentalized neuronal Ca^{2+} domains *in vivo* (Chen et al., 2013). Another approach that was tested on acute brain slices is based on targeting GCaMP sensor to astrocytic plasma membrane which improves signal to noise ratio and improves resolution when Ca^{2+} signals in PAPs are monitored (Shigetomi et al., 2013(a)). Combination of these two approaches may lead to a creation of Ca^{2+} sensor optimized for *in vivo* TPEM on PAPs.

4.4.1.2. In vivo microscopy on anaesthetized animals

An intact entire brain provides much more appropriate preparation for studying its function than organotypic cultures or acute brain slices. Moreover, some aspects of brain functionality cannot be studied in those simplified preparations. These aspects include cerebral blood flow and brain pH regulation by glial cells (Attwell et al., 2010; Gourine et al., 2010), long term structural changes related to experience (Hofer et al., 2009), network activity (Svoboda et al., 1997; Kuchibhotla et al., 2009) and pathology development (Bacskai et al., 2002). All these questions could be addressed in anaesthetized animals. To make complex things simpler, it was shown recently that use of conventional laser scanning microscope for *in vivo* imaging is also possible (Perez-Alvarez et al., 2013). We can conclude that the main advantage of *in vivo* microscopy performed on

anaesthetized animals is a combination of its relatively high relevance with relative simplicity, although its results should be interpreted with caution since the brain is the main site of action for all general anesthetics.

4.4.1.3. In vivo microscopy on awake animals

General anesthetics cause disruption of Ca^{2+} signaling in astrocytes and may have a strong impact on data obtained on anesthetized animals (Schummers et al., 2008; Thrane et al., 2012). Moreover some experimental paradigm such as different cognitive and behavioral tasks or coupling between cerebellar neuronal activity and Bergman glia Ca^{2+} signaling during exploratory behavior (Nimmerjahn et al., 2009) is impossible to perform on unconscious animals.

The first approach for imaging in freely moving animals came from the idea of miniaturized microscopic device that can be mounted on animal's head without restricting its movements (Helmchen et al., 2001; Flusberg et al., 2008; Ghosh et al., 2011). These microscopes can be placed in cortical as well as in hippocampal regions of the brain and allow imaging of individual cells, blood vessels and measure a blood flow dynamics. The main limitation for these systems is their narrow field of view, stationary positioning, high level of customization and relatively low numerical aperture restricted by limited optics dimensions.

As an alternative, commercially available upright microscopes can be used in combination with systems that compensates for an animal's movements. The first prototype of a spherical treadmill was shown to be effective for microscopy on awake mice (Dombeck et al., 2007) as well as for complex behavioral paradigms utilizing virtual reality (Harvey et al., 2009). The main advantages for this strategy are the use of commercially available stock optics, higher resolution and better signal to noise ratio due to the possibility to use high numerical aperture objectives. On the other hand this approach is difficult to apply for hippocampus or deeper brain regions without affecting sensitivity.

Another obstacle that concerns both approaches are artifacts caused by animal movements that always appear when *in vivo* microscopy on awake animal is performed. Indeed, there are several software modules available that help to overcome this problem.

The possibility to perform automated short term repetitive imaging sessions was reported recently on behaving rats (Scott et al., 2013). Short image acquisition time (up to 8 seconds), firm attachment of the head plate to microscope stage for the moment of image acquisition and animal comfort can provide better image quality than in long term sessions when the animal is forced by unnatural stimuli to be head restrained.

In vivo microscopy on non-anaesthetized animals is the most natural microscopic approach that allows combining micrometer spatial resolution, millisecond temporal resolution and lack of artifacts caused by general anesthetics. The set of these methodological applications might help us to understand how commonly used anesthetics affect an entire brain and each of its components, and what is happening in the brain during behavior.

4.5 Gene delivery to astrocytes

Even though transgenic animals provide a wide range of ready-to-use solutions or can be generated for particular purposes, they lack flexibility – the characteristic feature of acute gene delivery approaches. For instance, it is very difficult to create a transgenic mouse line that will express different genes in two or more similar brain regions. In contrast, such an option is feasible with focal multiple point gene delivery by means of viral vectors. Another issue we will address here is astroglia as a prospective target for gene therapy approaches in clinical cases. In this part we will discuss some methodological approaches allowing transient or stable transgene expression in astrocytes *in vitro* and *in vivo*, especially focusing on their expression efficiency and specificity.

4.5.1. Specificity and efficiency of gene delivery to astrocytes

Both specificity and efficiency of expression are always an issue for *in vivo* gene delivery, since there are many different cell types in the brain that sometimes are difficult to distinguish and there are many chemical and mechanical barriers that are difficult to overcome. For *in vitro* applications, when the dissociated cell culture primarily contains cells of interest or cells which can be distinguished easily by their morphology or other linage markers, the efficiency of transfection maybe the major problem. Cell-type specificity and expression efficiency *in vitro* and *in vivo* is determined by factors which only partially overlap. Thus, use of astrocyte specific promoters such as hGFAP, gfaABC1D (Lee et al., 2006; Lee et al., 2008) or ubiquitous cytomegalovirus (CMV) promoter (Thomsen et al., 1984; Jeang et al., 1984) in combination with standard transfection protocols is usually the best and simplest choice for dissociated astrocytic cultures. Both CMV and enhanced versions of hGFAP promoter, such as gfaABC1D, result in relatively high expression levels in astrocytes *in vitro*.

The situation is changed if there is a need to transfect astrocytes in organotypic cultures that contain other cell types in addition to astrocytes, as well as extracellular matrix proteins. Moreover, presence of several cellular layers may prevent gene delivery to the lower parts of the slice. These obstacles can be

effectively overcome with ballistic gene delivery using different types of available gene guns (O'Brien et al., 2001; Benediktsson et al., 2005; Shefi et al., 2006) or by using electroporation combined with electro diffusion (Murphy and Messer, 2000; Kawabata et al., 2004).

The most demanding case appears to be the *in vivo* applications when specificity of promoters is not same as in the *in vitro* situation and may be determined by several factors including the age of the animal, particular brain region, source of exogenous DNA encoding the transgene and promoter. Such factors as microglial and astroglial activation caused by application of exogenous biomaterial should be also considered. Side by side with efficient viral vector strategies (reviewed by Merienne et al., 2013) there are non-viral alternatives based on postnatal (Barnabe-Heider et al., 2008; Molotkov et al., 2010; De la Rossa et al., 2013) and prenatal *in utero* electroporation (Saito, 2006; Dixit et al., 2011). In the case of viral vector mediated expression the specificity and efficiency is determined by nature of the virus and the promoter; while in the case of electroporation it is determined by the promoter and to a lesser degree by the source of DNA, its methylation and conformation. Further we will go through some approaches that were shown to be more or less effective for targeting transgene expression to astrocytes.

4.5.2. Gene-gun mediated gene delivery (biolistics)

The principle of the gene-gun or ballistic mediated delivery system is relatively simple. It utilizes gas pressure to accelerate small (around 1 μm in diameter), heavy (made of gold or tungsten), dispersed particles – "bullets". Accelerated particles work as carriers for different molecules that can be attached to their surface such as DNA, proteins or small molecules like dyes. The relatively high speed and density of particles allow them to penetrate biological tissues to a depth of up to 100 microns, depending on preparation type. This penetration depth is ideal for organotypic brain slices, for instance, in which the normal thickness rarely exceeds 100 microns (Biewenga et al., 1997; O'Brien and Lummis, 2004).

There are two approaches available for particle acceleration. In the classical gene gun that is used for gene delivery and immunization both accelerated particles and the accelerating gas stream reach the transducing tissue (reviewed by Johnston and Tang 1994). The blast wave of accelerated gas may cause tissue damage, thus several approaches were developed to protect biological sample from outflowing compressed gas (O'Brien et al., 2001). Alternative method solving this problem was developed by Shefi and colleagues and is based on the idea that the bullets do not need accelerating gas flow after they left the accelerating channel of the gene gun. Their system is designed in way that the exhausting gas is scavenged before it

reaches the tissue (Shefi et al., 2006). This method minimizes tissue damage caused by gas pressure and improves penetration depth for particles by decreasing the distance between the gene gun accelerating channel and tissue sample.

4.5.3. *In vivo* electroporation

Electroporation is based on a principle that a charged DNA molecule in a form of highly compacted circular plasmid can migrate fast in electrical field created by application of rectangular electrical pulses and cross cellular plasma membrane. The fate of the DNA that entered the cell is unclear; it is also unclear how plasmid is able to cross nuclear envelope to be transcribed (Nishikawa and Huang, 2001; Wells, 2010). It was shown, indeed, that compounds improving the DNA migration into the nucleus also improve efficiency of its expression in non-dividing cell types after *in vivo* electroporation (De la Rossa et al., 2013).

There are two main subtypes of electroporation technique: the one that is done prenatally on rodent uterus on prenatal days 14 – 16 targets efficiently dividing and migrating neuronal precursors and was not reported to target astroglial cells. The plasmid solution in this case is injected into the embryo brain ventricles and targeting of brain region is determined by positioning of electrodes (Saito, 2006; Dixit et al., 2011). Postnatal electroporation that can be done on the first postnatal week or even on adult animals (Boutin et al., 2008; Molotkov et al., 2010; Barnabe-Heider et al., 2008; De la Rossa et al., 2013) allows delivering the DNA to the site of interest by mean of stereotaxic injection and, thus facilitates targeting non-migrating cell types such as cortical astrocytes.

Choosing the correct promoter is a critical step for successful *in vivo* electroporation both for *in utero* and postnatal variants. Thus, CMV enhancer/chicken β actin (CAG) promoter was shown to be the most effective to target variety of cell types and cause high and stable expression for several weeks (Matsuda and Cepko, 2007). Expression in astrocytes and radial glia in neonatal rat cortex can be also targeted by CAG promoter (Molotkov et al., 2010). On the other hand, mouse GFAP 1.7 kb promoter facilitates expression in neuronal cells of neonatal rat cortex when delivered by postnatal electroporation (Molotkov et al., 2011). Some common promoters such as CMV do not cause efficient gene expression when delivered by *in utero* or postnatal electroporation for unknown reason. The plasmid quality and purification method are very critical for the efficiency of transgene expression as well for minimizing inflammation response in the brain that can be caused by bacterial polysaccharides that are common contaminants for bacteria-derived plasmid DNA. Thus, individual testing for each

genetic construct is recommended to be done *in vivo* in order to determine its expression efficiency and specificity.

4.5.4. Viral gene delivery methods to get astrocyte specificity *in vivo*

Specificity or tropism of the virus is determined by interaction between the virus envelope or capsid proteins with receptors on cell surface. Additionally, each viral vector type has its characteristic features determining the mechanism of their entry, intracellular transport and expression (Davidson and Breakefield, 2003). Pseudotyping for lentiviral vectors (Page et al., 1990) or different combinations of capsid proteins for Adeno and Adeno-associated viruses (AAV) (Shevtsova et al., 2005) as well directed molecular engineering of AAVs (Gray et al., 2011) allows changing the tropism to desirable cell types and creation of novel highly specialized vectors. Unfortunately the tropism of a particular viral vector cannot be predicted from results obtained *in vitro* on primary dissociated cells, it requires testing and titration of viral vector by *in vivo* applications (reviewed in Merienne et al., 2013).
The viral vector for *in vivo* application should match several minimal terms: being able to transduce both dividing and non-dividing cells, have ability to cause little or no immune response and be non-cytotoxic, additionally it should have good spreading properties determined by viral particles size and their diffusion in the brain. Currently there are several viral vector systems available that in a grater or in a lesser extent facilitate astrocyte-specific gene delivery *in vivo* (reviewed in Merienne et al., 2013).

4.5.4.1. Adeno-associated viral vectors for astrocytes

Small, 20 nm in diameter, AAV particles have several advantages and limitations: they spread easily by diffusion in the brain and cause small or no immune response. On the other hand, their vector capacity is limited to 4.5-5 kb of genetic material (Concalves, 2005); the space that can accommodate a small promoter, couple of genes and a regulatory element (for instance internal ribosome entry site). AAV vectors are derived from naturally replication deficient viruses which can reproduce themselves only in a presence of adenoviral infection. Transgene expression delivered by AAV is mainly originated from episomal viral DNA (Nakai et al., 2001).
The broad spectrum of natural AAV serotypes, chimeric vectors that combine features of different serotypes, and new AAV-based vectors generated by random mutagenesis with consequent tropism screening, underlie the flexibility of AAV based vector systems and make them promising vectors for clinical gene therapy.

Indeed, some AAVs have natural tropism to astroglial cells but transduction conditions and vector purification method should always be considered. Thus, AAV serotype 2 is able to efficiently target astrocytes *in vitro* but lack this ability *in vivo* (Gong et al., 2004). Systemic injection of AAV9 with CAG promoter results in neuronal and astrocytic expression in an age-dependent manner; in neonatal mice it has neuronal expression pattern (Klein et al., 2008; Foust et al., 2009), but when administered to an adult mice expression was detected mainly in astrocytes (Foust et al., 2009). AAV4 and AAVrh43 in combination with CAG promoter can target astrocytes and ependymal cells in sub ventricular zone *in vivo* (Liu et al., 2005) that is very similar to results obtained by *in vivo* electroporation on neonatal rats with expression of CAG-EGFP cassette (Molotkov et al., unpublished observation). Chimeric AAVs, carrying the serotype 5 capsid proteins in a combination with hGFAP 2.2 kb and gfaABC1D promoters, were shown to be efficient for astrocyte transduction *in vivo* (Drinkut et al., 2012; Xie et al., 2010).
Due to absence of immune response and their small size facilitating diffusion, sometimes even penetration through BBB in case of serotype 9, the AAV based vectors have a good potential to become carriers for future gene therapy approaches.

4.5.4.2. Lentiviral vectors for astrocytes

These relatively big enveloped viral particles of around 60-80 nm in diameter are derived from the human immunodeficiency virus 1 (HIV-1), they facilitate integration of transgene into the host cell genome and are able to escape immune response. The first packaging and efficient expression of the lentiviral vector in a rat brain was done by Naldini and colleagues who have demonstrated efficiency of lentiviral vector in transduction of neuronal cells (Naldini et al., 1996), that made this vector a popular tool for gene delivery into the brain. The vector integration into genome has a potential risk of insertional mutagenesis. In order to decrease risks and made these vectors suitable for gene therapy applications, integration-deficient lentiviral vectors with an integrase mutation were developed (Wanisch and Yanez-Munoz, 2009).
Ability of the lentiviral vectors to be pseudotyped with different viral glycoproteins provides additional flexibility in adjusting vector tropism that should also be considered with *in vivo* virus application. Thus, transduction of primary neuronal and glial co-cultures by vesicular stomatitis virus glycoprotein (VSVG) pseudotyped lentiviral vectors showed preferential expression in astrocytes (Englund et al., 2000). At the same time, application of the same VSVG-pseudotyped vectors demonstrated their preference to infect neuronal cell rather

than astroglia (Naldini et al., 1996; Deglon et al., 2000). Pseudotyping lentiviral vector with Mokola virus or lymhocytic choriomeningitis virus (LCMV) envelope proteins results in preferable transduction of astrocytes *in vivo* (Colin et al., 2009; Cannon et al., 2011). However switching specificity by pseudotyping may result also in lowering virus stability and titer. Thus, both LCMV and Mokola pseudotyped lentiviral particles demonstrate decreased infectivity and lower titer when vector was produced and purified (Miletic et al., 2004; Colin et al., 2009).

An interesting model that took an advantage of GFAP gene up-regulation in reactive astrocytes was developed. Using lentiviral vector with the GFAP promoter it was shown that expression can be up to 8 times higher under pathological conditions when astrocytes are activated compare to undamaged brain (Jakobsson et al., 2004). This model provides evidence that the promoter introduced by mean of lentiviral vector is regulated similarly to the endogenous promoter. It also opens a prospective strategy for using astrocytes as pathology-activated gene therapy targets.

It is worth mentioning an elegant detargeting approach used by Colin and colleagues. In order to obtain nearly absolute astrocyte specificity they introduced an RNA sequence in the vector that suppresses gene expression in neurons by interaction with the neuron-specific micro RNA miR124 (Colin et al., 2009). This approach made possible astrocyte-specific expression from the lentiviral vector.

4.5.4.3. Adenoviral vectors for astrocytes

The surprisingly unpopular for *in vivo* CNS applications adenoviral vector has natural astroglial tropism, is an emerging tool to study astroglial cells and has variety of promoters determining its expression efficiency rather than specificity. Natural specificity of the adenoviral vector serotype 5 is caused by its ability to bind the coxsackie and adenoviral vector receptor (CAR). Those cells that do not express CAR on their surface cannot be targeted by the virus. This explains ability of astrocytes and also some dopaminergic neurons that express CAR to be transduced with adenoviral vectors (Lewis et al., 2010).

The relatively big size (80-100 nm in diameter) of adenoviral particles and an absence of membrane envelope are limiting factors for diffusion of adenoviral particles when injected into the brain *in vivo* (Merienne et al., 2013). This problem of the vector is compensated by the fact that it can be applied locally to transduce a subset of cells within the well-defined spatial territory. Genetic material delivered by the vector is always episomal thus has no risk of insertional mutagenesis and provides transient expression of which productivity can be manipulated by the promoter. In that sense adenoviral vectors represent the most predictable delivery

system in which specificity is determined by natural tropism and in which efficiency can be adjusted by mean of different genetic insulating elements (promoters, enhancers, suppressors etc.) in the transgene.

For twenty years adenoviral vectors have been used to transduce brain cells *in vivo* (Le Gal La Salle et al., 1993; Horellou et al., 1994). A novel packaging system (AdEasy system) that simplified vector production and improved its safety was developed by He and colleagues (He et al., 1998). The system allows rapid generation of replication deficient adenoviral vectors, which made them a popular tool for gene delivery to a variety of cell types. Recently, there have been several studies where successful *in vivo* application of adenoviral vectors, both in the neonatal and in the adult rodent brain, for astrocyte targeting in different brain regions was demonstrated (Gourine et al., 2010; Molotkov et al., *in preparation (study III)*).

Adenoviral vectors are quite immunogenic when applied systemically in a blood flow. Thus, a strong immune response induced by adenoviral vectors was reported in humans when the effect of adenovirus based vaccine was studied (White et al., 2011). On the other hand, such immunogenic properties could make adenovirus a promising vector for glioma therapy (Candolfi et al., 2006).

AIMS

The major aim of the Thesis was to find out whether astrocytes *in vivo* are subjected to morphological changes and investigate if these changes are involved in the functionality of the neuronal network.

The specific aims were to:

1. Develop and validate *in vitro* a molecular genetically encodable tool for suppression of activity-dependent astroglial motility and fine morphological tracing (**study I**).

2. Design and implement the gene delivery system that can facilitate astrocyte-specific gene expression in rodent brain cortex *in vivo* (**studies II and III**).

3. Find out if there are spontaneous morphological changes in astrocytes *in vivo* in the mouse brain and check the effect of their suppression on neuronal activity (**study III**).

METHODS

All materials and methods used in this Thesis work are described in detail in the original publications (I-III). An overview of the methodological approaches and some highlights are provided below.

Image analysis (I, II and III)

For the summary of software solutions and methodological approaches for the image analysis and quantification used in the original studies I, II and III of the Thesis please see the table below.

