Astrocytes in Alzheimer's Disease

Alexei Verkhratsky,*† Markel Olabarria,* Harun N. Noristani,* Chia-Yu Yeh,* and Jose Julio Rodriguez†‡§

*Faculty of Life Sciences, The University of Manchester, Manchester, United Kingdom, M13 9PT; †Institute of Experimental Medicine, ASCR, Prague, 142 20 Czech Republic; ‡Ikerbasque, Basque Foundation for Science, Bilbao, 48011 Spain; and §Department of Neurosciences, University of the Basque Country UPV/EHU, Leioa, 48940 Spain

Summary: The circuitry of the human brain is formed by neuronal networks embedded into astroglial syncytia. The astrocytes perform numerous functions, providing for the overall brain homeostasis, assisting in neurogenesis, determining the micro-architecture of the grey matter, and defending the brain through evolutionary conserved astrogliosis programs.

Astroglial cells are engaged in neurological diseases by determining the progression and outcome of neuropathological process. Astrocytes are specifically involved in various neurodegenerative diseases, including Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and various forms of dementia. Recent evidence suggest that early stages of neurodegenerative processes are associated with atrophy of astroglia, which causes disruptions in synaptic connectivity, disbalance in neurotransmitter homeostasis, and neuronal death through increased excitotoxicity. At the later stages, astrocytes become activated and contribute to the neuroinflammatory component of neurodegeneration. **Key Words:** Astrocytes, neuroglia, neurodegeneration, Alzheimer's disease, dementia, Parkinson's disease.

GLIAL EXPLOSION FORMS THE HUMAN BRAIN

The human brain is the most sophisticated and complex system in the universe, as far as we are aware. Indeed, nature compacted ~1.5 trillions of cells connected by hundreds of trillions of contacts within the strictly limited volume of the skull, and orchestrated concerted development of neuronal circuits that produce human intellect, which is unparalleled in its computational and creative power by any other device, be it natural or artificial.

The evolution of the nervous system began with an appearance of multicellular organisms, which required coordination of their remote parts to achieve maximal biological success. At the very core of neural elements lies the excitability and intercellular signaling, both appearing very early in the evolution. The very first and primitive forms of life needed to perceive the environmental changes and preserve their internal homeostasis; this function was achieved by membrane ion channels regulating transmembrane ion movements. These transmembrane ion movements formed the basis for intercellular signaling, epitomized in the Ca^{2+} signaling system.[1] Transmembrane ion gradients and selectively permeable plasmalemma generated uneven distribution of charges in the very vicinity of the plasmalemma, thus stimulating the appearance of voltage-dependent gating mechanisms that laid the foundations for electrical excitability. The voltage-gated channels exist in virtually all living species, and we can find several types of them in bacteria, with the Ca^{2+}- and K^+-selective channels being the most ancient.[2–7] The bacteria also evolved the first precursor of the Na^+ channel, the NaChBac expressed, for example, in *Bacillus halodurans*.[8] In the eukaryotes, the excitable molecules developed further; in single-cell organisms, the waves of plasmalemmal excitation began to generate through the spreading opening/closures of voltage-gated channels.[9] In parallel eukaryotic organisms acquired intracellular organelles, intracellular channels dwelling in the endomembranes, and exocytotic machinery formed the basis for chemical intercellular transmission.[1,10–12]

Address correspondence and reprint requests to: Prof. Alexei Verkhratsky, The University of Manchester, Oxford Road, Manchester, M13 9PT UK. E-mail: alex.verkhratsky@manchester.ac.uk; or Prof. J. J. Rodriguez, Ikerbasque, Basque Foundation for Science, Department of Neuroscience, The University of the Basque Country UPV/EHU, Technological Park, Bldg. 205, Floor -1, Laida Bidea, Zamudio, Vizcaya, 48170, Spain. E-mail: j.rodriguez-arellano@ikerbasque.org.