Software	Developer	Plug-ins used	Main applications	Study
Cell-R	Olympus		Image acquisition, calibration, quantification of Ca^{2+} responses and basic measurements	I
ImagePro 5.1	Media Cybernetics	Custom macros	Application of filters, masking, quantification of filaments	I
Image J	Open source	Image calculator, built-in filter functions	Binarization, background reduction, automatic and manual object tracing and quantification, image subtraction, image skeletonization	I
LSM image browser	Zeiss		Image acquisition, calibration and basic analysis and measurements	I and II
Fiji	Open source	Image stabilizer, 3D drift correction, Maximum entropy threshold, Image calculator, Gaussian filter	Image stabilization, drift correction, automatic thresholding, binarization, filtering, image subtraction and quantification	III
AutoQuant X3	Media Cybernetics		3D non-blind deconvolution	III
Imaris	Bitplane		Surface rendering, surface and volume quantification, masking	III
MatLab	The MathWorks	Custom scripts	BOLD signal analysis; Fourier transform, filtering in frequency domain, quantification and representation of amplitude and phase maps	III

Primary cortical astrocyte cultures (I)

Cortical astrocytes were obtained from 2-3-day-old Wistar rats. After dissection and cell dissociation they were plated on glass bottom MatTek dishes precoated with poly-L-lysine or bovine plasma fibronectin at a density of $5x10^4$ cells per square centimeter. Cells were maintained in culture with high-glucose DMEM supplemented with 10% fetal bovine serum and antibiotics at +37 ^oC, 5% CO_2. Plasmids were transfected into cultured astrocytes 2-3 days after plating using Lipofectamine reagent according to manufacturer recommendations.

Photolysis of caged calcium and live cell imaging (I)

Prior to the Ca^{2+} uncaging experiments cultured cortical astrocytes were loaded with caged 10 μM Ca^{2+} compound o-nitrophenyl (NP)-EGTA AM ester with 4 μM Fura-2 AM and 0.025% Pluronic F-127. The ultraviolet light beam (350-400 nm) flash (200 ms) from 150 W xenon lamp focused by Olympus PlanApo 60x/NA 1.45 oil objective was used for the photolysis of NP-EGTA compound. Single wavelength (380 nm) Fura-2 signal and generated ΔF/F ratio (Grynkiewicz et al., 1985) were used to evaluate effectiveness and the slope of Ca^{2+} responses and to perform calibration for estimation of peak intracellular concentrations of Ca^{2+} caused by uncaging.

For live cell imaging of astrocytes in culture the inverted Olympus Cell-R microscope equipped with Olympus PlanApo 60x/NA 1.45 oil objective and CCD camera was used. For fine morphological tracing the maximum camera resolution 1344x1024 pixels was used. Frames with an exposure time of 500 ms were collected at a frequency of 1 to 0.2 Hz depending on particular experimental procedure. During imaging cells were maintained at +34 ^oC, 5% CO_2 humidified atmosphere.

Postnatal *in vivo* electroporation (II)

Gene delivery using postnatal electroporation technique was done on newborn Wistar rat pups (1-2 days old). 50 to 100 nl of Plasmid solution with a high concentration of endotoxin-free DNA (1.5-3.0 mg/ml) was injected into the brain cortex through the pooled glass needle using stereotaxic manipulator and automatic microinjector. Plasmid injection was followed by the train of 5 rectangular electrical pulses (100-150 V/cm, 50 ms, 1 Hz). All surgical procedures were done under the isoflurane anesthesia (4% for induction, 2% for maintenance). Operated pups were recovered from anesthesia in warm chamber and returned to their mother within 15-20 minutes after operation was completed.

Preparation and *in vivo* application of adenoviral vectors (III)

Serotype 5 adenoviral vectors were prepared according to AdEasy protocol (He et al., 1998). Expression cassettes containing LckGFP and abdProf-1-IRES-LckGFP were cloned into pShuttle vector and then transferred into pAdEasy-1 vector using homologous recombination approach. Viral particles were produced in 293A cell line and were subjected for 3-4 replication rounds in order to obtain high titer viral stock. Viral particles were concentrated and purified by high-speed centrifugation (175.000 g) in CsCl density gradient for 24 hours at +10 ^{o}C and then dialyzed versus sterile 1X PBS pH 7.4 supplemented with 5% glycerol using 20K MWCO membrane at +4 ^{o}C in order to get rid of CsCl in the virus preparation. Each viral vector batch was tested to contain no replication effective particles by reinfection of BHK21 cells. Virus titer was determined on 293A cells 24 hours post infection.
For *in vivo* application the optimal dose for each adenoviral vector was adjusted by injecting serial virus dilutions (5-fold increment) into the adult mouse cortex through the sharped glass needle (Fig. 3A). The optimal topical virus application parameters for transduction of cortical astrocytes were determined and used further as follows: injection rate 1-2 nl/s, injection volume 500 nl, amount of virus in a range of $2*10^4$ to $5*10^4$ infectious units per injection. For all surgical procedures animals were anesthetized with isoflurane or with ketamine/xylazine mixture.

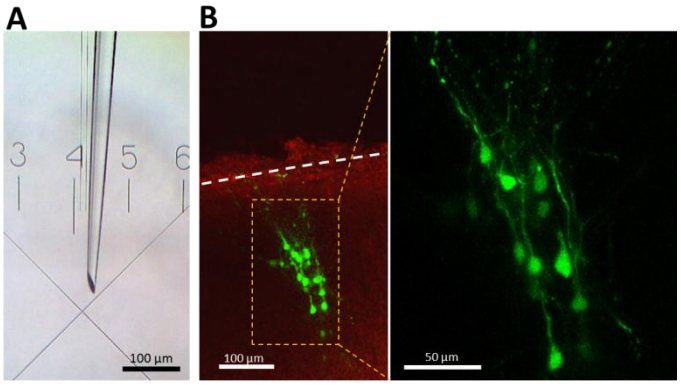

Figure 3. Gene delivery to the rodent brain cortex. Sharped borosilicate glass needle used for topical virus *injections* (A).Neuronal cells in the rat neonatal pup visual cortex expressing GFP under the control of GFAP promter from the plasmid delivered using *in vivo* electroporation technique (B).

In vivo two-photon microscopy through the cranial window (III)

In vivo microscopy was done using Olympus FV1000MPE microscope equipped with Olympus 25X NA 1.05 XLPLN water objective and Mai Tai DeepSee mode locked tunable infrared laser. Prior every *in vivo* microscopy experiment 70.000MW Texas Red – conjugated dextran was injected into the tail vein for fluorescent tracing of cortical vasculature. For both GFP and Texas Red the same

excitation beam of 860 nm was used, emitted light was filtered through 515-560 BP filter and 590-650 BP filter for GFP and Texas Red respectively. Image stacks were collected for astrocytes located at cortical layers 2/3 using the constant voxel dimensions of 0.167x0.167x0.5 microns (XYZ) and 5 minutes time increment. All animals were maintained anesthetized with ketamine/xylazine mixture during *in vivo* microscopy experiments.

Intrinsic BOLD signal optical imaging (III)

Images with a resolution of 1312x1082 pixels from primary visual cortex illuminated with red light (filter 630BP30) were collected using fast monochrome CMOS camera equipped with Nikkor 135 f/2 and Nikkor 50 f/1.2 objectives. Visual stimulus was presented by the vertical 5^{o}-thick moving (0.2111 Hz) bright bar covering 90^{o} and 50^{o} of single eye visual field in horizontal and vertical planes respectively. Frames were collected in a rate of 10 Hz for 6 minutes in order to accumulate BOLD signal in a response to stimulation. During BOLD signal acquisition animals were maintained under mild isoflurane anesthesia (0.8-1.0%) with continuous monitoring of the respiration, heart beat rates and blood oxygenation level.

Experimental animals (I, II and III)

All the procedures were performed in accordance with the guidance for animal care (EU directive 2010/63/EU) and University of Helsinki animal practice. Animal license (ESAVI/2857/04.10.03/2012) was obtained from local authority (ELÄINKOELAUTKUNTA-ELLA). Newborn (1-3 days old) Wistar rat pups (study I and II) and adult (2-4 month old) C56BL mice (study III) were bred in the certified animal facility managed by the University of Helsinki. Animals were maintained in controlled temperature, humidity, constant 12/12 light-dark cycle and provided with *ad libitum* water and food.

RESULTS AND DISCUSSION

1. The role of Profilin-1 in the activity-dependent outgrowth of astrocytic peripheral processes (study I).

1.1 Actin turnover as the major mediator for Ca^{2+}-dependent PAPs motility *in vitro*

Even though actin dependent mechanisms for astrocytic morphological plasticity and for the outgrowth of PAPs was proposed by several studies (Reichenbach et al., 2010; Haber et al., 2006; Lavialle et al., 2011) there was no direct demonstration of actin in PAPs, and also its functional role in PAPs remodeling was not fully proved. On the other hand it is logical for actin to be involved in morphological plasticity of astrocytes and their peripheral processes since actin cytoskeleton remodeling is well known to be a driving force in cell edge motility and filopodia building process (Pollard and Borisy, 2003; Faix and Rottner, 2006). Using co-expression of membrane bound LckGFP (Benediktsson et al., 2005) and Lifeact-RFP (Riedl et al., 2008) in study I we demonstrated directly the presence of the filamentous actin within peripheral processes of cultured astrocytes (Study I, Fig. 1). This finding demonstrates that actin cytoskeleton has the potential to be involved in astrocytic morphological plasticity. The fact that filamentous actin is present within PAPs allowed us to suggest also that PAPs have a filopodia-like nature and their motility is regulated by the same fundamental mechanisms.

Actin remodeling machinery is known to be differentially regulated in a variety of cellular compartments (reviewed by Pollard and Borisy, 2003). Since Profilin-1 is widely accepted as one of the major mediators of actin remodeling especially in filopodia formation and extension (Small, 1995; Witke et al., 2004; Faix and Rottner, 2006; Mattila and Lappalainen, 2008, Nemethova et al., 2008) it can also be involved in regulation of morphological changes in astrocytes and their peripheral processes. We next examined the functional role of Profilin-1 in the maintenance of astrocytic morphology, basal cell edge motility and activity-dependent PAPs outgrowth using overexpression of actin binding deficient form of Profilin-1 (abdProf-1) (Suetsugu et al., 1999) in cultured astrocytes. We found that 5-fold overexpression of abdProf-1 in transfected astrocytes, with regard to the basal Profilin-1 level in surrounding astrocytes, (Study I, Suppl. fig. 1) had no significant effect on the general complexity of cell morphology (Study I, Fig. 2) and basal motility rates of cell edge (Study I, Fig. 3). However, the effect of abdProf-1 overexpression has a significant impact on the activity-dependent

morphological plasticity of PAPs that was induced by Ca^{2+} uncaging in cultured astrocytes (Study I, Fig. 4). Control astrocytes did respond to the Ca^{2+} increase by the extensive PAPs outgrowth that resulted in doubling the PAPs density from 1 to 2 PAPs per 10 μm of cell perimeter 400 second after the Ca^{2+} uncaging was performed. However, astrocytes that express abdProf-1 were not able to increase the PAPs density in a response to Ca^{2+} uncaging; despite they had the same basal level density of PAPs (Study I, Fig. 4G). Since the density of processes in the cell at the certain time point reflects the dynamic equilibrium between filopodia extension and retraction events we can assume that overexpression of abdProf-1 prevented the shift of equilibrium towards the prevalence of PAPs formation over the PAPs retraction or in the other words damped the PAPs outgrowth rate to the basal level silencing the effect of Ca^{2+} stimulation. These findings demonstrate that first – astrocytes are able to form new filopodia-like processes in a response to the increase of intracellular Ca^{2+} and second – that Profilin-1 interaction with actin monomers is critical for the rapid extensive formation of astrocytic processes caused by the increase of intracellular Ca^{2+} but not for the maintenance of static morphology and base level motility of the cell edge.

Profilin-1 binds to actin monomers, catalyzes the ADP-to-ATP exchange and facilitates shuttling of G-actin to the filamentous actin barbed ends promoting its polymerization (Pollard and Borisy, 2003). At the same time both actin and Profilin-1, as participants of the cyclic process of actin treadmilling, are subjected to feedback inhibition caused by monomer deficiency in case of extensive polymerization (Yarmola et al., 2009). Once we had found the damping effect of abdProf-1 overexpression on extensive *de novo* PAPs formation in Ca^{2+} stimulated astrocytes, we asked the question if there is any difference in properties of individual PAPs. Using time-lapse microscopy on astrocytes in culture we measured the kinetics for the appearance and extension of individual PAPs induced by Ca^{2+} uncaging. In addition to quantitative differences described above we found also qualitative differences between PAPs formed by control and abdProf-1 expressing astrocytes. Indeed, the average outgrowth rate for PAPs formed by astrocytes expressing abdProf-1 was almost 2 times lower than in control astrocytes that expressed LckGFP only (1.89 μm/min in abdProf-1 *versus* 3.26 μm/min in control). In addition to decreased outgrowth rates PAPs from astrocytes expressing abdProf-1 had on average significantly decreased maximum length (2.66 μm in abdProf-1 *versus* 4.61 μm in control) (Study I, Fig. 4H, I and J). These observations pointed out the Profilin-1 interaction with actin as a factor affecting the dynamics of activity-dependent PAPs outgrowth, which together with the presence of F-actin in PAPs suggests an actin-dependent mechanism underlying PAPs remodeling.

1.2 The novel tool to suppress activity-dependent PAPs motility

In several recent studies cellular motility has been manipulated by affecting cytoskeleton-regulating signaling cascades on the level of small GTPases. Indeed, it was implemented as a photactivatable form of Rac that allowed control of cell migration (Wu et al., 2009) also as an overexpression of dominant negative form of Rac that was shown to suppress PAPs motility rates in organotypic slices (Nishida and Okabe, 2007). The main drawback for the GTPase-based approaches is caused by divergence of the Rac signaling and the side effects on other motility types like lamellipodia formation and general ability of the cell to migrate rather than to local activity-dependent morphological changes (Pollard and Borisy, 2003). In order to overcome these limitations and affect specifically astrocytic filopodia-like processes in activity-dependent manner, rather than cell migration in general, we utilized the Profilin-1 as a direct effector of actin polymerization which is well known for its role in filopodia building process (Witke, 2004; Faix and Rottner, 2006; Mattila and Lappalainen, 2009) and was demonstrated to be critical especially for microspikes formation (Suetsugu et al., 1998; 1999).
We propose that overexpression of abdProf-1 that was shown to suppress activity dependent outgrowth of PAPs *in vitro* (Study I, Fig. 4) can also be used as a genetically encodable tool to manipulate the morphological plasticity of astrocytes *in vivo* and study the effect of its suppression on neuronal function. However, in order to use the abdProf-1 *in vivo* the proper gene delivery system for astrocyte-specific targeting should be implemented.

2. Gene delivery to a postnatal rodent brain (studies II and III).

2.1 Stereotaxic plasmid microinjection and electroporation on neonatal rat brain (study II)

Gene delivery using electro-mediated nucleic acid transfer such as electroporation or iontophoresis is a powerful alternative to time consuming creation of transgenic animals or approaches based on viral vectors. Although the exact mechanism for nucleic acid entering the cell is not still revealed, electro-mediated nucleic acid transfer is widely used in CNS research (reviewed by De Vry et al., 2010(a)). The principal idea of the method is to inject genetic material such as DNA, RNA or shRNA into the brain and then facilitate its uptake by applying several electrical pulses that create directed electrical field and electro-driving force for charged nucleic acid molecules and also influence properties of cellular membranes. There are two main modifications for this technique; the one that is performed prenatally

by mean of surgical removal of the embryos from the uterus, plasmid injection into the lateral ventricles followed by electroporation (Saito and Nakatsuji, 2001; Tabata and Nakajima, 2001; Saito, 2006; Dixit et al., 2011; Sato et al., 2013) and another one that is performed on the different postnatal stages with a help of stereotaxic manipulator (Barnabe-Heider et al., 2008; Boutin et al., 2008; Borrell, 2010; Molotkov et al., 2010 (study II); 2011; De la Rossa et al., 2013) or without it (Feliciano et al., 2013). Indeed, postnatal electroporation has such advantages as possibility to target spatially restricted region of interest regarding to the stereotaxic coordinates and overcome developmental compensatory effects of exogenous gene transfer. However, it also was limited mainly to the transduction of cells in a sub ventricular zone (SVZ). There are now several recent modifications of the postnatal electroporation technique allowing targeting of cells in brain regions outside the SVZ (Barnabe-Heider et al., 2008; Molotkov et al., 2010 (study II); De al Rossa et al., 2013).

Using plasmid with ubiquitously expressed CAG promoter and EGFP as a reporter gene (Matsuda and Cepko, 2004; 2007) we modified the postnatal electroporation technique in order to apply it to non-SVZ brain regions such as cortical areas. We found that rapid injection (within 10 seconds) of the small volume (50 nl) of the highly concentrated plasmid (3 µg/µl) though the thin glass capillary (outlet diameter 20-30 µm) that was immediately followed by the application of 5 rectangular (50 ms, 1 Hz) electrical pulses in a voltage controlled mode (120 V/cm) via forceps-like electrodes placed directly on the skull surface (Study II, movie) produces a well-defined pattern of EGFP expression in cortex and striatum (Study II, Fig. 1). We also found that the age between 1 and 3 postnatal days is the most suitable for the performance of the described method likely due to relatively low myelin content and not completely formed cranial bones which provide lower electrical resistance and thus the same applied voltage results in generation of higher current that improve transduction efficiency. Developed protocol also implemented application of the electro conductive gel for the best electrical contact between electrodes and skull bones and for the uniform distribution of the electrical field between the electrodes that was shown before to be one of the critical factors that can affect electroporation efficiency (Ivorra et al., 2008).

The method described in study II facilitates spatially restricted expression of genes delivered in a form of plasmid DNA into neonatal brain parenchyma *in vivo* by mean of stereotaxic injection and subsequent electroporation. There was one previous study describing non-ventricular plasmid delivery in postnatal animals, where the targeting of the hippocampal *dentate gyrus* neuronal stem cells was shown on adult mice (Barnabe-Heider et al., 2008). In study II as well as in our following experiments (Molotkov et al., 2011) we met the problem caused by low

transduction efficiency in cortex and hippocampus that is likely a characteristic feature of non-dividing cells in the postnatal brain. The problem could be overcame by use of new-born pups as described in study II or facilitation of DNA uptake by application of complex-forming agents such as trans-cyclodextrane-1,2-diol that provides DNA entry to the nucleus in the non-dividing cell (De la Rossa et al., 2013). It is also possible that recently proposed improvements for the electroporation procedure replacing voltage controlled pulses by current controlled pulses (De Vry et al., 2010(b)) and use of local needle-like electrodes generating higher electrical field density compared to plate electrodes (reviewed by De Vry et al., 2010(a)) may improve postnatal electroporation efficiency.

The spatio-temporal restriction of the transgene expression is one of the main issues considered in genetic manipulation *in vivo*. It provides cell type specificity of expression, minimizing severe compensatory changes caused by prolonged expression during early developmental stages and allows expressing the transgene only in the defined region of interest within the brain. There are several approaches that could be implemented with electroporation techniques in order to get spatio-temporal expression selectivity (reviewed by LoTurco et al., 2009; Huh et al., 2010). The use of cell-type specific promoters is the easiest way to get expression in a certain subpopulations of cells *in vitro* or in case of creation of the transgenic animals, but can meet some limitations when naked DNA is applied *in vivo* by electroporation. Thus, if the expression of EGFP driven by ubiquitous CAG promoter appeared in different cell types (study II, Fig. 1) use of astroglia-specific GFAP promoter delivered into postnatal rat cortex by electroporation resulted in EGFP expression in neuronal cells rather than in astrocytes despite its expression specificity *in vitro* (Molotkov et al., 2011) (Fig. 3B). It is demonstrating the flexibility of GFAP promoter expression pattern which is likely caused by bacterial rather than eukaryotic methylation pattern of the plasmid DNA used for electroporation. This observation is a hinderance to validate transgene expression specificity *in vivo* when it is delivered in a form of naked bacterial plasmid DNA, whilst at the same time it promotes *in vivo* electroporation technique for studying regulatory elements influencing gene expression and effects of their epigenetic modifications such as methylation using synthetic constructs *in vivo*.

2.2 Astrocyte-specific gene delivery using adenoviral vectors (study III).

An intrinsic feature of adenoviruses is their ability to interact with CAR on the cell surface that determines a wide tropism of adenovirus-derived vectors (reviewed by Merienne et al., 2013). In the brain the CAR expression is restricted to astroglial

cells and dopaminergic neurons (Lewis et al., 2010) that proposes adenoviral vectors to be efficient and specific astrocyte transducers when they are applied topically in the brain areas lacking dopaminergic neurons.

Using stereotaxic manipulator we applied topically to the adult mouse visual cortex the adenoviral vector of serotype 5 that expresses the membrane form of GFP as a fluorescent reporter under the control of CAG promoter. We checked *ex vivo* on fixed brain slices and *in vivo* through the cranial window distribution of GFP-expressing cells, expression selectivity, transduction efficiency and expression timeline (Study III, Fig. 1). We found that although adenoviral particles are relatively big (80-100 nm in diameter) they can diffuse 200-500 nm away from the injection site causing efficient transduction in the total volume of 0.3-0.4 mm^3 in mouse cortex. Also single injection of $2*10^4$ to $5*10^4$ viral particles produces an expression pattern occupying all cortical layers with densely packed GFP expressing cells suggesting high transfection rate. Well defined borders of the transduced zone are also suggests that effective expression could be achieved by few viral particle per cell and proving the high infectivity known for adenoviral vectors. Astrocyte labelling using immunostaining with anti-GFAP antibody on frozen brain sections derived from transduced animals showed that all GFP expressing cells were GFAP positive and that there are just a few non-transduced GFAP positive cells in the region. This demonstrates astrocyte specificity and further shows high transduction efficiency for the virus. Finally, we found that the virus can facilitate long term expression which can be used in a period from 1 week up to at least 6 weeks after the injection was done, providing an excellent timeline for most experimental paradigms.

Although adenoviral vectors have been used to induce transgene expression in astrocytes both in organotypic slices (Nishida and Okabe, 2007) and *in vivo* for transduction of astroglia in the brainstem (Gourine et al., 2010) their effectiveness and specificity is under debate (Merienne et al., 2013). Relatively high immunogenic properties of adenovirus in comparison to other widely used vectors such as lenti and adeno-associated viruses as well as assumptions regarding their non-selectivity based on systemic adenovirus application (reviewed by Wirth et al., 2013) stimulated the development of alternative viral vectors for astrocyte-specific genetic manipulations. Indeed, there are at least two viral vectors used *on a par* with adenoviruses that were shown to have high astrocytic expression specificity *in vivo*. It was shown that pseudotyping of lentiviral vectors with MOKOLA envelope protein can enhance their astrocytic tropism that could be further improved by insertion of mir124T neuronal silencer sequence in the vector genome (Colin et al., 2009). Another alternative to facilitate astrocyte-specific targeting is based on the application of the AAV2/5 chimeric vector in combination with astroglia-specific

gfaABC1D promoter (Xie et al., 2010). Since AAV5 is known to have a wide tropism in the brain (Burger et al., 2004; Aschauer et al., 2013) which also varies depending on the developmental stage (Chakrabarty et al., 2013) it is possible to assume that astroglial expression of AAV2/5 is likely caused by the promoter rather than by virus transduction selectivity.

Based on the data we have obtained for adenovirus-mediated astrocyte transduction and transgene expression we can conclude that the unfairly under evaluated adenoviral vectors can be used in basic research as well is in some clinical applications (reviewed by Wirth et al., 2013) as a safe episomal vector causing high levels of transient expression. Naturally, limited virus tropism in the brain allows fine tuning of the expression by use of different promoters without losing cell-type specificity.

3. Motility of cortical astrocytes and neuronal function (study III).

3.1 Astroglia spontaneous morphological changes are Profilin-1 dependent.

In primary cultures cortical astrocytes display both spontaneous and activity-dependent morphological changes (see the study I). PAPs were also demonstrated to be motile at active synaptic terminals in acute brain slices (Hirrlinger et al., 2004) and to be involved in morphological interplay with dendritic spines in organotypic brain slices (Haber et al., 2006; Nishida and Okabe, 2007). At the same time spontaneous as well as activity-dependent morphological plasticity in astrocytes *in vivo* has not been described yet.

Using the adenoviral vector expressing a membrane form of GFP that allows high contrast labeling of PAPs we traced and quantified the rate of astrocytic motility at basal conditions, after induction of microstroke and with the overexpression of abdProf-1 (Study III, Fig. 3). We quantified the net changes of astrocyte peripheral processes and found that under resting conditions they are subjected to continuous relatively uniform morphological changes. This spontaneous motility results in 1.3-1.5 % of astrocyte peripheral volume shift during 5 minutes interval. Our measurements consider the overall motility rates which unfortunately difficult to compare with the data obtained for the linear outgrowth rates or volume changes of individual astrocytic processes near the active synaptic terminals (Hirrlinger et al., 2004) or during synaptogenesis (Haber et al., 2006). Interestingly, we found that overexpression of abdProf-1 caused suppression of spontaneous astrocyte motility

rates by 35-40% with respect to astrocytes expressing LckGFP only and used as a control. This finding demonstrates that similarly to astrocytes in culture (Study I) motility of PAPs *in vivo* is likely driven by actin dependent mechanism that is dependent on actin binding by Profilin-1. Although *in vitro* Profilin-1 overexpression affects exclusively activity-dependent morphological changes of astrocytes and, particularly, rapid PAPs outgrowth induced by Ca^{2+} uncaging, *in vivo* it affects spontaneous motility of astrocytes. There are several key features of *in vivo* astrocyte physiology that can cause different sensitivity to the abdProf-1 overexpression. First, astrocytes *in vivo* have remarkably increased compartmentalization due to their highly ramified structure, small internal PAPs volume limiting diffusion inside the PAP and thus, preventing local compensation effects. Second, increased external signal inputs caused by complex environment and also point-like localization of incoming signals sources, such as active synaptic terminals, that create highly localized gradients of neurotransmitters. Third, it should be considered that astrocytes *in vivo* are under the continuous baseline stimulation caused by background neuronal activity even in the anesthetized brain. Indeed, it would be extremely interesting to check whether spontaneous astrocytic motility differs in awake and anesthetized brain and what are the key mechanisms regulating motility of astrocytes *in vivo*. As a future development and improvement for the current study we propose the use of dual membrane and cytoplasmic fluorescent reporters that would allow more precise tracing of the surface of astrocyte together with its internal volume that would further increase signal to noise ratio for the microscopic investigation of PAPs *in vivo*.

3.2 Structure-functional interplay: the effect of suppressed astrocytic motility on neuronal processing.

Astrocytes were recently shown to affect neuronal processing in several ways. They can control the synaptic activity and basal synaptic transmission (Araque et al., 1999; Perea et al., 2009; Fellin et al., 2009; Navarette and Araque, 2011), affect metabolic coupling between neurons and astroglia (Magistretti, 2006; Pellerin et al., 2007) and participate in local control of blood flow in an activity-dependent manner (Zonta et al., 2003; Mulligan and McVicar, 2004; Takano et al., 2006). At the same time some major aspects of glia-neuronal signaling are still controversial (Fiacco et al., 2007; Petravicz et al., 2008; Agulhon et al., 2008; Agulhon et al., 2010). There are several potential sources for the controversy in the field of neuro-glial research. First, it became evident recently that signaling linking astroglia and neurons is not as simple as was assumed before and can include some alternative

pathways as well as subjected for developmental changes (Sun et al., 2013; Shigetomi et al., 2013(b)) and thus, have to be revised. Second, different results were obtained under different experimental conditions (reviewed by Nedergaard and Verkhratsky, 2012). Additionally some experimental tools cannot be assumed as fully physiological relevant. Thus, Ca^{2+} uncaging differs from receptor mediated Ca^{2+} spike by its amplitude, kinetics and localization providing a good solution for general astrocyte activation but incomparable in physiological sense with glia-neuronal signaling occurring *in vivo* (Wang et al., 2013). In this situation shading the light on the role of astrocytes in neuronal function using physiologically relevant tools *in vivo* is an extremely important issue.

Existing *in vitro* and *ex vivo* data demonstrate that astrocytes are highly motile cells and can respond to a variety of external and internal stimuli by changing their morphology in a short term manner (reviewed by Reichenbach et al., 2010). Particularly it was shown in ultrastructural studies that PAPs and synapses are closely associated (Witcher et al., 2007), and there is a correlation between long-term functional changes in synapses and their coverage by PAPs (Lushnikova et al., 2009). Using computational approaches to simulate changes in synaptic geometry it was predicted that PAP-synapse morphological interplay as well as GluT density in astrocytic compartment of the synapse could be the main determinants for synaptic efficacy and kinetics of postsynaptic neurotransmitter action (Freche et al., 2011) allowing us to assume that astroglial morphological changes could lead to functional changes in synapses. Several studies also demonstrated other possible molecular players involved in astrocytic morphological changes such as Ezrin, mGluRs, AQP4 and Kir4.1 (Lavialle et al., 2011; Nagelhus et al., 2004). Indeed, there is accumulating evidence that PAPs structural changes are driven by actin dependent mechanism (Haber et al., 2006, Nishida and Okabe, 2007; Lavialle et al., 2011).

We used abdProf-1 overexpression from the adenoviral vector, a molecular tool previously demonstrated to moderately suppress spontaneous PAPs motility *in vivo* (see part 3.1 and Study III, Fig. 3). By measuring BOLD signal propagation in response to visual stimulation as the readout of net neuronal activity in the primary visual cortex of adult anesthetized mice we tested whether suppressed astrocytic motility can affect neuronal processing. We found no signs of any distortions in activated cortical areas, magnitude maps or temporal phase maps as recorded from the visual cortices of animals transduced with control adenovirus expressing LckGFP or adenovirus expressing abdProf-1 (Study III, Fig. 4). In order to test whether optical fBOLD signal can be used for detecting functional changes in this experimental paradigm we quantified changes in fBOLD signal caused by laser induced thrombosis of single blood vessels and found that our acquisition and

analysis protocol is suitable for detecting functional changes caused by the microstroke (Study III, Fig. 5). These sets of data indicate that although microstroke caused functional changes in the cortex, the suppression of astrocytic motility by 35-40% (via abdProf-1) had no effect on net neuronal activity and corresponding oxygen metabolism in primary visual cortex of the adult anesthetized mouse.

Despite that there are many reasons for astrocytic motility to be closely related with neuronal activity and metabolism, there are also aspects which can dissociate the link between morphological plasticity of astrocytes and synaptic function.

Astrocytes are the first line to react on anesthesia since they have higher sensitivity for general anesthetics and lower threshold level to block their Ca^{2+} signalization (Schummers et al., 2008; Thrane et al., 2012). Silencing of astroglial Ca^{2+} signaling could also result in increased neuronal firing rates in the anesthetized brain (Thrane et al., 2012) which should cause metabolic changes and affect BOLD signal. Moreover developmental changes in the brain were shown to affect neuro-astrocyte crosstalk (Sun et al., 2013) and particularly caused decreased astrocytic sensitivity to general anesthetics in the developing brain (Hirase et al., 2004). Thus, investigating effect of suppressed astrocytic motility on net neuronal activity and synaptic function in the awake brain could be proposed as the next step to continue this study. It would also be useful to compare the obtained results with results gathered via more drastic PAPs motility suppression and also probe the functional effect of local astrocytic GluT blockade in awake and anesthetized brain. We also propose that the BOLD signal imaging could be accompanied with macro scale Ca^{2+} or voltage sensitive dye imaging in the cortex in order to separate neuronal activity and hemodynamic signal. Further, use of plasticity induction paradigms might reveal some changes caused by the suppression of astrocytic motility in the brain.

CONCLUSIONS

We developed and validated on primary astroglial culture a molecular genetically encodable tool for suppression of activity-dependent PAPs motility and fine morphological tracing of astrocytic morphology (**study I**).

We developed postnatal *in vivo* electroporation technique applicable for the rodent brain cortical areas (**Study II**) but found it to be unreliable for cases when absolute cell-type specificity is a prerequisite. We found out the gene delivery based on adenovirus serotype 5 with the CAG promoter to be a reliable astrocyte-specific gene delivery tool *in vivo* in the rodent brain cortex (**study III**).

Results obtained in this work demonstrate that although astrocytes *in vivo* are subjected for spontaneous morphological changes, suppression of the astroglial motility rates by 35% does not cause detectable changes in functionality of the neuronal network in mouse visual cortex under light isoflurane anesthesia. We did not find the direct link between astrocytic motility and functional performance of the brain under particular conditions (**Study III**).

ACKNOWLEDGEMENTS

Five years of PhD studies was a saturated period of my life. There were asperities as well as a lot of very bright moments. It would be merely impossible for me to complete my thesis project without practical help, mental support and friendship from people around.

I want to thank my supervisor Dr. Leonard Khirug for giving me freedom in project design and selection of experimental approaches. I have learnt a lot while working with you, Leo. Special thanks to my co-supervisor Prof. Eero Castren for giving me possibility to complete the project and write the thesis. It was also a great experience to work in your lab.

Special thanks to members of the thesis advisory board; Prof. Kai Kaila and Prof. Pekka Lappalainen. I appreciate your guidance and your advices during all five years of my PhD. Even though, I did not follow all of your comments and advices, with no doubt they influenced my current outlook.

I am grateful to Dr. Nathalie Rouach and Dr. Sarah Coleman for reviewing my thesis manuscript and for their professional and useful comments on my dissertation.

Special thanks to FGSN coordinator Dr. Katri Wegelius, an excellent organizer and person who care about students, for guiding me on my way to PhD.

I want to thank Maria Anastasina, Svetlana Zobova, Mikhail Kislin, Evgeny Pryazhnikov, Mikhail Paveliev, Stanislav Khirug, Elena Vaschinkina, Julia Kolikova, Pavel Uvarov, Alexey Adamov, Anastasia Ludwig, Mikhail Yuriev, Ramil Afzalov, Aleksandr Surin, Dmytro Toptunov, Eva Zusinaite, Lev Levanov, Alesya Romanovskaya, Andrey Golubtsov, Rashid Giniatullin, Ilya Kirilkin, Natalia Kulesskaya, Ekaterina Mugantseva, Evgeny Kulessky, Vera Shirokova, Pedro Pereira, Ilya Lesnichy and Roman Brulgart. These people helped me in performing scientific work, supported me by their friendship and gave me the will to win.

I want also to thank current and former members of Prof. Kai Kaila and Prof. Eero Castren's labs for their practical help, useful advices and friendship, especially Martin Pushkarjov, Else Tolner, Alexey Yukin, Peter Blaesse, Britta Haas, Merle Kampura, Faraz Ahmad, Nina Karpova and Ramon Guirado.

There are several funding sources which I am very grateful for supporting my research financially: Finnish Graduate School in Neuroscience, Center for International Mobility of Finland, Research Foundation of the University of Helsinki, Sigrid Juselius Foundation and Academy of Finland.

Completion of this work would not be possible without my family members, actually without them I would absolutely another person, and thus this thesis has their significant input as well. I am really thankful to my wife Karina and my daughter Marina for being with me, supporting me and tolerating my not ideal nature. Special thanks to my mom, my dad and my brother for their patience and for making me what I am.

LIST OF REFERENCES

Abbott, N.J., 2005. Dynamics of CNS barriers: evolution, differentiation, and modulation. Cell. Mol. Neurobiol. 25, 5–23.

Abbott, N.J., Rönnbäck, L., Hansson, E., 2006. Astrocyte-endothelial interactions at the blood-brain barrier. Nat. Rev. Neurosci. 7, 41–53.

Afshari, F.T., Kwok, J.C., White, L., Fawcett, J.W., 2010. Schwann cell migration is integrin-dependent and inhibited by astrocyte-produced aggrecan. Glia 58, 857–69.

Agulhon, C., Fiacco, T., McCarthy, K.D., 2010. Hippocampal short- and long-term plasticity are not modulated by astrocyte Ca2+ signaling. Science 327, 1250–4.

Agulhon, C., Petravicz, J., McMullen, A.B., Sweger, E.J., Minton, S.K., Taves, S.R., Casper, K.B., Fiacco, T. a, McCarthy, K.D., 2008. What is the role of astrocyte calcium in neurophysiology? Neuron 59, 932–46.

Aguzzi, A., Barres, B., Bennett, M.L., 2013. Microglia: scapegoat, saboteur, or something else? Science 339, 156–61.

Ahmed, N., Ramjeesingh, M., Wong, S., Varga, A., Garami, E., Bear, C.E., 2000. Chloride channel activity of ClC-2 is modified by the actin cytoskeleton. Biochem. J. 352 Pt 3, 789–94.

Airan, R.D., Thompson, K.R., Fenno, L.E., Bernstein, H., Deisseroth, K., 2009. Temporally precise in vivo control of intracellular signalling. Nature 458, 1025–9.

Akerman, K.E., 1982. Ca2+ transport and cell activation. Med. Biol. 60, 168–82.

Allam, S.L., Ghaderi, V.S., Bouteiller, J.-M.C., Legendre, A., Ambert, N., Greget, R., Bischoff, S., Baudry, M., Berger, T.W., 2012. A computational model to investigate astrocytic glutamate uptake influence on synaptic transmission and neuronal spiking. Front. Comput. Neurosci. 6, 70.

Allaman, I., Bélanger, M., Magistretti, P.J., 2011. Astrocyte-neuron metabolic relationships: for better and for worse. Trends Neurosci. 34, 76–87.

Angulo, M.C., Kozlov, A.S., Charpak, S., Audinat, E., 2004. Glutamate released from glial cells synchronizes neuronal activity in the hippocampus. J. Neurosci. 24, 6920–7.

Araque, A, Parpura, V., Sanzgiri, R.P., Haydon, P.G., 1999. Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci. 22, 208–15.

Armbruster, B.N., Li, X., Pausch, M.H., Herlitze, S., Roth, B.L., 2007. Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proc. Natl. Acad. Sci. U. S. A. 104, 5163–8.

Aroeira, R.I., Sebastião, A.M., Valente, C., 2013. GlyT1 and GlyT2 in brain astrocytes: expression, distribution and function. Brain Struct. Funct.

Arriza, J.L., Fairman, W.A., Amara, S.G., Kavanaugh, P., Wadiche, I., Murdoch, H., 1994. Functional Comparisons of Three Glutamate Cloned from Human Motor Cortex Transporter 14, 5559–5569.

Aschauer, D.F., Kreuz, S., Rumpel, S., 2013. Analysis of transduction efficiency, tropism and axonal transport of AAV serotypes 1, 2, 5, 6, 8 and 9 in the mouse brain. PLoS One 8, e76310.

Asztely, F., Erdemli, G., Kullmann, D.M., 1997. Extrasynaptic glutamate spillover in the hippocampus: dependence on temperature and the role of active glutamate uptake. Neuron 18, 281–93.