The perception of environmental chemical signals is in all likelihood inseparable from life existence, and the proto-cells certainly needed to detect the most basic chemical clues indicating changes in the immediate neighbourhood. We do not know what the very first chemical receptor was originally, yet we do know that bacteria have chemosensitivity and chemotaxis. Even more important, bacteria have developed the sensitivity to biologically produced chemicals, which are accumulating in the cytosol of the living cells and are released when damaged. This sensitivity is strictly survivalism, which allowed the cells to detect the danger signal produced by their dying relatives; yet this formed the basis for future neurotransmission. The first intercellular signaling molecules were ATP and glutamate, which are highly concentrated in the cytosol of living cells.[13]

Therefore, the very first multicellular organisms were in possession of several signaling systems associated with plasmalemmal channels, plasmalemmal receptors, and exocytotic machinery. The multicellularity brought with it another signaling mechanism made by transcellular channels (generally known as gap junctions), which established the direct communication route in cellular syncytia. The development of the multicellular species induced specialization of cellular layers and appearance of tissues; the surface layer developed into epithelial cells. The epithelial cells were endowed with ion channels; many of these cells possessed exocytotic vesicles; these epithelial cells were connected by gap junctions, and, most importantly, these cells were in direct contact with the environment. Therefore, it is not surprising that the epithelial cells became the ancestors of the nervous system.

The very first neural elements were diffusely scattered throughout the outer surface of the body of *Cnidaria* (i.e, jelly fishes, box gellies, sea anemones, and Hydrozoa). These nervous elements are primarily sensory and they are already attaining a degree of specialization. For example, in Hydra the nervous elements are represented by touch-sensitive and photosensitive cells, and they are connected in a simple nervous net through neuritis interwoven in between the epithelial cells.[14] With an increased complexity of organisms the first neuronal conglomerates represented by sensory organs and primordial nerve ganglia have evolved, and this increase in complexity and neuronal specialization coincided with the appearance of neuroglia, which already at this early stage controlled development and functional activity of the neuronal networks.[15,16]

The appearance of the CNS, with its clearly distinct central and peripheral parts was associated with further specialization of neurons and evolutionary progression of neuroglia. The latter became more complex, evolving into several functionally idiosyncratic cellular populations, represented by astroglia, NG-2 glia, and myelinating cells, represented by oligodendrocytes and Schwann cells. The astrocytes and NG-2 cells populate the grey matter, whereas oligodendrocytes and Schwann cells cover and myelinate the axons providing the latter with insulation that greatly increased action potential conduction velocity. The microglial cells are migrants, the cells of myeloid origin that invade the brain in the early postnatal period and form the neural immune/defence system. Therefore, the neuroglia assumes full responsibility for the nervous system homeostasis and defense.

The evolution of the primate brain and the emergence of intellect coincided with dramatic changes in neuroglia. First, the numbers exploded, and the neuroglia became the most numerous cell type in the human brain, outnumbering neurons by several times.[17] Second, the morphology had also changed; the human protoplasmic and fibrous astrocytes are 2 to 3 times larger as compared to rodents.[18,19] Even more importantly, the human astrocytes are immensely more complex[18]; each human astrocytes has ~10 times more primary processes than the rodent one; the arborization is also infinitely more complex and human protoplasmic astrocyte covers and integrates ~2 million synapses, whereas rodent astrocytes cover ~20,000 to 120,000 synaptic contacts. In addition, several primate specific astrocytes (e.g. interlaminar and polarized astrocytes) are involved in interlayer integration in the cortex.[18,19]

FUNCTIONS OF ASTROGLIA

Structural function

All neural elements develop from the neuroepithelial cells that at the very beginning of the embryogenesis gave birth to radial glia, which served as both the source of neural precursors and the scaffold that allowed neural cells to reach their final destination in the grey matter. The astrocytes, being direct descendants of the radial glia, shape the grey matter through the process of tiling.[20–22] Every protoplasmic astrocyte occupies it own territory, where its processes cover neuronal membranes and synaptic contacts. The astrocytes also send processes to the neighboring blood vessels, and in this way they form a neurovascular unit.[22]

Metabolic support

The neurovascular unit provides for a metabolic connection between blood vessels and parenchyma of the brain. First, astrocytes integrate the neuronal activity with the local blood flow being responsible for the functional hyperaemia, which is manifested by a rapid vasodilatation after a local increase in neuronal firing. The increased synaptic transmission induces astroglial Ca^{2+} signaling that travel to the perivascular processes of astroglial cells and triggers release of vasoactive substances from the endfeet.[23–26] Second, astrocytes provide

active neurons with metabolic substrates via a glucose-lactate shuttle. Increased neuronal activity leads to an increase in glutamate release, which in turn activates astroglial Na^+-dependent glutamate transporters. The latter mediate substantial Na^+ influx, and thus increase in cytosolic Na^+ concentration in astrocytes. In turn, increased Na^+ stimulates glycolysis and lactate synthesis. The lactate is subsequently transported to neurons through specific transporters.[27,28]

Brain homeostasis

The brain function is impossible without tight control over the extracellular environment, which includes regulation of extracellular concentrations of ions, metabolites, and neuroactive molecules. Extracellular ion homeostasis is particularly important for K^+ ions, because the latter are accumulated in quantities during neuronal activity, due to repetitive opening of neuronal K^+ channels with subsequent K^+ efflux. Increase in extracellular K^+ concentration in turn depolarizes neuronal membranes, thus altering their excitability. In physiological conditions, extracellular K^+ can rise from ~5 mM to 10 to 12 mM during periods of robust neuronal activity; in pathology, K^+ can rise much higher, attaining levels of up to 50 mM.[29] Extracellular K^+ homeostasis is mainly carried out by astrocytes through local K^+ uptake (via inward rectifier K^+ channels) and spatial K^+ buffering.[29,30] The spatial K^+ buffering is achieved through redistribution of K^+ within glial syncytia or even within single polarized glial cells from the areas with elevated $[K^+]_o$ to the regions with low $[K^+]_o$. This K^+ uptake and spatial buffering is coupled with astroglial water transport. Increases in synaptic activity are associated with local decreases in extracellular volume, which is regulated by water transport across astroglial membranes and water redistribution through the glial syncytium. Astroglial water transport is functionally linked to activation of water channels aquaporins that are concentrated in perisynaptic processes and in the astroglial endfeet structures.[31]

Astroglial cells are central elements of homeostasis of neurotransmitters in the brain. They are particularly important for homeostasis and turnover of the main excitatory neurotransmitter glutamate being the main sink of glutamate in the brain; from the bulk of glutamate released during synaptic transmission, approximately 20% is accumulated into neurons, whereas the remaining 80% is taken up by perisynaptic astrocytes.[32,33] Removal of extracellular glutamate from the extracellular space is vitally important for preventing its excitotoxicity. Astroglial glutamate transport is the function of specific glutamate transporters excitatory amino-acid transporter 1 and excitatory amino-acid transporter 2, which are expressed exclusively in astrocytes.[34] Glutamate transport is driven by transmembrane gradient for Na^+ translocation of every glutamate molecule, which is accompanied by an influx of 3 Na^+ ions and 1 H^+ ion, coupled with the efflux of 1 K^+ ion, making this transport electrogenic.[35] Activation of glutamate transporters is associated with substantial Na^+ fluxes and increase in $[Na^+]_i$,[36] which serves as a signal for a glucose-lactate shuttle described in the previous section. The excess of intracellular Na^+ is removed by sodium-calcium exchanger, which is conveniently co-localized with glutamate transporters in perisynaptic processes; increased $[Na^+]_i$ turns the exchanger into the reverse mode, thus rapidly reducing cytosolic Na^+ loads.[36–38]

The glutamate accumulated by astrocytes is critically important for the overall glutamate turnover in the brain. Glutamate after entering astrocytes is converted into glutamine by the glutamine synthetase.[39] The nontoxic glutamine is then transported back to the presynaptic terminal through the extracellular space; in the neuronal cytoplasm glutamine is converted back into glutamate, which is accumulated by synaptic vesicles, thus accomplishing the glutamate–glutamine shuttle.