Attwell, D., Buchan, A.M., Charpak, S., Lauritzen, M., Macvicar, B., Newman, E., 2010. Glial and neuronal control of brain blood flow. Nature 468, 232–43.

Baba, H., Nakahira, K., Morita, N., Tanaka, F., Akita, H., Ikenaka, K., 1997. GFAP gene expression during development of astrocyte. Dev. Neurosci. 19, 49–57.

Bacskai, B.J., Klunk, W.E., Mathis, C., Hyman, B.T., 2002. Imaging amyloid-beta deposits in vivo. J. Cereb. Blood Flow Metab. 22, 1035–41.

Bal-Price, A., Moneer, Z., Brown, G.C., 2002. Nitric oxide induces rapid, calcium-dependent release of vesicular glutamate and ATP from cultured rat astrocytes. Glia 40, 312–23.

Bandettini, P., 2012. Twenty years of functional MRI: the science and the stories. Neuroimage 62, 575–88.

Barnabé-Heider, F., Meletis, K., Eriksson, M., Bergmann, O., Sabelström, H., Harvey, M.A., Mikkers, H., Frisén, J., 2008. Genetic manipulation of adult mouse neurogenic niches by in vivo electroporation. Nat. Methods 5, 189–96.

Barres, B., 2008. The mystery and magic of glia: a perspective on their roles in health and disease. Neuron 60, 430–40.

Benediktsson, A.M., Schachtele, S.J., Green, S.H., Dailey, M.E., 2005. Ballistic labeling and dynamic imaging of astrocytes in organotypic hippocampal slice cultures 141, 41–53.

Bennett, M.V., Contreras, J.E., Bukauskas, F.F., Sáez, J.C., 2003. New roles for astrocytes: gap junction hemichannels have something to communicate. Trends Neurosci. 26, 610–7.

Bennett, M.V.L., Garré, J.M., Orellana, J., Bukauskas, F.F., Nedergaard, M., Sáez, J.C., 2012. Connexin and pannexin hemichannels in inflammatory responses of glia and neurons. Brain Res. 1487, 3–15.

Bergles, D.E., Jahr, C.E., 1998. Glial contribution to glutamate uptake at Schaffer collateral-commissural synapses in the hippocampus. J. Neurosci. 18, 7709–16.

Bergmann, K., 1857. Notiz über einige Structurverhältnisse des Cerebellum und Rückenmarks. Zeitschrift für rationelle Medizin.

Bernardinelli, Y., Magistretti, P.J., Chatton, J.-Y., 2004. Astrocytes generate Na+-mediated metabolic waves. Proc. Natl. Acad. Sci. U. S. A. 101, 14937–42.

Bernardinelli, Y., Salmon, C., Jones, E. V, Farmer, W.T., Stellwagen, D., Murai, K.K., 2011. Astrocytes display complex and localized calcium responses to single-neuron stimulation in the hippocampus. J. Neurosci. 31, 8905–19.

Berretta, S., 2012. Extracellular matrix abnormalities in schizophrenia. Neuropharmacology 62, 1584–97.

Bessis, A., Echade, C.B., Bernard, D., Roumier, A., 2007. Microglial Control of Neuronal Death and Synaptic Properties 238, 233–238.

Bezzi, P., Gundersen, V., Galbete, J.L., Seifert, G., Steinhäuser, C., Pilati, E., Volterra, A., 2004. Astrocytes contain a vesicular compartment that is competent for regulated exocytosis of glutamate. Nat. Neurosci. 7, 613–20.

Bhattacharjee, A., Kaczmarek, L.K., 2005. For K+ channels, Na+ is the new Ca2+. Trends Neurosci. 28, 422–8.

Biber, K., Laurie, D.J., Berthele, A., Sommer, B., Tölle, T.R., Gebicke-Härter, P.J., van Calker, D., Boddeke, H.W., 1999. Expression and signaling of group I metabotropic glutamate receptors in astrocytes and microglia. J. Neurochem. 72, 1671–80.

Biewenga, J.E., Destrée, O.H., Schrama, L.H., 1997. Plasmid-mediated gene transfer in neurons using the biolistics technique. J. Neurosci. Methods 71, 67–75.

Binder, D.K., Nagelhus, E., Ottersen, O.P., 2012. Aquaporin-4 and epilepsy. Glia 60, 1203–14.

Bittar, P.G., Charnay, Y., Pellerin, L., Bouras, C., Magistretti, P.J., 1996. Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain. J. Cereb. Blood Flow Metab. 16, 1079–89.

Borrell, V., 2010. In vivo gene delivery to the postnatal ferret cerebral cortex by DNA electroporation. J. Neurosci. Methods 186, 186–95.

Boucsein, C., Zacharias, R., Färber, K., Pavlovic, S., Hanisch, U.-K., Kettenmann, H., 2003. Purinergic receptors on microglial cells: functional expression in acute brain slices and modulation of microglial activation in vitro. Eur. J. Neurosci. 17, 2267–76.

Boutin, C., Diestel, S., Desoeuvre, A., Tiveron, M.-C., Cremer, H., 2008. Efficient in vivo electroporation of the postnatal rodent forebrain. PLoS One 3, e1883.

Bowman, C.L., Kimelberg, H.K., 1984. Excitatory amino acids directly depolarize rat brain astrocytes in primary culture. Nature 311, 656–9.

Bowser, D.N., Khakh, B.S., 2004. ATP excites interneurons and astrocytes to increase synaptic inhibition in neuronal networks. J. Neurosci. 24, 8606–20.

Brenner, M., Kisseberth, W.C., Su, Y., Besnard, F., Messing, A., 1994. GFAP promoter directs astrocyte-specific expression in transgenic mice. J. Neurosci. 14, 1030–7.

Bruzzone, R., Giaume, C., 1999. Connexins and information transfer through glia. Adv. Exp. Med. Biol. 468, 321–37.

Burden, S.J., 1998. The formation of neuromuscular synapses. Genes Dev. 12, 133–48.

Burger, C., Gorbatyuk, O.S., Velardo, M.J., Peden, C.S., Williams, P., Zolotukhin, S., Reier, P.J., Mandel, R.J., Muzyczka, N., 2004. Recombinant AAV viral vectors pseudotyped with viral capsids from serotypes 1, 2, and 5 display differential efficiency and cell tropism after delivery to different regions of the central nervous system. Mol. Ther. 10, 302–17.

Bushong, E., Martone, M.E., Jones, Y.Z., Ellisman, M.H., 2002. Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. J. Neurosci. 22, 183–92.

Cahoy, J.D., Emery, B., Kaushal, A., Foo, L.C., Zamanian, J.L., Christopherson, K.S., Xing, Y., Lubischer, J.L., Krieg, P., Krupenko, S., Thompson, W.J., Barres, B., 2008. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J. Neurosci. 28, 264–78.

Candolfi, M., Curtin, J.F., Xiong, W.-D., Kroeger, K.M., Liu, C., Rentsendorj, A., Agadjanian, H., Medina-Kauwe, L., Palmer, D., Ng, P., Lowenstein, P.R., Castro, M.G., 2006. Effective high-capacity gutless adenoviral vectors mediate transgene expression in human glioma cells. Mol. Ther. 14, 371–81.

Cannon, J.R., Sew, T., Montero, L., Burton, E.A., Greenamyre, J.T., 2011. Pseudotype-dependent lentiviral transduction of astrocytes or neurons in the rat substantia nigra. Exp. Neurol. 228, 41–52.

Chakrabarty, P., Rosario, A., Cruz, P., Siemienski, Z., Ceballos-Diaz, C., Crosby, K., Jansen, K., Borchelt, D.R., Kim, J.-Y., Jankowsky, J.L., Golde, T.E., Levites, Y., 2013. Capsid serotype and timing of injection determines AAV transduction in the neonatal mice brain. PLoS One 8, e67680.

Charles, A.C., Merrill, J.E., Dirksen, E.R., Sanderson, M.J., 1991. Intercellular signaling in glial cells: calcium waves and oscillations in response to mechanical stimulation and glutamate. Neuron 6, 983–92.

Chaudhry, F., Lehre, K.P., van Lookeren Campagne, M., Ottersen, O.P., Danbolt, N.C., Storm-Mathisen, J., 1995. Glutamate transporters in glial plasma membranes: highly differentiated localizations revealed by quantitative ultrastructural immunocytochemistry. Neuron 15, 711–20.

Chen, M.J., Kress, B., Han, X., Moll, K., Peng, W., Ji, R.-R., Nedergaard, M., 2012. Astrocytic CX43 hemichannels and gap junctions play a crucial role in development of chronic neuropathic pain following spinal cord injury. Glia 60, 1660–70.

Chen, T.-W., Wardill, T.J., Sun, Y., Pulver, S.R., Renninger, S.L., Baohan, A., Schreiter, E.R., Kerr, R.A., Orger, M.B., Jayaraman, V., Looger, L.L., Svoboda, K., Kim, D.S., 2013. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 499, 295–300.

Chiu, F.C., Goldman, J.E., 1985. Regulation of glial fibrillary acidic protein (GFAP) expression in CNS development and in pathological states. J. Neuroimmunol. 8, 283–92.

Choi, B.H., 1986. Glial fibrillary acidic protein in radial glia of early human fetal cerebrum: a light and electron microscopic immunoperoxidase study. J. Neuropathol. Exp. Neurol. 45, 408–18.

Choi, B.H., Kim, R.C., 1985. Expression of glial fibrillary acidic protein by immature oligodendroglia and its implications. J. Neuroimmunol. 8, 215–35.

Choi, M., Yoon, J., Ku, T., Choi, K., Choi, C., 2011. Label-free optical activation of astrocyte in vivo. J. Biomed. Opt. 16, 075003.

Christopherson, K.S., Ullian, E.M., Stokes, C.C., Mullowney, C.E., Hell, J.W., Agah, A., Lawler, J., Mosher, D.F., Bornstein, P., Barres, B. a, 2005. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. Cell 120, 421–33.

Cingolani, L., Goda, Y., 2008. Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy. Nat. Rev. Neurosci. 9, 344–56.

Coco, S., Calegari, F., Pravettoni, E., Pozzi, D., Taverna, E., Rosa, P., Matteoli, M., Verderio, C., 2003. Storage and release of ATP from astrocytes in culture. J. Biol. Chem. 278, 1354–62.

Colin, A., Faideau, M., Dufour, N., Auregan, G., Hassig, R., Andrieu, T., Brouillet, E., Hantraye, P., Bonvento, G., Déglon, N., 2009. Engineered lentiviral vector targeting astrocytes in vivo. Glia 57, 667–79.

Collingridge, G.L., Isaac, J.T.R., Wang, Y.T., 2004. Receptor trafficking and synaptic plasticity. Nat. Rev. Neurosci. 5, 952–62.

Connor, I.O., Kimelberg, K., 1993. Role of Calcium in Astrocyte of Ions and Amino Acids Volume Regulation Release 13.

Cornell-Bell, A.H., Finkbeiner, S.M., Cooper, M.S., Smith, S.J., 1990. Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. Science 247, 470–3.

Cua, R.C., Lau, L.W., Keough, M.B., Midha, R., Apte, S.S., Yong, V.W., 2013. Overcoming neurite-inhibitory chondroitin sulfate proteoglycans in the astrocyte matrix. Glia 61, 972–84.

Danbolt, N.C., 2001. Glutamate uptake. Prog. Neurobiol. 65, 1–105.

Danbolt, N.C., Storm-Mathisen, J., Kanner, B.I., 1992. An [Na++ K+]coupled L-glutamate transporter purified from rat brain is located in glial cell processes. Neuroscience 51, 295–310.

Daneman, R., Zhou, L., Kebede, A. a, Barres, B., 2010. Pericytes are required for blood-brain barrier integrity during embryogenesis. Nature 468, 562–6.

Dani, J.W., Chernjavsky, A., Smith, S.J., 1992. Neuronal activity triggers calcium waves in hippocampal astrocyte networks. Neuron 8, 429–40.

Davalos, D., Grutzendler, J., Yang, G., Kim, J. V, Zuo, Y., Jung, S., Littman, D.R., Dustin, M.L., Gan, W.-B., 2005. ATP mediates rapid microglial response to local brain injury in vivo. Nat. Neurosci. 8, 752–8.

Davidson, B.L., Breakefield, X.O., 2003. Viral vectors for gene delivery to the nervous system. Nat. Rev. Neurosci. 4, 353–64.

Davson, H., 1976. Review lecture. The blood-brain barrier. J. Physiol. 255, 1–28.

De la Rossa, A., Bellone, C., Golding, B., Vitali, I., Moss, J., Toni, N., Lüscher, C., Jabaudon, D., 2013. In vivo reprogramming of circuit connectivity in postmitotic neocortical neurons. Nat. Neurosci. 16, 193–200.

De Vry, J., Martínez-Martínez, P., Losen, M., Temel, Y., Steckler, T., Steinbusch, H.W.M., De Baets, M.H., Prickaerts, J., 2010a. In vivo electroporation of the central nervous system: a non-viral approach for targeted gene delivery. Prog. Neurobiol. 92, 227–44.

De Vry, J., Martínez-Martínez, P., Losen, M., Bode, G.H., Temel, Y., Steckler, T., Steinbusch, H.W., Baets, M. De, Prickaerts, J., 2010b. Low Current-driven Micro-electroporation Allows Efficient In Vivo Delivery of Nonviral DNA into the Adult Mouse Brain. Mol. Ther.

Déglon, N., Tseng, J.L., Bensadoun, J.C., Zurn, A.D., Arsenijevic, Y., Pereira de Almeida, L., Zufferey, R., Trono, D., Aebischer, P., 2000. Self-inactivating lentiviral vectors with enhanced transgene expression as potential gene transfer system in Parkinson's disease. Hum. Gene Ther. 11, 179–90.

Denk, W., Strickler, J.H., Webb, W.W., 1990. Two-photon laser scanning fluorescence microscopy. Science 248, 73–6.

Derouiche, A, Anlauf, E., Aumann, G., Mühlstädt, B., Lavialle, M., 2002. Anatomical aspects of glia-synapse interaction: the perisynaptic glial sheath consists of a specialized astrocyte compartment. J. Physiol. Paris 96, 177–82.

Derouiche, A, Frotscher, M., 2001. Peripheral astrocyte processes: monitoring by selective immunostaining for the actin-binding ERM proteins. Glia 36, 330–41.

Derouiche, A., Frotscher, M., 1991. Astroglial processes around identified glutamatergic synapses contain glutamine synthetase: evidence for transmitter degradation. Brain Res. 552, 346–50.

Di Castro, M.A., Chuquet, J., Liaudet, N., Bhaukaurally, K., Santello, M., Bouvier, D., Tiret, P., Volterra, A., 2011. Local Ca2+ detection and modulation of synaptic release by astrocytes. Nat. Neurosci. 14, 1276–84.

Dityatev, A., Rusakov, D., 2011. Molecular signals of plasticity at the tetrapartite synapse. Curr. Opin. Neurobiol. 21, 353–9.

Dityatev, A., Schachner, M., 2003. Extracellular matrix molecules and synaptic plasticity. Nat. Rev. Neurosci. 4, 456–68.

Dixit, R., Lu, F., Cantrup, R., Gruenig, N., Langevin, L.M., Kurrasch, D.M., Schuurmans, C., 2011. Efficient gene delivery into multiple CNS territories using in utero electroporation. J. Vis. Exp. 3–9.

Dombeck, D. a, Harvey, C.D., Tian, L., Looger, L.L., Tank, D.W., 2010. Functional imaging of hippocampal place cells at cellular resolution during virtual navigation. Nat. Neurosci. 13, 1433–40.

Dombeck, D. a, Khabbaz, A.N., Collman, F., Adelman, T.L., Tank, D.W., 2007. Imaging large-scale neural activity with cellular resolution in awake, mobile mice. Neuron 56, 43–57.

Dow, K.E., Wang, W., 1998. Cell biology of astrocyte proteoglycans. Cell. Mol. Life Sci. 54, 567–81.

Drew, P.J., Shih, A.Y., Driscoll, J.D., Knutsen, P.M., Blinder, P., Davalos, D., Akassoglou, K., Tsai, P.S., Kleinfeld, D., 2010. Chronic optical access through a polished and reinforced thinned skull. Nat. Methods 7, 981–4.

Drinkut, A., Tereshchenko, Y., Schulz, J.B., Bähr, M., Kügler, S., 2012. Efficient gene therapy for Parkinson's disease using astrocytes as hosts for localized neurotrophic factor delivery. Mol. Ther. 20, 534–43.

Ehlers, M.D., 2005. Synapse formation: astrocytes spout off. Curr. Biol. 15, R134–7.

Elias, L. B., Wang, D.D., Kriegstein, A.R., 2007. Gap junction adhesion is necessary for radial migration in the neocortex. Nature 448, 901–7.

Englund, U., Ericson, C., Rosenblad, C., Mandel, R.J., Trono, D., Wictorin, K., Lundberg, C., 2000. The use of a recombinant lentiviral vector for ex vivo gene transfer into the rat CNS. Neuroreport 11, 3973–7.

Engqvist-Goldstein, A.E.Y., Drubin, D.G., 2003. Actin assembly and endocytosis: from yeast to mammals. Annu. Rev. Cell Dev. Biol. 19, 287–332.

Enkvist, M.O., McCarthy, K.D., 1994. Astroglial gap junction communication is increased by treatment with either glutamate or high K+ concentration. J. Neurochem. 62, 489–95.

Eroglu, C., Allen, N.J., Susman, M.W., O'Rourke, N. a, Park, C.Y., Ozkan, E., Chakraborty, C., Mulinyawe, S.B., Annis, D.S., Huberman, A.D., Green, E.M., Lawler, J., Dolmetsch, R., Garcia, K.C., Smith, S.J., Luo, Z.D., Rosenthal, A., Mosher, D.F., Barres, B., 2009. Gabapentin receptor alpha2delta-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. Cell 139, 380–92.

Eugenin, E. a, Clements, J.E., Zink, M.C., Berman, J.W., 2011. Human immunodeficiency virus infection of human astrocytes disrupts blood-brain barrier integrity by a gap junction-dependent mechanism. J. Neurosci. 31, 9456–65.

Evers, M.R., Salmen, B., Bukalo, O., Rollenhagen, A., Bösl, M.R., Morellini, F., Bartsch, U., Dityatev, A., Schachner, M., 2002. Impairment of L-type Ca2+ channel-dependent forms of hippocampal synaptic plasticity in mice deficient in the extracellular matrix glycoprotein tenascin-C. J. Neurosci. 22, 7177–94.

Faissner, A., Pyka, M., Geissler, M., Sobik, T., Frischknecht, R., Gundelfinger, E.D., Seidenbecher, C., 2010. Contributions of astrocytes to synapse formation and maturation - Potential functions of the perisynaptic extracellular matrix. Brain Res. Rev. 63, 26–38.

Faix, J., Rottner, K., 2006. The making of filopodia. Curr. Opin. Cell Biol. 18, 18–25.

Fallier-Becker, P., Sperveslage, J., Wolburg, H., Noell, S., 2011. The impact of agrin on the formation of orthogonal arrays of particles in cultured astrocytes from wild-type and agrin-null mice. Brain Res. 1367, 2–12.

Fedele, E., Foster, A.C., 1992. [3H]glycine uptake in rat hippocampus: kinetic analysis and autoradiographic localization. Brain Res. 572, 154–63.

Feliciano, D.M., Lafourcade, C.A., Bordey, A., 2013. Neonatal subventricular zone electroporation. J. Vis. Exp.