Signaling in neuronal-glial circuits

1) Glial cells express neurotransmitter receptors. Glial expression of neurotransmitter receptors was discovered in 1984 when glutamate and GABA-induced electrical responses were recorded from cultured astrocytes and oligodendrocytes.[40–42] Subsequent *in vitro* experiments have demonstrated that glial cells express the very same diverse variety of neurotransmitter receptors and ion channels as do neurons,[43–55] thus raising the question of the role for neuroglia in information processing in the brain. Further experiments have found that the expression pattern of neurotransmitter receptors *in situ* is very much restricted by the immediate neurotransmitter environment; as a consequence glial cells are properly endowed to sense the neurotransmitters released in their territorial domains.[56–59] The expression of neurotransmitter receptors in astrocytes from different brain regions is extremely heterogeneous; although most of astroglial cells express receptors to purines and to glutamate.[33,60–62] Importantly, astrocytes and oligodendrocytes possess a special type of NMDA glutamate receptors, which, in contrast to neurons, are devoid of Mg^{2+} block[63–65] and therefore can be activated at characteristically negative glial resting potentials (approximately ~80 to -90 mV).

2) The tripartite synapse. The synapses in the CNS are formed by three elements: by the pre- and postsynaptic neuronal compartments and by the astroglial perisynaptic processes. This structure is generally known as a tripartite synapse.[66,67] The neurotransmitters released in the course of synaptic transmission from the neuronal terminal are stimulating the astroglial receptors

of both ionotropic and metabotropic varieties,[33,62] thus providing the information input to neuroglial circuitry.

3) Signaling in astroglial syncytia. In contrast to neuronal networks, which are constructed from physically separated neuronal cells, astrocytes are integrated into physically continuous structures known as astroglial syncytia. This integration is achieved through the gap junctions expressed in the peripheral portions of astroglial processes. The gap junctions are formed by intercellular channels, the connexons.[68] The latter create relatively big pores, which span through the plasmalemma of adjacent cells. The connexon pore is permeable to molecules with molecular weight ~1 KD and it is instrumental for long-range glial signaling. Astroglial syncytia are anatomically localized and segregated; for example, in somatosensory cortex these syncytia are confined to individual barrels and do not have inter-barrel connectivity.[69,70]

The intercellular communication route provides the substrate for astroglial long-range signaling. Indeed, the glial cells are electrically nonexcitable and are unable to generate propagating action potential. Nonetheless, astrocytes are using the intracellular organelle, the endoplasmic reticulum (ER) to generate intra- and intercellular signals. The ER has many functions, which include protein synthesis and post-translational protein modification, as well as intracellular transport of various molecules. In addition, the ER acts as a universal dynamic intracellular Ca^{2+} store,[71–75] which plays the central role in Ca^{2+}-signal generation in both nonexcitable and excitable cells.