Fellin, T., Carmignoto, G., 2004. Neurone-to-astrocyte signalling in the brain represents a distinct multifunctional unit. J. Physiol. 559, 3–15.

Fellin, T., Halassa, M.M., Terunuma, M., Succol, F., Takano, H., Frank, M., Moss, S.J., Haydon, P.G., 2009. Endogenous nonneuronal modulators of synaptic transmission control cortical slow oscillations in vivo. Proc. Natl. Acad. Sci. U. S. A. 106, 15037–42.

Fellin, T., Pascual, O., Gobbo, S., Pozzan, T., Haydon, P.G., Carmignoto, G., 2004. Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. Neuron 43, 729–43.

Fellows, L.K., Boutelle, M.G., Fillenz, M., 1993. Physiological stimulation increases nonoxidative glucose metabolism in the brain of the freely moving rat. J. Neurochem. 60, 1258–63.

Fiacco, T., McCarthy, K., 2004. Intracellular astrocyte calcium waves in situ increase the frequency of spontaneous AMPA receptor currents in CA1 pyramidal neurons. J. Neurosci. 24, 722–32.

Fiacco, T., Agulhon, C., Taves, S.R., Petravicz, J., Casper, K.B., Dong, X., Chen, J., McCarthy, K.D., 2007. Selective stimulation of astrocyte calcium in situ does not affect neuronal excitatory synaptic activity. Neuron 54, 611–26.

Fiala, J.C., Kirov, S., Feinberg, M.D., Petrak, L.J., George, P., Goddard, C.A., Harris, K.M., 2003. Timing of neuronal and glial ultrastructure disruption during brain slice preparation and recovery in vitro. J. Comp. Neurol. 465, 90–103.

Fields, R.D., Stevens, B., 2000. ATP: an extracellular signaling molecule between neurons and glia. Trends Neurosci. 23, 625–33.

Figueiredo, M., Lane, S., Tang, F., Liu, B.H., Hewinson, J., Marina, N., Kasymov, V., Souslova, E., Chudakov, D.M., Gourine, A.V., Teschemacher, A.G., Kasparov, S., 2011. Optogenetic experimentation on astrocytes. Exp. Physiol. 96, 40–50.

Filosa, A., Paixão, S., Honsek, S.D., Carmona, M., Becker, L., Feddersen, B., Gaitanos, L., Rudhard, Y., Schoepfer, R., Klopstock, T., Kullander, K., Rose, C.R., Pasquale, E.B., Klein, R., 2009. Neuron-glia communication via EphA4/ephrin-A3 modulates LTP through glial glutamate transport. Nat. Neurosci. 12, 1285–92.

Flusberg, B.A., Nimmerjahn, A., Cocker, E.D., Mukamel, E.A., Barretto, R.P.J., Ko, T.H., Burns, L.D., Jung, J.C., Schnitzer, M.J., 2008. High-speed, miniaturized fluorescence microscopy in freely moving mice. Nat. Methods 5, 935–8.

Fossat, P., Turpin, F.R., Sacchi, S., Dulong, J., Shi, T., Rivet, J.-M., Sweedler, J. V, Pollegioni, L., Millan, M.J., Oliet, S.H.R., Mothet, J.-P., 2012. Glial D-serine gates NMDA receptors at excitatory synapses in prefrontal cortex. Cereb. Cortex 22, 595–606.

Foust, K.D., Nurre, E., Montgomery, C.L., Hernandez, A., Chan, C.M., Kaspar, B.K., 2009. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. Nat. Biotechnol. 27, 59–65.

Freche, D., Lee, C.-Y., Rouach, N., Holcman, D., 2012. Synaptic transmission in neurological disorders dissected by a quantitative approach. Commun. Integr. Biol. 5, 448–52.

Freche, D., Pannasch, U., Rouach, N., Holcman, D., 2011. Synapse geometry and receptor dynamics modulate synaptic strength. PLoS One 6, e25122.

Frischknecht, R., Heine, M., Perrais, D., Seidenbecher, C.I., Choquet, D., Gundelfinger, E.D., 2009. Brain extracellular matrix affects AMPA receptor lateral mobility and short-term synaptic plasticity. Nat. Neurosci. 12, 897–904.

Gähwiler, B.H., 1981. Organotypic monolayer cultures of nervous tissue. J. Neurosci. Methods 4, 329–42.

Gähwiler, B.H., 1984. Slice cultures of cerebellar, hippocampal and hypothalamic tissue. Experientia 40, 235–43.

Gähwiler, B.H., Capogna, M., Debanne, D., McKinney, R., Thompson, S.M., 1997. Organotypic slice cultures: a technique has come of age. Trends Neurosci. 20, 471–7.

Gähwiler, B.H., Thompson, S.M., Muller, D., 2001. Preparation and maintenance of organotypic slice cultures of CNS tissue. Curr. Protoc. Neurosci. Chapter 6, Unit 6.11.

Gallo, V., Ghiani, C., 2000. Glutamate receptors in glia: new cells, new inputs and new functions. Trends Pharmacol. Sci. 21, 252–8.

Gardner, P., 1989. Calcium and T lymphocyte activation. Cell 59, 15–20.

Gautreau, A., Louvard, D., Arpin, M., 2002. ERM proteins and NF2 tumor suppressor: the Yin and Yang of cortical actin organization and cell growth signaling. Curr. Opin. Cell Biol. 14, 104–9.

Geissler, M., Gottschling, C., Aguado, A., Rauch, U., Wetzel, C.H., Hatt, H., Faissner, A., 2013. Primary hippocampal neurons, which lack four crucial extracellular matrix molecules, display abnormalities of synaptic structure and function and severe deficits in perineuronal net formation. J. Neurosci. 33, 7742–55.

Ghosh, K.K., Burns, L.D., Cocker, E.D., Nimmerjahn, A., Ziv, Y., Gamal, A. El, Schnitzer, M.J., 2011. Miniaturized integration of a fluorescence microscope. Nat. Methods 8, 871–8.

Giaume, C., 2010. Astroglial Wiring is Adding Complexity to Neuroglial Networking. Front. Neuroenergetics 2, 1–7.

Giaume, C., Tabernero, A., Medina, J.M., 1997. Metabolic trafficking through astrocytic gap junctions. Glia 21, 114–23.

Giordano, G., Guizzetti, M., Dao, K., Mattison, H., Costa, L.G., 2011. Ethanol impairs muscarinic receptor-induced neuritogenesis in rat hippocampal slices: Role of astrocytes and extracellular matrix proteins. Biochem. Pharmacol. 82, 1792–9.

Gogolla, N., Galimberti, I., DePaola, V., Caroni, P., 2006. Long-term live imaging of neuronal circuits in organotypic hippocampal slice cultures. Nat. Protoc. 1, 1223–6.

Golgi C., 1903. Opera Omnia. Ulrico Hoepli, Milan

Gomes, F.C., Garcia-Abreu, J., Galou, M., Paulin, D., Moura Neto, V., 1999. Neurons induce GFAP gene promoter of cultured astrocytes from transgenic mice. Glia 26, 97–108.

Gonçalves, M., 2005. Adeno-associated virus: from defective virus to effective vector. Virol. J. 2, 43.

Gong, Y., Chen, S., Sonntag, C.F., Sumners, C., Klein, R.L., King, M.A., Hughes, J.A., Meyer, E.M., 2004. Recombinant adeno-associated virus serotype 2 effectively transduces primary rat brain astrocytes and microglia. Brain Res. Brain Res. Protoc. 14, 18–24.

Gordon, G.R.J., Baimoukhametova, D. V, Hewitt, S., Rajapaksha, W., Fisher, T.E., Bains, J.S., 2005. Norepinephrine triggers release of glial ATP to increase postsynaptic efficacy. Nat. Neurosci. 8, 1078–86.

Gordon, G.R.J., Howarth, C., MacVicar, B., 2011. Bidirectional control of arteriole diameter by astrocytes. Exp. Physiol. 96, 393–9.

Gordon, G.R.J., Iremonger, K.J., Kantevari, S., Ellis-Davies, G.C.R., MacVicar, B., Bains, J.S., 2009. Astrocyte-mediated distributed plasticity at hypothalamic glutamate synapses. Neuron 64, 391–403.

Gourine, A. V, Kasymov, V., Marina, N., Tang, F., Figueiredo, M.F., Lane, S., Teschemacher, A.G., Spyer, K.M., Deisseroth, K., Kasparov, S., 2010. Astrocytes control breathing through pH-dependent release of ATP. Science 329, 571–5.

Graeber, M.B., 2010. Changing face of microglia. Science 330, 783–8.

Gray, S.J., Foti, S.B., Schwartz, J.W., Bachaboina, L., Taylor-Blake, B., Coleman, J., Ehlers, M.D., Zylka, M.J., McCown, T.J., Samulski, R.J., 2011. Optimizing promoters for recombinant adeno-associated virus-mediated gene expression in the peripheral and central nervous system using self-complementary vectors. Hum. Gene Ther. 22, 1143–53.

Grewer, C., Gameiro, A., Zhang, Z., Tao, Z., Braams, S., Rauen, T., 2008. Glutamate forward and reverse transport: from molecular mechanism to transporter-mediated release after ischemia. IUBMB Life 60, 609–19.

Griffiths, I., Klugmann, M., Anderson, T., Thomson, C., Vouyiouklis, D., Nave, K., 1998. Current concepts of PLP and its role in the nervous system. Microsc. Res. Tech. 41, 344–58.

Gris, P., Tighe, A., Levin, D., Sharma, R., Brown, A., 2007. Transcriptional Regulation of Scar Gene Expression in Primary Astrocytes 1155, 1145–1155.

Grosche, J., Kettenmann, H., Reichenbach, A., 2002. Bergmann glial cells form distinct morphological structures to interact with cerebellar neurons. J. Neurosci. Res. 68, 138–49.

Grosche, J., Matyash, V., Möller, T., Verkhratsky, A., Reichenbach, A., Kettenmann, H., 1999. Microdomains for neuron-glia interaction: parallel fiber signaling to Bergmann glial cells. Nat. Neurosci. 2, 139–43.

Grynkiewicz, G., Poenie, M., Tsien, R.Y., 1985. A new generation of Ca2+ indicators with greatly improved fluorescence properties. J. Biol. Chem. 260, 3440–50.

Haber, M., Zhou, L., Murai, K.K., 2006. Cooperative astrocyte and dendritic spine dynamics at hippocampal excitatory synapses. J. Neurosci. 26, 8881–91.

Halassa, M.M., Fellin, T., Takano, H., Dong, J.-H., Haydon, P.G., 2007. Synaptic islands defined by the territory of a single astrocyte. J. Neurosci. 27, 6473–7.

Hamilton, N.B., Attwell, D., 2010. Do astrocytes really exocytose neurotransmitters? Nat. Rev. Neurosci. 11, 227–38.

Harris, A.L., 2001. Emerging issues of connexin channels: biophysics fills the gap. Q. Rev. Biophys. 34, 325–472.

Harris, A.L., 2007. Connexin channel permeability to cytoplasmic molecules. Prog. Biophys. Mol. Biol. 94, 120–43.

Harvey, C.D., Collman, F., Dombeck, D.A., Tank, D.W., 2009. Intracellular dynamics of hippocampal place cells during virtual navigation. Nature 461, 941–6.

Hatton, G.I., 1997. Function-related plasticity in hypothalamus. Annu. Rev. Neurosci. 20, 375–97.

Hawkins, B.T., Gu, Y.-H., Izawa, Y., Del Zoppo, G.J., 2013. Disruption of dystroglycan-laminin interactions modulates water uptake by astrocytes. Brain Res. 1503, 89–96.

Hawrylak, N., Fleming, J.C., Salm, A. K., 1998. Dehydration and rehydration selectively and reversibly alter glial fibrillary acidic protein immunoreactivity in the rat supraoptic nucleus and subjacent glial limitans. Glia 22, 260–71.

Haydon, P.G., 2001. GLIA: listening and talking to the synapse. Nat. Rev. Neurosci. 2, 185–93.

Haydon, P.G., Carmignoto, G., 2006. Astrocyte control of synaptic transmission and neurovascular coupling. Physiol. Rev. 86, 1009–31.

He, T.C., Zhou, S., da Costa, L.T., Yu, J., Kinzler, K.W., Vogelstein, B., 1998. A simplified system for generating recombinant adenoviruses. Proc. Natl. Acad. Sci. U. S. A. 95, 2509–14.

Heck, N., Garwood, J., Schütte, K., Fawcett, J., Faissner, A., 2003. Astrocytes in culture express fibrillar collagen. Glia 41, 382–92.

Helmchen, F., Denk, W., 2005. Deep tissue two-photon microscopy. Nat. Methods 2, 932–40.

Helmchen, F., Fee, M.S., Tank, D.W., Denk, W., 2001. A miniature head-mounted two-photon microscope. high-resolution brain imaging in freely moving animals. Neuron 31, 903–12.

Henneberger, C., Bard, L., King, C., Jennings, A., Rusakov, D., 2013. NMDA receptor activation: two targets for two co-agonists. Neurochem. Res. 38, 1156–62.

Henneberger, C., Papouin, T., Oliet, S.H.R., Rusakov, D., 2010. Long-term potentiation depends on release of D-serine from astrocytes. Nature 463, 232–6.

Hirano, S., Yonezawa, T., Hasegawa, H., Hattori, S., Greenhill, N.S., Davis, P.F., Sage, E.H., Ninomiya, Y., 2004. Astrocytes express type VIII collagen during the repair process of brain cold injury. Biochem. Biophys. Res. Commun. 317, 437–43.

Hirase, H., Qian, L., Barthó, P., Buzsáki, G., 2004. Calcium dynamics of cortical astrocytic networks in vivo. PLoS Biol. 2, E96.

Hirota, S., Liu, Q., Lee, H.S., Hossain, M.G., Lacy-Hulbert, A., McCarty, J.H., 2011. The astrocyte-expressed integrin αvβ8 governs blood vessel sprouting in the developing retina. Development 138, 5157–66.

Hirrlinger, J., Hülsmann, S., Kirchhoff, F., 2004. Astroglial processes show spontaneous motility at active synaptic terminals in situ. Eur. J. Neurosci. 20, 2235–9.

Hirrlinger, J., Scheller, A., Hirrlinger, P.G., Kellert, B., Tang, W., Wehr, M.C., Goebbels, S., Reichenbach, A., Sprengel, R., Rossner, M.J., Kirchhoff, F., 2009. Split-cre complementation indicates coincident activity of different genes in vivo. PLoS One 4, e4286.

Hirrlinger, P.G., Scheller, A., Braun, C., Hirrlinger, J., Kirchhoff, F., 2006. Temporal control of gene recombination in astrocytes by transgenic expression of the tamoxifen-inducible DNA recombinase variant CreERT2. Glia 54, 11–20.

Hofer, S.B., Mrsic-Flogel, T.D., Bonhoeffer, T., Hübener, M., 2009. Experience leaves a lasting structural trace in cortical circuits. Nature 457, 313–7.

Holen, T., 2011. The ultrastructure of lamellar stack astrocytes. Glia 59, 1075–83.

Holopainen, I., Kontro, P., 1989. Uptake and release of glycine in cerebellar granule cells and astrocytes in primary culture: potassium-stimulated release from granule cells is calcium-dependent. J. Neurosci. Res. 24, 374–83.

Holtmaat, A., Bonhoeffer, T., Chow, D.K., Chuckowree, J., De Paola, V., Hofer, S.B., Hübener, M., Keck, T., Knott, G., Lee, W.-C. a, Mostany, R., Mrsic-Flogel, T.D., Nedivi, E., Portera-Cailliau, C., Svoboda, K., Trachtenberg, J.T., Wilbrecht, L., 2009. Long-term, high-resolution imaging in the mouse neocortex through a chronic cranial window. Nat. Protoc. 4, 1128–44.

Hoogland, T.M., Kuhn, B., Göbel, W., Huang, W., Nakai, J., Helmchen, F., Flint, J., Wang, S.S.-H., 2009. Radially expanding transglial calcium waves in the intact cerebellum. Proc. Natl. Acad. Sci. U. S. A. 106, 3496–501.

Horellou, P., Vigne, E., Castel, M.N., Barnéoud, P., Colin, P., Perricaudet, M., Delaère, P., Mallet, J., 1994. Direct intracerebral gene transfer of an adenoviral vector expressing tyrosine hydroxylase in a rat model of Parkinson's disease. Neuroreport 6, 49–53.

Houades, V., Koulakoff, A., Ezan, P., Seif, I., Giaume, C., 2008. Gap junction-mediated astrocytic networks in the mouse barrel cortex. J. Neurosci. 28, 5207–17.

Housley, G.D., Bringmann, A., Reichenbach, A., 2009. Purinergic signaling in special senses. Trends Neurosci. 32, 128–41.

Hsieh, H.-L., Wu, C.-Y., Yang, C.-M., 2008. Bradykinin induces matrix metalloproteinase-9 expression and cell migration through a PKC-delta-dependent ERK/Elk-1 pathway in astrocytes. Glia 56, 619–32.

Hsu, J.-Y.C., Bourguignon, L.Y.W., Adams, C.M., Peyrollier, K., Zhang, H., Fandel, T., Cun, C.L., Werb, Z., Noble-Haeusslein, L.J., 2008. Matrix metalloproteinase-9 facilitates glial scar formation in the injured spinal cord. J. Neurosci. 28, 13467–77.

Huber, J.D., Egleton, R.D., Davis, T.P., 2001. Molecular physiology and pathophysiology of tight junctions in the blood-brain barrier. Trends Neurosci. 24, 719–25.

Huh, Y., Oh, M.S., Leblanc, P., Kim, K.-S., 2010. Gene transfer in the nervous system and implications for transsynaptic neuronal tracing. Expert Opin. Biol. Ther. 10, 763–72.

Hyder, F., 2009. Dynamic imaging of brain function. Methods Mol. Biol. 489, 3–21.

Illes, P., Verkhratsky, A., Burnstock, G., Franke, H., 2012. P2X receptors and their roles in astroglia in the central and peripheral nervous system. Neuroscientist 18, 422–38.

Ivorra, A., Al-Sakere, B., Rubinsky, B., Mir, L.M., 2008. Use of conductive gels for electric field homogenization increases the antitumor efficacy of electroporation therapies. Phys. Med. Biol. 53, 6605–18.

Jakobsson, J., Georgievska, B., Ericson, C., Lundberg, C., 2004. Lesion-dependent regulation of transgene expression in the rat brain using a human glial fibrillary acidic protein-lentiviral vector. Eur. J. Neurosci. 19, 761–5.

Jeang, K.T., Cho, M.S., Hayward, G.S., 1984. Abundant constitutive expression of the immediate-early 94K protein from cytomegalovirus (Colburn) in a DNA-transfected mouse cell line. Mol. Cell. Biol. 4, 2214–23.

Jensen, A.M., Chiu, S.Y., 1990. Fluorescence measurement of changes in intracellular calcium induced by excitatory amino acids in cultured cortical astrocytes. J. Neurosci. 10, 1165–75.

Johnston, G.A., Kennedy, S.M., Twitchin, B., 1979. Action of the neurotoxin kainic acid on high affinity uptake of L-glutamic acid in rat brain slices. J. Neurochem. 32, 121–7.

Johnston, S.A., Tang, D.C., 1994. Gene gun transfection of animal cells and genetic immunization. Methods Cell Biol. 43 Pt A, 353–65.

Jourdain, P., Bergersen, L.H., Bhaukaurally, K., Bezzi, P., Santello, M., Domercq, M., Matute, C., Tonello, F., Gundersen, V., Volterra, A., 2007. Glutamate exocytosis from astrocytes controls synaptic strength. Nat. Neurosci. 10, 331–9.

Kafitz, K.W., Meier, S.D., Stephan, J., Rose, C.R., 2008. Developmental profile and properties of sulforhodamine 101--Labeled glial cells in acute brain slices of rat hippocampus. J. Neurosci. Methods 169, 84–92.

Kang, J., Jiang, L., Goldman, S., Nedergaard, M., 1998. Astrocyte-mediated potentiation of inhibitory synaptic transmission. Nat. Neurosci. 1, 683–92.

Kang, J., Kang, N., Lovatt, D., Torres, A., Zhao, Z., Lin, J., Nedergaard, M., 2008. Connexin 43 hemichannels are permeable to ATP. J. Neurosci. 28, 4702–11.

Kang, J., Kang, N., Yu, Y., Zhang, J., Petersen, N., Tian, G.F., Nedergaard, M., 2010. Sulforhodamine 101 induces long-term potentiation of intrinsic excitability and synaptic efficacy in hippocampal CA1 pyramidal neurons. Neuroscience 169, 1601–9.

Kang, N., Peng, H., Yu, Y., Stanton, P.K., Guilarte, T.R., Kang, J., 2013. Astrocytes release D-serine by a large vesicle. Neuroscience 240, 243–57.

Karpova, N.N., Pickenhagen, A., Lindholm, J., Tiraboschi, E., Kulesskaya, N., Agústsdóttir, A., Antila, H., Popova, D., Akamine, Y., Bahi, A., Sullivan, R., Hen, R., Drew, L.J., Castrén, E., 2011. Fear erasure in mice requires synergy between antidepressant drugs and extinction training. Science 334, 1731–4.

Kartvelishvily, E., Shleper, M., Balan, L., Dumin, E., Wolosker, H., 2006. Neuron-derived D-serine release provides a novel means to activate N-methyl-D-aspartate receptors. J. Biol. Chem. 281, 14151–62.

Karus, M., Denecke, B., ffrench-Constant, C., Wiese, S., Faissner, A., 2011. The extracellular matrix molecule tenascin C modulates expression levels and territories of key patterning genes during spinal cord astrocyte specification. Development 138, 5321–31.

Kasischke, K., Vishwasrao, H.D., Fisher, P.J., Zipfel, W.R., Webb, W.W., 2004. Neural activity triggers neuronal oxidative metabolism followed by astrocytic glycolysis. Science 305, 99–103.

Katakowski, M., Buller, B., Wang, X., Rogers, T., Chopp, M., 2010. Functional microRNA is transferred between glioma cells. Cancer Res. 70, 8259–63.

Kato, F., Kawamura, M., Shigetomi, E., Tanaka, J., Inoue, K., 2004. Forum Mini review ATP- and Adenosine-Mediated Signaling in the Central Nervous System : Synaptic Purinoceptors : the Stage for ATP to Play Its " Dual-Role " 111, 107–111.

Kawabata, I., Umeda, T., Yamamoto, K., Okabe, S., 2004. Electroporation-mediated gene transfer system applied to cultured CNS neurons. Neuroreport 15, 971–5.

Kettenmann, H., Backus, K.H., Schachner, M., 1984. Aspartate, glutamate and gamma-aminobutyric acid depolarize cultured astrocytes. Neurosci. Lett. 52, 25–9.

Kettenmann, H., Hanisch, U., Noda, M., Verkhratsky, A., 2011. Physiology of Microglia 461–553.

Kettenmann, H., Kirchhoff, F., Verkhratsky, A., 2013. Microglia: new roles for the synaptic stripper. Neuron 77, 10–8.

Kettenmann, H., Ransom, B., 1995. Neuroglia. Oxford University Press.

Kirischuk, S., Parpura, V., Verkhratsky, A., 2012. Sodium dynamics: another key to astroglial excitability? Trends Neurosci. 35, 497–506.

Kirov, S., Petrak, L.J., Fiala, J.C., Harris, K.M., 2004. Dendritic spines disappear with chilling but proliferate excessively upon rewarming of mature hippocampus. Neuroscience 127, 69–80.

Kirov, S., Sorra, K.E., Harris, K.M., 1999. Slices have more synapses than perfusion-fixed hippocampus from both young and mature rats. J. Neurosci. 19, 2876–86.

Kitamura, K., Judkewitz, B., Kano, M., Denk, W., Häusser, M., 2008. Targeted patch-clamp recordings and single-cell electroporation of unlabeled neurons in vivo. Nat. Methods 5, 61–7.

Klein, R.L., Dayton, R.D., Tatom, J.B., Henderson, K.M., Henning, P.P., 2008. AAV8, 9, Rh10, Rh43 vector gene transfer in the rat brain: effects of serotype, promoter and purification method. Mol. Ther. 16, 89–96.

Kochlamazashvili, G., Henneberger, C., Bukalo, O., Dvoretskova, E., Senkov, O., Lievens, P.M.-J., Westenbroek, R., Engel, A.K., Catterall, W., Rusakov, D., Schachner, M., Dityatev, A., 2010. The extracellular matrix molecule hyaluronic acid regulates hippocampal synaptic plasticity by modulating postsynaptic L-type Ca(2+) channels. Neuron 67, 116–28.

Koehler, R.C., Roman, R.J., Harder, D.R., 2009. Astrocytes and the regulation of cerebral blood flow. Trends Neurosci. 32, 160–9.

Kofuji, P., Newman, E., 2004. Potassium buffering in the central nervous system. Neuroscience 129, 1045–56.

Koizumi, S., Fujishita, K., Tsuda, M., Shigemoto-Mogami, Y., Inoue, K., 2003. Dynamic inhibition of excitatory synaptic transmission by astrocyte-derived ATP in hippocampal cultures. Proc. Natl. Acad. Sci. U. S. A. 100, 11023–8.

Koulakoff, A., Mei, X., Orellana, J., Sáez, J.C., Giaume, C., 2012. Glial connexin expression and function in the context of Alzheimer's disease. Biochim. Biophys. Acta 1818, 2048–57.

Koyama, Y., Baba, A., Iwata, H., 1991. L-glutamate-induced swelling of cultured astrocytes is dependent on extracellular Ca2+. Neurosci. Lett. 122, 210–2.

Kuchibhotla, K., Lattarulo, C.R., Hyman, B.T., Bacskai, B.J., 2009. Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice. Science 323, 1211–5.

Kuffler, S.W., Nicholls, J.G., Orkand, R.K., 1966. Physiological properties of glial cells in the central nervous system of amphibia. J. Neurophysiol. 29, 768–87.

Kullmann, D.M., Asztely, F., 1998. Extrasynaptic glutamate spillover in the hippocampus: evidence and implications. Trends Neurosci. 21, 8–14.

Langle, S.L., Poulain, D., Theodosis, D.T., 2003. Induction of rapid, activity-dependent neuronal-glial remodelling in the adult rat hypothalamus in vitro. Eur. J. Neurosci. 18, 206–214.

Lascola, C.D., Nelson, D.J., Kraig, R.P., 1998. Cytoskeletal actin gates a Cl- channel in neocortical astrocytes. J. Neurosci. 18, 1679–92.

Lavialle, M., Aumann, G., Anlauf, E., Pröls, F., Arpin, M., Derouiche, A., 2011. Structural plasticity of perisynaptic astrocyte processes involves ezrin and metabotropic glutamate receptors. Proc. Natl. Acad. Sci. U. S. A. 108, 12915–9.

Le Gal La Salle, G., Robert, J.J., Berrard, S., Ridoux, V., Stratford-Perricaudet, L.D., Perricaudet, M., Mallet, J., 1993. An adenovirus vector for gene transfer into neurons and glia in the brain. Science 259, 988–90.

Lee, S.-W., Kim, W.J., Choi, Y.K., Song, H.S., Son, M.J., Gelman, I.H., Kim, Y.-J., Kim, K.-W., 2003. SSeCKS regulates angiogenesis and tight junction formation in blood-brain barrier. Nat. Med. 9, 900–6.

Lee, Y., Su, M., Messing, A., Brenner, M., 2006. Astrocyte heterogeneity revealed by expression of a GFAP-LacZ transgene. Glia 53, 677–87.

Lehre, K.P., Rusakov, D., 2002. Asymmetry of glia near central synapses favors presynaptically directed glutamate escape. Biophys. J. 83, 125–34.

Levy, L.M., Warr, O., Attwell, D., 1998. Stoichiometry of the glial glutamate transporter GLT-1 expressed inducibly in a Chinese hamster ovary cell line selected for low endogenous Na+-dependent glutamate uptake. J. Neurosci. 18, 9620–8.

Lewis, T.B., Glasgow, J.N., Glandon, A.M., Curiel, D.T., Standaert, D.G., 2010. Transduction of brain dopamine neurons by adenoviral vectors is modulated by CAR expression: rationale for tropism modified vectors in PD gene therapy. PLoS One 5.

Li, D., Hérault, K., Silm, K., Evrard, A., Wojcik, S., Oheim, M., Herzog, E., Ropert, N., 2013. Lack of evidence for vesicular glutamate transporter expression in mouse astrocytes. J. Neurosci. 33, 4434–55.

Li, L., Welser, J. V, Dore-Duffy, P., del Zoppo, G.J., Lamanna, J.C., Milner, R., 2010. In the hypoxic central nervous system, endothelial cell proliferation is followed by astrocyte activation, proliferation, and increased expression of the alpha 6 beta 4 integrin and dystroglycan. Glia 58, 1157–67.

Liang, S.-L., Carlson, G.C., Coulter, D., 2006. Dynamic regulation of synaptic GABA release by the glutamate-glutamine cycle in hippocampal area CA1. J. Neurosci. 26, 8537–48.

Lin, J.H.C., Takano, T., Cotrina, M.L., Arcuino, G., Kang, J., Liu, S., Gao, Q., Jiang, L., Li, F., Lichtenberg-Frate, H., Haubrich, S., Willecke, K., Goldman, S., Nedergaard, M., 2002. Connexin 43 enhances the adhesivity and mediates the invasion of malignant glioma cells. J. Neurosci. 22, 4302–11.

Liu, G., Martins, I., Wemmie, J.A., Chiorini, J.A., Davidson, B.L., 2005. Functional correction of CNS phenotypes in a lysosomal storage disease model using adeno-associated virus type 4 vectors. J. Neurosci. 25, 9321–7.

Liu, T., Sun, L., Xiong, Y., Shang, S., Guo, N., Teng, S., Wang, Y., Liu, B., Wang, C., Wang, L., Zheng, L., Zhang, C.X., Han, W., Zhou, Z., 2011. Calcium triggers exocytosis from two types of organelles in a single astrocyte. J. Neurosci. 31, 10593–601.

Livet, J., Weissman, T., Kang, H., Draft, R.W., Lu, J., Bennis, R., Sanes, J.R., Lichtman, J.W., 2007. Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. Nature 450, 56–62.

LoTurco, J., Manent, J.-B., Sidiqi, F., 2009. New and improved tools for in utero electroporation studies of developing cerebral cortex. Cereb. Cortex 19 Suppl 1, i120–5.

Lovatt, D., Sonnewald, U., Waagepetersen, H.S., Schousboe, A., He, W., Lin, J.H.-C., Han, X., Takano, T., Wang, S., Sim, F.J., Goldman, S., Nedergaard, M., 2007. The transcriptome and metabolic gene signature of protoplasmic astrocytes in the adult murine cortex. J. Neurosci. 27, 12255–66.

Lu, D.-Y., Yu, W.-H., Yeh, W.-L., Tang, C.-H., Leung, Y.-M., Wong, K.-L., Chen, Y.-F., Lai, C.-H., Fu, W.-M., 2009. Hypoxia-induced matrix metalloproteinase-13 expression in astrocytes enhances permeability of brain endothelial cells. J. Cell. Physiol. 220, 163–73.

Lushnikova, I., Skibo, G., Muller, D., Nikonenko, I., 2009. Synaptic potentiation induces increased glial coverage of excitatory synapses in CA1 hippocampus. Hippocampus 19, 753–62.

Lutz, S.E., Zhao, Y., Gulinello, M., Lee, S.C., Raine, C.S., Brosnan, C.F., 2009. Deletion of astrocyte connexins 43 and 30 leads to a dysmyelinating phenotype and hippocampal CA1 vacuolation. J. Neurosci. 29, 7743–52.

MacAulay, N., Gether, U., Klaerke, D.A., Zeuthen, T., 2001. Water transport by the human Na+-coupled glutamate cotransporter expressed in Xenopus oocytes. J. Physiol. 530, 367–78.

MacVicar, B., Thompson, R.J., 2010. Non-junction functions of pannexin-1 channels. Trends Neurosci. 33, 93–102.

Magistretti, P.J., 2006. Neuron-glia metabolic coupling and plasticity. J. Exp. Biol. 209, 2304–11.

Magistretti, P.J., Pellerin, L., 1999. Cellular mechanisms of brain energy metabolism and their relevance to functional brain imaging. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 354, 1155–63.

Malatesta, P., Hack, M., Hartfuss, E., Kettenmann, H., Klinkert, W., Kirchhoff, F., Götz, M., 2003. Neuronal or glial progeny: regional differences in radial glia fate. Neuron 37, 751–64.

Malinow, R., Malenka, R.C., 2002. AMPA receptor trafficking and synaptic plasticity. Annu. Rev. Neurosci. 25, 103–26.

Marchaland, J., Calì, C., Voglmaier, S.M., Li, H., Regazzi, R., Edwards, R.H., Bezzi, P., 2008. Fast subplasma membrane Ca2+ transients control exo-endocytosis of synaptic-like microvesicles in astrocytes. J. Neurosci. 28, 9122–32.

Marrero, H., Orkand, R.K., 1996. Nerve impulses increase glial intercellular permeability. Glia 16, 285–9.

Martin, D.L., 1992. Synthesis and release of neuroactive substances by glial cells. Glia 5, 81–94.

Martinowich, K., Mendelowitz, D., Williams, S., Sutherland M., 2001. EAAT2 overexpression plays a neuroprotective role in models of temporal lobe epilepsy. SfN Abstracts. 27(2): 1584.

Matsuda, T., Cepko, C.L., 2004. Electroporation and RNA interference in the rodent retina in vivo and in vitro. Proc. Natl. Acad. Sci. U. S. A. 101, 16–22.

Matsuda, T., Cepko, C.L., 2007. Controlled expression of transgenes introduced by in vivo electroporation. Proc. Natl. Acad. Sci. U. S. A. 104, 1027–32.

Mattila, P.K., Lappalainen, P., 2008. Filopodia: molecular architecture and cellular functions. Nat. Rev. Mol. Cell Biol. 9, 446–54.

Mauch, D.H., Nägler, K., Schumacher, S., Göritz, C., Müller, E.C., Otto, A., Pfrieger, F.W., 2001. CNS synaptogenesis promoted by glia-derived cholesterol. Science 294, 1354–7.

Maya Vetencourt, J.F., Sale, A., Viegi, A., Baroncelli, L., De Pasquale, R., O'Leary, O.F., Castrén, E., Maffei, L., 2008. The antidepressant fluoxetine restores plasticity in the adult visual cortex. Science 320, 385–8.

Mckenna, M.C., 2007. Review The Glutamate-Glutamine Cycle Is Not Stoichiometric : Fates of Glutamate in Brain 3358, 3347–3358.

Merienne, N., Le Douce, J., Faivre, E., Déglon, N., Bonvento, G., 2013. Efficient gene delivery and selective transduction of astrocytes in the mammalian brain using viral vectors. Front. Cell. Neurosci. 7, 106.

Messing, A., Brenner, M., 2003. GFAP: functional implications gleaned from studies of genetically engineered mice. Glia 43, 87–90.

Middeldorp, J., Hol, E.M., 2011. GFAP in health and disease. Prog. Neurobiol. 93, 421–43.

Miletic, H., Fischer, Y.H., Neumann, H., Hans, V., Stenzel, W., Giroglou, T., Hermann, M., Deckert, M., Von Laer, D., 2004. Selective transduction of malignant glioma by lentiviral vectors pseudotyped with lymphocytic choriomeningitis virus glycoproteins. Hum. Gene Ther. 15, 1091–100.

Min, R., Nevian, T., 2012. Astrocyte signaling controls spike timing-dependent depression at neocortical synapses. Nat. Neurosci. 15, 746–53.

Minelli, A., Brecha, N.C., Karschin, C., DeBiasi, S., Conti, F., 1995. GAT-1, a high-affinity GABA plasma membrane transporter, is localized to neurons and astroglia in the cerebral cortex. J. Neurosci. 15, 7734–46.

Molotkov, D., Zobova, S., Arcas, J.M., Khiroug, L., 2013. Calcium-induced outgrowth of astrocytic peripheral processes requires actin binding by Profilin-1. Cell Calcium 53, 338–48.

Molotkov, D., Zobova, S., Khiroug, L., 2011. Glia-specific mGFAP promoter directs expression preferentially to neurons in rat visual cortex in vivo. Glia 59(S1), S55.

Molotkov, D.A., Yukin, A.Y., Afzalov, R.A., Khiroug, L.S., 2010. Gene delivery to postnatal rat brain by non-ventricular plasmid injection and electroporation. J. Vis. Exp.

Moore, C.S., Milner, R., Nishiyama, A., Frausto, R.F., Serwanski, D.R., Pagarigan, R.R., Whitton, J.L., Miller, R.H., Crocker, S.J., 2011. Astrocytic tissue inhibitor of metalloproteinase-1 (TIMP-1) promotes oligodendrocyte differentiation and enhances CNS myelination. J. Neurosci. 31, 6247–54.

Moore, R.Y., 1993. Principles of synaptic transmission. Ann. N. Y. Acad. Sci. 695, 1–9.

Morgello, S., Uson, R.R., Schwartz, E.J., Haber, R.S., 1995. The human blood-brain barrier glucose transporter (GLUT1) is a glucose transporter of gray matter astrocytes. Glia 14, 43–54.

Morioka, N., Abdin, J.M., Morita, K., Kitayama, T., Nakata, Y., Dohi, T., 2008. The regulation of glycine transporter GLYT1 is mainly mediated by protein kinase Calpha in C6 glioma cells. Neurochem. Int. 53, 248–54.

Muallem, S., Kwiatkowska, K., Xu, X., Yin, H.L., 1995. Actin filament disassembly is a sufficient final trigger for exocytosis in nonexcitable cells. J. Cell Biol. 128, 589–98.

Mucke, L., Oldstone, M.B., Morris, J.C., Nerenberg, M.I., 1991. Rapid activation of astrocyte-specific expression of GFAP-lacZ transgene by focal injury. New Biol. 3, 465–74.

Mulligan, S.J., Macvicar, B.A., 2004. Calcium transients in astrocyte endfeet cause cerebrovascular constrictions 431, 195–199.

Murai, K.K., Nguyen, L.N., Irie, F., Yamaguchi, Y., Pasquale, E.B., 2003. Control of hippocampal dendritic spine morphology through ephrin-A3/EphA4 signaling. Nat. Neurosci. 6, 153–60.

Murai, K.K., Pasquale, E.B., 2011. Eph receptors and ephrins in neuron-astrocyte communication at synapses. Glia 59, 1567–78.

Murphy, R.C., Messer, A., 2001. Gene transfer methods for CNS organotypic cultures: a comparison of three nonviral methods. Mol. Ther. 3, 113–21.