Ca^{2+} ions are universal and ubiquitous intracellular second messengers that control an exceedingly wide range of cellular reactions. The Ca^{2+}-signaling system is one of the most ancient, and it is operative in virtually all living forms.[1,76,77] The ER participates in Ca^{2+}- signaling through Ca^{2+} release and Ca^{2+} accumulation.[78,79] The ER membrane contains Ca^{2+} pumps (the sarco[endo]plasmic reticulum ATP-ases (or SERCAs) that transport Ca^{2+} into the ER lumen.[80] The intra-ER Ca^{2+} concentration is very high (range, 0.5 to 1 mM),[81,82] which creates a steep concentration gradient aimed at the cytosol. Importantly, the lumen of the ER is internally continuous and Ca^{2+} can rapidly equilibrate within the organelle through unopposed diffusion.[83–85] The ER membrane is also endowed with two classes of Ca^{2+} release channels (i.e, the Ca^{2+}-gated Ca^{2+} channels, generally known as ryanodine receptors, or RyRs, and Inositol 1,4,5-trisphosphate [$InsP_3$]-gated channels, or $InsP_3$ receptors).[86,87] Both channels are sensitive to cytosolic Ca^{2+} (thus being able to produce Ca^{2+}-induced Ca^{2+} release). In addition, the $InsP_3$ receptors are sensitive to intracellular second messenger $InsP_3$. The $InsP_3$ is produced by phospholypase C, which in turn is linked to plasmalemmal metabotropic receptors via G proteins. The $InsP_3$-meidated Ca^{2+} release is central for astroglial Ca^{2+} signaling, and activation of metabotropic glial receptors triggers both local and propagating Ca^{2+} release from the ER.[88,89] Importantly, glial Ca^{2+} signals are capable of propagating though glial syncytia,[90–92] using several complimentary mechanisms that include diffusion of $InsP_3$ through gap junctions, or release and extracellular diffusion of gliotransmitter.[93–96] These propagating glial Ca^{2+} waves are the most thoroughly investigated mechanism of long-range glial signaling; nonetheless, many other molecules (e.g., metabolic substrates, ATP, or other second messengers) can also participate in signaling within astroglial circuits.

4) The gliotransmission. Excitation of astroglial cells and astroglial Ca^{2+} waves trigger the release of gliotransmitters. These gliotransmitters include glutamate, ATP, D-serine, GABA, taurine, and mediate glial-neuronal and glial-glial signaling.[97–103] The leading mechanism for gliotransmitters release is exocytotic,[104–106] although diffusion though large-pore plasmalemmal channels can also be involved.[107–110]

5) Glia and information processing in the brain. The ability of neuroglia to detect neurotransmitters, to produce active responses after stimulation of various receptors to generate propagating signals and to release gliotransmitters, naturally questioned their role in the information processing in the brain. We already know that astrocytes may actively modulate transmission in neuronal networks and affect synaptic plasticity[111]; we may also assume that astroglial circuits can, together with neurons, participate in cognition, learning, and memory. However, this remains an assumption, and more experimental data are required to understand the role of glial cells in higher brain functions.

NEUROLOGICAL DISORDERS AS GLIOPATHOLOGY: THE ROLE OF ASTROGLIA

Diseases of the nervous system remain the most difficult to handle and to cure; the therapeutic advances in neurology are at best modest when compared to other branches of medicine. The reason is simple; it is the singular complexity of the human brain and its connections, both morphological and functional.

For a long time the neurocentric view dominated the neuropathological theories, although the pathological potential of glia was already acknowledged by prominent neuropathologists of the 19th century, such as Alzheimer,[112] Frommann,[113] and Nissl.[114] Nonetheless, it is now clear that it is neuroglia, which determines the progression and outcome of most, if not all, neurological diseases.[115,116] Indeed, the brain homeostasis is managed solely by the neuroglia, and the failure of neuroglia to maintain this homeostasis is fatal for the nervous tissue.

This is particularly manifest in the ischemic insult in which performance of astroglia very much determines the development of the ischemic core and its relations with penumbra.[117] In addition, the astroglia possess a specific defensive mechanism, (i.e., the astrogliosis that is activated in response to brain insults).[118,119] The astrogliosis is fundamental for limiting the areas of damage (by scar formation through anisomorphic astrogliosis) and for the post-insult remodeling and recovery of neural function (by isomorphic astrogliosis).