Nagelhus, E., Mathiisen, T.M., Ottersen, O.P., 2004. Aquaporin-4 in the central nervous system: cellular and subcellular distribution and coexpression with KIR4.1. Neuroscience 129, 905–13.

Nägler, K., Mauch, D.H., Pfrieger, F.W., 2001. Glia-derived signals induce synapse formation in neurones of the rat central nervous system. J. Physiol. 533, 665–79.

Nakai, H., Yant, S.R., Storm, T.A., Fuess, S., Meuse, L., Kay, M.A., 2001. Extrachromosomal recombinant adeno-associated virus vector genomes are primarily responsible for stable liver transduction in vivo. J. Virol. 75, 6969–76.

Naldini, L., Blömer, U., Gage, F.H., Trono, D., Verma, I.M., 1996. Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. Proc. Natl. Acad. Sci. U. S. A. 93, 11382–8.

Nase, G., Helm, P.J., Enger, R., Ottersen, O.P., 2008. Water entry into astrocytes during brain edema formation. Glia 56, 895–902.

Navarrete, M., Araque, A., 2008. Endocannabinoids mediate neuron-astrocyte communication. Neuron 57, 883–93.

Navarrete, M., Araque, A., 2011. Basal synaptic transmission: astrocytes rule! Cell 146, 675–7.

Nave, K.-A., 2010. Myelination and support of axonal integrity by glia. Nature 468, 244–52.

Nedergaard, M., Verkhratsky, A., 2012. Artifact versus reality--how astrocytes contribute to synaptic events. Glia 60, 1013–23.

Nemethova, M., Auinger, S., Small, J.V., 2008. Building the actin cytoskeleton: filopodia contribute to the construction of contractile bundles in the lamella. J. Cell Biol. 180, 1233–44.

Nicchia, G.P., Frigeri, A., Liuzzi, G.M., Svelto, M., 2003. Inhibition of aquaporin-4 expression in astrocytes by RNAi determines alteration in cell morphology, growth, and water transport and induces changes in ischemia-related genes. FASEB J. 17, 1508–10.

Nicholls, D., Attwell, D., 1990. The release and uptake of excitatory amino acids. Trends Pharmacol. Sci. 11, 462–8.

Nicholls, J., Martin, R., Wallace, B., Fuchs, P., 2001. From neuron to brain, 4th edition. Sinauer Associates, Inc. Publishing.

Nico, B., Frigeri, A., Nicchia, G.P., Corsi, P., Ribatti, D., Quondamatteo, F., Herken, R., Girolamo, F., Marzullo, A., Svelto, M., Roncali, L., 2003. Severe alterations of endothelial and glial cells in the blood-brain barrier of dystrophic mdx mice. Glia 42, 235–51.

Nico, B., Ribatti, D., 2012. Morphofunctional aspects of the blood-brain barrier. Curr. Drug Metab. 13, 50–60.

Nimmerjahn, A., Helmchen, F., 2012. In vivo labeling of cortical astrocytes with sulforhodamine 101 (SR101). Cold Spring Harb. Protoc. 2012, 326–34.

Nimmerjahn, A., Kirchhoff, F., Helmchen, F., 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308, 1314–8.

Nimmerjahn, A., Kirchhoff, F., Kerr, J.N.D., Helmchen, F., 2004. Sulforhodamine 101 as a specific marker of astroglia in the neocortex in vivo. Nat. Methods 1, 31–7.

Nimmerjahn, A., Mukamel, E., Schnitzer, M.J., 2009. Motor behavior activates Bergmann glial networks. Neuron 62, 400–12.

Nishida, H., Okabe, S., 2007. Direct astrocytic contacts regulate local maturation of dendritic spines. J. Neurosci. 27, 331–40.

Nishikawa, M., Huang, L., 2001. Nonviral vectors in the new millennium: delivery barriers in gene transfer. Hum. Gene Ther. 12, 861–70.

Nishimura, N., Schaffer, C.B., Friedman, B., Tsai, P.S., Lyden, P.D., Kleinfeld, D., 2006. Targeted insult to subsurface cortical blood vessels using ultrashort laser pulses: three models of stroke. Nat. Methods 3, 99–108.

Noda, M., Nakanishi, H., Nabekura, J., Akaike, N., 2000. AMPA-kainate subtypes of glutamate receptor in rat cerebral microglia. J. Neurosci. 20, 251–8.

Noell, S., Wolburg-Buchholz, K., Mack, A.F., Beedle, A.M., Satz, J.S., Campbell, K.P., Wolburg, H., Fallier-Becker, P., 2011. Evidence for a role of dystroglycan regulating the membrane architecture of astroglial endfeet. Eur. J. Neurosci. 33, 2179–86.

Noguchi, J., Nagaoka, A., Watanabe, S., Ellis-Davies, G.C.R., Kitamura, K., Kano, M., Matsuzaki, M., Kasai, H., 2011. In vivo two-photon uncaging of glutamate revealing the structure-function relationships of dendritic spines in the neocortex of adult mice. J. Physiol. 589, 2447–57.

Nong, Y., Huang, Y.-Q., Ju, W., Kalia, L. V, Ahmadian, G., Wang, Y.T., Salter, M.W., 2003. Glycine binding primes NMDA receptor internalization. Nature 422, 302–7.

O'Brien, J., Holt, M., Whiteside, G., Lummis, S.C., Hastings, M.H., 2001. Modifications to the hand-held Gene Gun: improvements for in vitro biolistic transfection of organotypic neuronal tissue. J. Neurosci. Methods 112, 57–64.

O'Brien, J., Lummis, S.C.R., 2004. Biolistic and diolistic transfection: using the gene gun to deliver DNA and lipophilic dyes into mammalian cells. Methods 33, 121–5.

Oberheim, N.A., Wang, X., Goldman, S., Nedergaard, M., 2006. Astrocytic complexity distinguishes the human brain. Trends Neurosci. 29, 547–53.

Ogata, K., Kosaka, T., 2002. Structural and quantitative analysis of astrocytes in the mouse hippocampus. Neuroscience 113, 221–33.

Ohtsuki, S., Terasaki, T., 2007. Contribution of carrier-mediated transport systems to the blood-brain barrier as a supporting and protecting interface for the brain; importance for CNS drug discovery and development. Pharm. Res. 24, 1745–58.

Oliet, S.H., Piet, R., Poulain, D., 2001. Control of glutamate clearance and synaptic efficacy by glial coverage of neurons. Science 292, 923–6.

Page, K.A., Landau, N.R., Littman, D.R., 1990. Construction and use of a human immunodeficiency virus vector for analysis of virus infectivity. J. Virol. 64, 5270–6.

Panatier, A., Theodosis, D.T., Mothet, J.-P., Touquet, B., Pollegioni, L., Poulain, D., Oliet, S.H.R., 2006. Glia-derived D-serine controls NMDA receptor activity and synaptic memory. Cell 125, 775–84.

Panatier, A., Vallée, J., Haber, M., Murai, K.K., Lacaille, J.-C., Robitaille, R., 2011. Astrocytes are endogenous regulators of basal transmission at central synapses. Cell 146, 785–98.

Pangrsic, T., Potokar, M., Stenovec, M., Kreft, M., Fabbretti, E., Nistri, A., Pryazhnikov, E., Khiroug, L., Giniatullin, R., Zorec, R., 2007. Exocytotic release of ATP from cultured astrocytes. J. Biol. Chem. 282, 28749–58.

Pannasch, U., Rouach, N., 2013. Emerging role for astroglial networks in information processing: from synapse to behavior. Trends Neurosci. 36, 405–17.

Pannasch, U., Vargová, L., Reingruber, J., Ezan, P., Holcman, D., Giaume, C., Syková, E., Rouach, N., 2011. Astroglial networks scale synaptic activity and plasticity. Proc. Natl. Acad. Sci. U. S. A. 108, 8467–72.

Pantazopoulos, H., Woo, T.-U.W., Lim, M.P., Lange, N., Berretta, S., 2010. Extracellular matrix-glial abnormalities in the amygdala and entorhinal cortex of subjects diagnosed with schizophrenia. Arch. Gen. Psychiatry 67, 155–66.

Papadopoulos, M.C., Verkman, A.S., 2013. Aquaporin water channels in the nervous system. Nat. Rev. Neurosci. 14, 265–77.

Papouin, T., Ladépêche, L., Ruel, J., Sacchi, S., Labasque, M., Hanini, M., Groc, L., Pollegioni, L., Mothet, J.-P., Oliet, S.H.R., 2012. Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. Cell 150, 633–46.

Pardridge, W.M., 2007. Drug targeting to the brain. Pharm. Res. 24, 1733–44.

Parri, H.R., Gould, T.M., Crunelli, V., 2001. Spontaneous astrocytic Ca2+ oscillations in situ drive NMDAR-mediated neuronal excitation. Nat. Neurosci. 4, 803–12.

Pascual, O., Casper, K.B., Kubera, C., Zhang, J., Revilla-Sanchez, R., Sul, J.-Y., Takano, H., Moss, S.J., McCarthy, K., Haydon, P.G., 2005. Astrocytic purinergic signaling coordinates synaptic networks. Science 310, 113–6.

Pellerin, L., Bouzier-Sore, A.-K., Aubert, A., Serres, S., Merle, M., Costalat, R., Magistretti, P.J., 2007. Activity-dependent regulation of energy metabolism by astrocytes: an update. Glia 55, 1251–62.

Pellerin, L., Magistretti, P.J., 1994. Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc. Natl. Acad. Sci. U. S. A. 91, 10625–9.

Penfield et al., 1932. Cytology and cellular pathology of the nervous system. New York: Hoeber.

Perea, G., Araque, A., 2005. Properties of synaptically evoked astrocyte calcium signal reveal synaptic information processing by astrocytes. J. Neurosci. 25, 2192–203.

Perea, G., Araque, A., 2007. Astrocytes potentiate transmitter release at single hippocampal synapses. Science 317, 1083–6.

Perea, G., Navarrete, M., Araque, A., 2009. Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci. 32, 421–31.

Pérez-Alvarez, A., Araque, A., Martín, E.D., 2013. Confocal microscopy for astrocyte in vivo imaging: Recycle and reuse in microscopy. Front. Cell. Neurosci. 7, 51.

Peters, A., Palay, S., Webster, H., 1991. The fine structure of the nervous system. Oxford University Press.

Petravicz, J., Fiacco, T., McCarthy, K.D., 2008. Loss of IP3 receptor-dependent Ca2+ increases in hippocampal astrocytes does not affect baseline CA1 pyramidal neuron synaptic activity. J. Neurosci. 28, 4967–73.

Petzold, G.C., Albeanu, D.F., Sato, T.F., Murthy, V.N., 2008. Coupling of neural activity to blood flow in olfactory glomeruli is mediated by astrocytic pathways. Neuron 58, 897–910.

Pfrieger, F.W., Barres, B.A., 1997. Synaptic efficacy enhanced by glial cells in vitro. Science 277, 1684–7.

Pfrieger, F.W., Slezak, M., 2012. Genetic approaches to study glial cells in the rodent brain. Glia 60, 681–701.

Piatkevich, K.D., Subach, F. V, Verkhusha, V. V, 2013. Far-red light photoactivatable near-infrared fluorescent proteins engineered from a bacterial phytochrome. Nat. Commun. 4, 2153.

Piet, R., Vargová, L., Syková, E., Poulain, D., Oliet, S.H.R., 2004. Physiological contribution of the astrocytic environment of neurons to intersynaptic crosstalk. Proc. Natl. Acad. Sci. U. S. A. 101, 2151–5.

Pollard, T.D., 2003. The cytoskeleton, cellular motility and the reductionist agenda. Nature 422, 741–5.

Pollard, T.D., Borisy, G.G., 2003. Cellular motility driven by assembly and disassembly of actin filaments. Cell 112, 453–65.

Porter, J.T., McCarthy, K.D., 1996. Hippocampal astrocytes in situ respond to glutamate released from synaptic terminals. J. Neurosci. 16, 5073–81.

Prakash, R., Yizhar, O., Grewe, B., Ramakrishnan, C., Wang, N., Goshen, I., Packer, A.M., Peterka, D.S., Yuste, R., Schnitzer, M.J., Deisseroth, K., 2012. Two-photon optogenetic toolbox for fast inhibition, excitation and bistable modulation. Nat. Methods 9, 1171–9.

Pryazhnikov, E., Khiroug, L., 2008. Sub-Micromolar Increase in [Ca2+]i Triggers Delayed Exocytosis of ATP in Cultured Astrocytes 49, 38–49.

Pyka, M., Wetzel, C., Aguado, A., Geissler, M., Hatt, H., Faissner, A., 2011. Chondroitin sulfate proteoglycans regulate astrocyte-dependent synaptogenesis and modulate synaptic activity in primary embryonic hippocampal neurons. Eur. J. Neurosci. 33, 2187–202.

Quaegebeur, A., Lange, C., Carmeliet, P., 2011. The neurovascular link in health and disease: molecular mechanisms and therapeutic implications. Neuron 71, 406–24.

Raichle, M.E., Mintun, M. A., 2006. Brain work and brain imaging. Annu. Rev. Neurosci. 29, 449–76.

Ralay Ranaivo, H., Hodge, J.N., Choi, N., Wainwright, M.S., 2012. Albumin induces upregulation of matrix metalloproteinase-9 in astrocytes via MAPK and reactive oxygen species-dependent pathways. J. Neuroinflammation 9, 68.

Ramon y Cajal S., 1995. Histology of the nervous system of man and vertebrates. Oxford University Press.

Ransom, B.R., 1991. Vertebrate glial classification, lineage, and heterogeneity. Ann. N. Y. Acad. Sci. 633, 19–26.

Ransom, C.B., Sontheimer, H., 2001. BK channels in human glioma cells. J. Neurophysiol. 85, 790–803.

Reichelt, W., Dettmer, D., Brückner, G., Brust, P., Eberhardt, W., Reichenbach, A., 1989. Potassium as a signal for both proliferation and differentiation of rabbit retinal (Müller) glia growing in cell culture. Cell. Signal. 1, 187–94.

Reichenbach, A., Derouiche, A., Kirchhoff, F., 2010. Morphology and dynamics of perisynaptic glia. Brain Res. Rev. 63, 11–25.

Riedl, J., Crevenna, A.H., Kessenbrock, K., Yu, J.H., Neukirchen, D., Bista, M., Bradke, F., Jenne, D., Holak, T.A., Werb, Z., Sixt, M., Wedlich-Soldner, R., 2008. Lifeact: a versatile marker to visualize F-actin. Nat. Methods 5, 605–7.

Rosenberg, D., Artoul, S., Segal, A.C., Kolodney, G., Radzishevsky, I., Dikopoltsev, E., Foltyn, V.N., Inoue, R., Mori, H., Billard, J.-M., Wolosker, H., 2013. Neuronal D-serine and glycine release via the Asc-1 transporter regulates NMDA receptor-dependent synaptic activity. J. Neurosci. 33, 3533–44.

Rossi, D.J., Brady, J.D., Mohr, C., 2007. Astrocyte metabolism and signaling during brain ischemia. Nat. Neurosci. 10, 1377–86.

Rossi, D.J., Oshima, T., Attwell, D., 2000. Glutamate release in severe brain ischaemia is mainly by reversed uptake. Nature 403, 316–21.

Rothstein, J.D., Dykes-Hoberg, M., Pardo, C., Bristol, L., Jin, L., Kuncl, R.W., Kanai, Y., Hediger, M., Wang, Y., Schielke, J.P., Welty, D.F., 1996. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16, 675–86.

Rouach, N., Glowinski, J., Giaume, C., 2000. Activity-dependent neuronal control of gap-junctional communication in astrocytes. J. Cell Biol. 149, 1513–26.

Rouach, N., Koulakoff, A., Abudara, V., Willecke, K., Giaume, C., 2008. Astroglial metabolic networks sustain hippocampal synaptic transmission. Science 322, 1551–5.

Roux, L., Benchenane, K., Rothstein, J.D., Bonvento, G., Giaume, C., 2011. Plasticity of astroglial networks in olfactory glomeruli. Proc. Natl. Acad. Sci. U. S. A.

Rowitch, D.H., Kriegstein, A.R., 2010. Developmental genetics of vertebrate glial-cell specification. Nature 468, 214–22.

Rusakov, D., 2001. The role of perisynaptic glial sheaths in glutamate spillover and extracellular Ca(2+) depletion. Biophys. J. 81, 1947–59.

Saito, T., 2006. In vivo electroporation in the embryonic mouse central nervous system. Nat. Protoc. 1, 1552–8.

Saito, T., Nakatsuji, N., 2001. Efficient gene transfer into the embryonic mouse brain using in vivo electroporation. Dev. Biol. 240, 237–46.

Santello, M., Bezzi, P., Volterra, A., 2011. TNFα controls glutamatergic gliotransmission in the hippocampal dentate gyrus. Neuron 69, 988–1001.

Santello, M., Volterra, A., 2009. Synaptic modulation by astrocytes via Ca2+-dependent glutamate release. Neuroscience 158, 253–9.

Sato, T., Muroyama, Y., Saito, T., 2013. Inducible gene expression in postmitotic neurons by an in vivo electroporation-based tetracycline system. J. Neurosci. Methods 214, 170–6.

Savtchenko, L.P., Rusakov, D., 2007. The optimal height of the synaptic cleft. Proc. Natl. Acad. Sci. U. S. A. 104, 1823–8.

Sbai, O., Ould-Yahoui, A., Ferhat, L., Gueye, Y., Bernard, A., Charrat, E., Mehanna, A., Risso, J.-J., Chauvin, J.-P., Fenouillet, E., Rivera, S., Khrestchatisky, M., 2010. Differential vesicular distribution and trafficking of MMP-2, MMP-9, and their inhibitors in astrocytes. Glia 58, 344–66.

Scemes, E., Giaume, C., 2006. Astrocyte Calcium Waves : What They Are and What They Do 725, 716–725.

Scharfman, H.E., Binder, D.K., 2013. Aquaporin-4 water channels and synaptic plasticity in the hippocampus. Neurochem. Int. 63, 702–11.

Schell, M.J., Molliver, M.E., Snyder, S.H., 1995. D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. Proc. Natl. Acad. Sci. U. S. A. 92, 3948–52.

Schipke, C.G., Ohlemeyer, C., Matyash, M., Nolte, C., Kettenmann, H., Kirchhoff, F., 2001. Astrocytes of the mouse neocortex express functional N-methyl-D-aspartate receptors. FASEB J. 15, 1270–2.

Schnell, C., Hagos, Y., Hülsmann, S., 2012. Active sulforhodamine 101 uptake into hippocampal astrocytes. PLoS One 7, e49398.

Schools, G.P., Kimelberg, H.K., 1999. mGluR3 and mGluR5 are the predominant metabotropic glutamate receptor mRNAs expressed in hippocampal astrocytes acutely isolated from young rats. J. Neurosci. Res. 58, 533–43.

Schubert, D., Herrera, F., Cumming, R., Read, J., Low, W., Maher, P., Fischer, W.H., 2009. Neural cells secrete a unique repertoire of proteins. J. Neurochem. 109, 427–35.

Schummers, J., Yu, H., Sur, M., 2008. Tuned responses of astrocytes and their influence on hemodynamic signals in the visual cortex. Science 320, 1638–43.

Schwartz, N.E., Alford, S., 2000. Physiological activation of presynaptic metabotropic glutamate receptors increases intracellular calcium and glutamate release. J. Neurophysiol. 84, 415–27.

Scimemi, A., Fine, A., Kullmann, D.M., Rusakov, D., 2004. NR2B-containing receptors mediate cross talk among hippocampal synapses. J. Neurosci. 24, 4767–77.

Scott, B.B., Brody, C.D., Tank, D.W., 2013. Cellular resolution functional imaging in behaving rats using voluntary head restraint. Neuron 80, 371–84.

Seidel, B., Zuschratter, W., Wex, H., Garner, C.C., Gundelfinger, E.D., 1995. Spatial and sub-cellular localization of the membrane cytoskeleton-associated protein alpha-adducin in the rat brain. Brain Res. 700, 13–24.

Semyanov, A., Kullmann, D.M., 2000. Modulation of GABAergic signaling among interneurons by metabotropic glutamate receptors. Neuron 25, 663–72.

Serrano, A., Haddjeri, N., Lacaille, J.-C., Robitaille, R., 2006. GABAergic network activation of glial cells underlies hippocampal heterosynaptic depression. J. Neurosci. 26, 5370–82.

Shefi, O., Simonnet, C., Baker, M.W., Glass, J.R., Macagno, E.R., Groisman, A., 2006. Microtargeted gene silencing and ectopic expression in live embryos using biolistic delivery with a pneumatic capillary gun. J. Neurosci. 26, 6119–23.

Shevtsova, Z., Malik, J.M.I., Michel, U., Bähr, M., Kügler, S., 2005. Promoters and serotypes: targeting of adeno-associated virus vectors for gene transfer in the rat central nervous system in vitro and in vivo. Exp. Physiol. 90, 53–9.

Shigetomi, E., Bushong, E. a, Haustein, M.D., Tong, X., Jackson-Weaver, O., Kracun, S., Xu, J., Sofroniew, M. V, Ellisman, M.H., Khakh, B.S., 2013a. Imaging calcium microdomains within entire astrocyte territories and endfeet with GCaMPs expressed using adeno-associated viruses. J. Gen. Physiol. 141, 633–47.

Shigetomi, E., Jackson-Weaver, O., Huckstepp, R.T., O'Dell, T.J., Khakh, B.S., 2013b. TRPA1 channels are regulators of astrocyte basal calcium levels and long-term potentiation via constitutive D-serine release. J. Neurosci. 33, 10143–53.

Shih, A.Y., Nishimura, N., Nguyen, J., Friedman, B., Lyden, P.D., Schaffer, C.B., Kleinfeld, D., 2013. Optically induced occlusion of single blood vessels in rodent neocortex. Cold Spring Harb. Protoc. 2013, 1153–60.

Simard, M., Nedergaard, M., 2004. The neurobiology of glia in the context of water and ion homeostasis. Neuroscience 129, 877–96.

Small, J. V, 1995. Getting the actin filaments straight: nucleation-release or treadmilling? Trends Cell Biol. 5, 52–5.

Spacek, J., Harris, K.M., 2004. Trans-endocytosis via spinules in adult rat hippocampus. J. Neurosci. 24, 4233–41.

Steinhäuser, C., Jabs, R., Kettenmann, H., 1994. Properties of GABA and glutamate responses in identified glial cells of the mouse hippocampal slice. Hippocampus 4, 19–35.

Stoffels, J.M.J., de Jonge, J.C., Stancic, M., Nomden, A., van Strien, M.E., Ma, D., Sisková, Z., Maier, O., Ffrench-Constant, C., Franklin, R.J.M., Hoekstra, D., Zhao, C., Baron, W., 2013. Fibronectin aggregation in multiple sclerosis lesions impairs remyelination. Brain 136, 116–31.

Stoppini, L., Buchs, P.A., Muller, D., 1991. A simple method for organotypic cultures of nervous tissue. J. Neurosci. Methods 37, 173–82.

Streit, W.J., Graeber, M.B., Kreutzberg, G.W., 1988. Functional plasticity of microglia: a review. Glia 1, 301–7.

Suadicani, S.O., Iglesias, R., Wang, J., Dahl, G., Spray, D.C., Scemes, E., 2012. ATP signaling is deficient in cultured Pannexin1-null mouse astrocytes. Glia 60, 1106–16.

Suetsugu, S., Miki, H., Takenawa, T., 1998. The essential role of profilin in the assembly of actin for microspike formation. EMBO J. 17, 6516–26.

Suetsugu, S., Miki, H., Takenawa, T., 1999. Distinct roles of profilin in cell morphological changes: microspikes, membrane ruffles, stress fibers, and cytokinesis. FEBS Lett. 457, 470–4.

Summers, L., Kangwantas, K., Nguyen, L., Kielty, C., Pinteaux, E., 2010. Adhesion to the extracellular matrix is required for interleukin-1 beta actions leading to reactive phenotype in rat astrocytes. Mol. Cell. Neurosci. 44, 272–81.

Sun, W., McConnell, E., Pare, J.-F., Xu, Q., Chen, M., Peng, W., Lovatt, D., Han, X., Smith, Y., Nedergaard, M., 2013. Glutamate-dependent neuroglial calcium signaling differs between young and adult brain. Science 339, 197–200.

Suzuki, A., Stern, S. a, Bozdagi, O., Huntley, G.W., Walker, R.H., Magistretti, P.J., Alberini, C.M., 2011. Astrocyte-neuron lactate transport is required for long-term memory formation. Cell 144, 810–23.

Suzuki, R., Watanabe, J., Arata, S., Funahashi, H., Kikuyama, S., Shioda, S., 2003. A transgenic mouse model for the detailed morphological study of astrocytes. Neurosci. Res. 47, 451–454.

Svoboda, K., Denk, W., Kleinfeld, D., Tank, D.W., 1997. In vivo dendritic calcium dynamics in neocortical pyramidal neurons. Nature 385, 161–5.

Svoboda, K., Yasuda, R., 2006. Principles of two-photon excitation microscopy and its applications to neuroscience. Neuron 50, 823–39.

Syková, E., 2001. Glial diffusion barriers during aging and pathological states. Prog. Brain Res. 132, 339–63.

Syková, E., 2004. Extrasynaptic volume transmission and diffusion parameters of the extracellular space. Neuroscience 129, 861–76.

Sylantyev, S., Savtchenko, L.P., Ermolyuk, Y., Michaluk, P., Rusakov, D., 2013. Spike-driven glutamate electrodiffusion triggers synaptic potentiation via a homer-dependent mGluR-NMDAR link. Neuron 77, 528–41.

Tabata, H., Nakajima, K., 2001. Efficient in utero gene transfer system to the developing mouse brain using electroporation: visualization of neuronal migration in the developing cortex. Neuroscience 103, 865–72.

Tabernero, A., Medina, J.M., Giaume, C., 2006. Glucose metabolism and proliferation in glia: role of astrocytic gap junctions. J. Neurochem. 99, 1049–61.

Takahashi, S., Driscoll, B.F., Law, M.J., Sokoloff, L., 1995. Role of sodium and potassium ions in regulation of glucose metabolism in cultured astroglia. Proc. Natl. Acad. Sci. U. S. A. 92, 4616–20.

Takano, T., Tian, G.-F., Peng, W., Lou, N., Libionka, W., Han, X., Nedergaard, M., 2006. Astrocyte-mediated control of cerebral blood flow. Nat. Neurosci. 9, 260–7.

Takata, N., Hirase, H., 2008. Cortical layer 1 and layer 2/3 astrocytes exhibit distinct calcium dynamics in vivo. PLoS One 3, e2525.

Tanaka, K., Watase, K., Manabe, T., Yamada, K., Watanabe, M., Takahashi, K., Iwama, H., Nishikawa, T., Ichihara, N., Kikuchi, T., Okuyama, S., Kawashima, N., Hori, S., Takimoto, M., Wada, K., 1997. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. Science 276, 1699–702.

Theis, M., Söhl, G., Eiberger, J., Willecke, K., 2005. Emerging complexities in identity and function of glial connexins. Trends Neurosci. 28, 188–95.

Theodosis, D.T., Montagnese, C., Rodriguez, F., Vincent, J.D., Poulain, D.A., 1986. Oxytocin induces morphological plasticity in the adult hypothalamo-neurohypophysial system. Nature 322, 738–40.

Theodosis, D.T., Piet, R., Poulain, D., Oliet, S.H.., 2004. Neuronal, glial and synaptic remodeling in the adult hypothalamus: functional consequences and role of cell surface and extracellular matrix adhesion molecules. Neurochem. Int. 45, 491–501.

Theodosis, D.T., Poulain, D., Oliet, S.H.R., 2008. Activity-dependent structural and functional plasticity of astrocyte-neuron interactions. Physiol. Rev. 88, 983–1008.

Theodosis, D.T., Poulain, D.A., 1989. Neuronal-glial and synaptic plasticity in the adult rat paraventricular nucleus. Brain Res. 484, 361–6.

Theodosis, D.T., Poulain, D.A., 1993. Activity-dependent neuronal-glial and synaptic plasticity in the adult mammalian hypothalamus. Neuroscience 57, 501–35.

Thomsen, D.R., Stenberg, R.M., Goins, W.F., Stinski, M.F., 1984. Promoter-regulatory region of the major immediate early gene of human cytomegalovirus. Proc. Natl. Acad. Sci. U. S. A. 81, 659–63.

Thrane, A.S., Rangroo Thrane, V., Zeppenfeld, D., Lou, N., Xu, Q., Nagelhus, E.A., Nedergaard, M., 2012. General anesthesia selectively disrupts astrocyte calcium signaling in the awake mouse cortex. Proc. Natl. Acad. Sci. U. S. A. 109, 18974–9.

Tremblay, M.-È., Majewska, A.K., 2011. A role for microglia in synaptic plasticity? Commun. Integr. Biol. 4, 220–2.

Ullian, E.M., Sapperstein, S.K., Christopherson, K.S., Barres, B.A., 2001. Control of synapse number by glia. Science 291, 657–61.

Valiunas, V., Polosina, Y.Y., Miller, H., Potapova, I. a, Valiuniene, L., Doronin, S., Mathias, R.T., Robinson, R.B., Rosen, M.R., Cohen, I.S., Brink, P.R., 2005. Connexin-specific cell-to-cell transfer of short interfering RNA by gap junctions. J. Physiol. 568, 459–68.

Van den Pol, A.N., Finkbeiner, S.M., Cornell-Bell, A.H., 1992. Calcium excitability and oscillations in suprachiasmatic nucleus neurons and glia in vitro. J. Neurosci. 12, 2648–64.

Ventura, R., Harris, K.M., 1999. Three-dimensional relationships between hippocampal synapses and astrocytes. J. Neurosci. 19, 6897–906.

Verkhratsky, A, Orkand, R.K., Kettenmann, H., 1998. Glial calcium: homeostasis and signaling function. Physiol. Rev. 78, 99–141.

Verkhratsky, A., Kettenmann, H., 1996. Calcium signaling in glial cells. Trends Neurosci. 19, 346–52.

Verleysdonk, S., Martin, H., Willker, W., Leibfritz, D., Hamprecht, B., 1999. Rapid uptake and degradation of glycine by astroglial cells in culture: synthesis and release of serine and lactate. Glia 27, 239–48.

Vermeiren, C., Najimi, M., Vanhoutte, N., Tilleux, S., de Hemptinne, I., Maloteaux, J.-M., Hermans, E., 2005. Acute up-regulation of glutamate uptake mediated by mGluR5a in reactive astrocytes. J. Neurochem. 94, 405–16.

Virchow, R., 1846. Über das granulierte Aussehen der Wandungen des Gehirnvenrtikels, Zeitschrift für Psychiatrie, Heft 2, 242ff.

Virchow, R., 1858. "Cellularpathologie", Hirschwald.

Volterra, A., Magistretti, P., Haydon, P., 2002. The tripartite synapse glia in synaptic transmission. Oxford University Press.

Volterra, A., Meldolesi, J., 2005. Astrocytes, from brain glue to communication elements: the revolution continues. Nat. Rev. Neurosci. 6, 626–40.

Wake, H., Moorhouse, A.J., Miyamoto, A., Nabekura, J., 2013. Microglia: actively surveying and shaping neuronal circuit structure and function. Trends Neurosci. 36, 209–17.

Wallraff, A., Köhling, R., Heinemann, U., Theis, M., Willecke, K., Steinhäuser, C., 2006. The impact of astrocytic gap junctional coupling on potassium buffering in the hippocampus. J. Neurosci. 26, 5438–47.

Wang, F., Smith, N., Xu, Q., Fujita, T., Baba, A., Matsuda, T., Takano, T., Bekar, L., Nedergaard, M., 2012. Astrocytes modulate neural network activity by Ca^{2}+-dependent uptake of extracellular K+. Sci. Signal. 5, ra26 1-15.

Wang, F., Smith, N.A., Xu, Q., Goldman, S., Peng, W., Huang, J.H., Takano, T., Nedergaard, M., 2013. Photolysis of caged Ca2+ but not receptor-mediated Ca2+ signaling triggers astrocytic glutamate release. J. Neurosci. 33, 17404–12.

Wang, X., Lou, N., Xu, Q., Tian, G.-F., Peng, W.G., Han, X., Kang, J., Takano, T., Nedergaard, M., 2006. Astrocytic Ca2+ signaling evoked by sensory stimulation in vivo. Nat. Neurosci. 9, 816–23.

Wang, Y., Haughey, N.J., Mattson, M.P., Furukawa, K., 2004. Dual effects of ATP on rat hippocampal synaptic plasticity. Neuroreport 15, 633–6.

Wanisch, K., Yáñez-Muñoz, R.J., 2009. Integration-deficient lentiviral vectors: a slow coming of age. Mol. Ther. 17, 1316–32.

Webb, S.E., Miller, A.L., 2003. Calcium signalling during embryonic development. Nat. Rev. Mol. Cell Biol. 4, 539–51.

Wells, D.J., 2010. Electroporation and ultrasound enhanced non-viral gene delivery in vitro and in vivo. Cell Biol. Toxicol. 26, 21–8.

White, E., Bienemann, A., Taylor, H., Castrique, E., Bunnun, C., Wyatt, M., Gill, S., 2011. An evaluation of site-specific immune responses directed against first-generation adenoviral vectors administered by convection-enhanced delivery. J. Gene Med. 13, 269–82.

Wiese, S., Karus, M., Faissner, A., 2012. Astrocytes as a source for extracellular matrix molecules and cytokines. Front. Pharmacol. 3, 120.

Willecke, K., Eiberger, J., Degen, J., Eckardt, D., Romualdi, A., Güldenagel, M., Deutsch, U., Söhl, G., 2002. Structural and functional diversity of connexin genes in the mouse and human genome. Biol. Chem. 383, 725–37.

Winship, I.R., Plaa, N., Murphy, T.H., 2007. Rapid astrocyte calcium signals correlate with neuronal activity and onset of the hemodynamic response in vivo. J. Neurosci. 27, 6268–72.

Wirth, M.J., Wahle, P., 2003. Biolistic transfection of organotypic cultures of rat visual cortex using a handheld device. J. Neurosci. Methods 125, 45–54.

Wirth, T., Parker, N., Ylä-Herttuala, S., 2013. History of gene therapy. Gene 525, 162–9.

Witcher, M.R., Kirov, S.A., Harris, K.M., 2007. Plasticity of Perisynaptic Astroglia During Synaptogenesis in the Mature Rat Hippocampus 23, 13–23.

Witke, W., 2004. The role of profilin complexes in cell motility and other cellular processes. Trends Cell Biol. 14, 461–9.

Witte, S., Negrean, A., Lodder, J.C., de Kock, C.P.J., Testa Silva, G., Mansvelder, H.D., Louise Groot, M., 2011. Label-free live brain imaging and targeted patching with third-harmonic generation microscopy. Proc. Natl. Acad. Sci. U. S. A. 108, 5970–5.

Wolff, J., 1970. Quantitative aspects of astroglia. In Proceedings of the 7th international congress of neuropathology pp. 324-52. Mason and Cie., Paris.

Wolff, J.R., Stuke, K., Missler, M., Tytko, H., Schwarz, P., Rohlmann, A., Chao, T.I., 1998. Autocellular coupling by gap junctions in cultured astrocytes: a new view on cellular autoregulation during process formation. Glia 24, 121–40.

Wolosker, H., 2006. D-serine regulation of NMDA receptor activity. Sci. STKE 2006, pe41.

Wu, Y.I., Frey, D., Lungu, O.I., Jaehrig, A., Schlichting, I., Kuhlman, B., Hahn, K.M., 2009. A genetically encoded photoactivatable Rac controls the motility of living cells. Nature 461, 104–8.

Xie, Y., Wang, T., Sun, G.Y., Ding, S., 2010. Specific disruption of astrocytic Ca2+ signaling pathway in vivo by adeno-associated viral transduction. Neuroscience 170, 992–1003.

Xu, H.-T., Pan, F., Yang, G., Gan, W.-B., 2007. Choice of cranial window type for in vivo imaging affects dendritic spine turnover in the cortex. Nat. Neurosci. 10, 549–51.

Yang, Y., Ge, W., Chen, Y., Zhang, Z., Shen, W., Wu, C., Poo, M., Duan, S., 2003. Contribution of astrocytes to hippocampal long-term potentiation through release of D-serine. Proc. Natl. Acad. Sci. U. S. A. 100, 15194–9.

Yarmola, E.G., Bubb, M.R., 2009. How depolymerization can promote polymerization: the case of actin and profilin. Bioessays 31, 1150–60.

Ye, Z., Sontheimer, H., 1999. Agonists Reduce Glutamate Release From Cultured Astrocytes 281, 270–281.

Ye, Z.-C., Wyeth, M.S., Baltan-Tekkok, S., Ransom, B.R., 2003. Functional hemichannels in astrocytes: a novel mechanism of glutamate release. J. Neurosci. 23, 3588–96.

Yu, S., Ding, W.G., 1998. The 45 kDa form of glucose transporter 1 (GLUT1) is localized in oligodendrocyte and astrocyte but not in microglia in the rat brain. Brain Res. 797, 65–72.

Zamecnik, J., Homola, A., Cicanic, M., Kuncova, K., Marusic, P., Krsek, P., Sykova, E., Vargova, L., 2012. The extracellular matrix and diffusion barriers in focal cortical dysplasias. Eur. J. Neurosci. 36, 2017–24.

Zhang, L.-H., Gong, N., Fei, D., Xu, L., Xu, T.-L., 2008. Glycine uptake regulates hippocampal network activity via glycine receptor-mediated tonic inhibition. Neuropsychopharmacology 33, 701–11.

Zhang, Q., Pangrsic, T., Kreft, M., Krzan, M., Li, N., Sul, J.-Y., Halassa, M., Van Bockstaele, E., Zorec, R., Haydon, P.G., 2004. Fusion-related release of glutamate from astrocytes. J. Biol. Chem. 279, 12724–33.

Zhuang, Z., Yang, B., Theus, M.H., Sick, J.T., Bethea, J.R., Sick, T.J., Liebl, D.J., 2010. EphrinBs regulate D-serine synthesis and release in astrocytes. J. Neurosci. 30, 16015–24.

Zhuo, L., Theis, M., Alvarez-Maya, I., Brenner, M., Willecke, K., Messing, A., 2001. hGFAP-cre transgenic mice for manipulation of glial and neuronal function in vivo. Genesis 31, 85–94.

Zlokovic, B. V, 2008. The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron 57, 178–201.

Zonta, M., Angulo, M.C., Gobbo, S., Rosengarten, B., Hossmann, K.-A., Pozzan, T., Carmignoto, G., 2003. Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. Nat. Neurosci. 6, 43–50.