Astroglia is involved in pathogenesis of many chronic neurological disorders.[67,120] For example, astrocytes undergo remodeling in the epileptic brain, which includes both morphological and functional changes.[121,122] Astrocytes are also important for pathogenesis of various psychiatric disorders. The astrocytes may play an important role in schizophrenia, because failures in astroglia-dependent glutamate homeostasis can result in neurotransmission disbalance.[123]

ASTROCYTES IN NEURODEGENERATIVE DISEASES

The neurodegenerative disorders are arguably the most fearsome human diseases because they destroy our intellect and reduce human beings to the animal state. The neurodegenerative diseases are also uniquely the property of mankind, because as a rule they do not occur in animals, making one wonder whether they may represent a price for the exclusive power of our brain. The neurodegenerative processes start with disruptions in the connectivity within the brain circuitry,[124–127] which affect cognitive functions and underlie the early stages of the disease. Further pathological development of the neurodegenerative process results in neural cell death and general atrophy of the brain, manifested by the disappearance of higher brain functions.

The pathological potential of astroglia in neurodegeneration began to be explored only very recently, as for a long time neurodegenerative diseases were associated primarily with neuronal death. Nonetheless, it is quite obvious now that the astroglia is invariably affected at the early stages of neurodegenerative process, and this determines to a large extent the progression and severity of the disease. Several recent investigations discovered astroglial atrophy, which appears at the very early stages of different neurodegenerative diseases. Conceptually atrophic changes in astrocytes may lie at the very core of initial disruption of neural circuitry, as reduced astroglial support affects maintenance and performance of synapses. Several articles, published in this special issue discuss the role of neuroglia in various neurodegenerative processes in detail; here we shall briefly overview evidence for astroglial atrophic changes in the most frequent forms of neurodegenerative diseases.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) described by Charcot[128] and Charcot and Joffroy[129] is manifested by the degeneration of motor neurons from the cortex, the brain stem, and the spinal cord. The causes and aetiology of ALS remain generally unknown, although approximately 20% of cases are associated with dominant mutations in the gene coding for Cu–Zn superoxide dismutase (SOD1).[130]

Neuroglial reactions play an important role in ALS pathology. Prominent astroglial degeneration and atrophy was found in the h(uman)SOD1^{G93A} transgenic mouse; this astrodegeneration preceded both neuronal death and the appearance of clinical symptoms.[120,131] Incidentally, the ALS astrocytes (expressing hSOD1) were specifically sensitive to glutamate, and contrary to healthy astrocytes they displayed glutamate excitotoxicity.[120,131] Even more importantly, selective silencing of the SOD1 mutant gene in astrocytes significantly slowed the progression of ALS in transgenic mice.[132] Late stages of ALS are characterized by significant astrogliosis and astroglial proliferation.[133,134]

Parkinson's disease

The symptoms of Parkinson's disease (akinesia, rigidity, tremor at rest, and postural abnormalities[135]) develop because of specific degeneration and demise of dopaminergic neurons in substantia nigra. The role of astrocytes in the pathogenesis of the Parkinson's disease has not been characterized; although astrogliosis was detected at the late stages of the disease.[136,137] At the same time substantia nigra, in which Parkinson's disease pathology primarily develops, has a low density of astrocytes compared to other brain regions and early astroglial atrophy may have a pathological significance; astrodegeneration can result in diminished support of domapinergic neurons associated with an increase of their vulnerability. However, this hypothesis has to be experimentally tested.

Non-AD dementia

Profound changes in astrocytes are observed in many types of non-AD dementia-related neurodegeneration. For example, the early stages of frontotemporal dementia are characterized by significant astroglial degeneration and apoptotic death of astrocytes.[138] Importantly, the depth of glial atrophy correlated with the severity of dementia. However, other studies found prominent astrogliosis in postmortem tissues form patients with frontotemporal dementia.[139] Similarly, prominent astrogliosis leads to the development of thalamic dementia, in which neuronal loss is secondary to pathological remodeling of astroglia.[140] Both astrogliosis and astroglial atrophy were observed in immunodeficiency virus-1 (HIV-1) associated dementia, in which astrogliosis in the initial phase is followed by significant astro-

cytic death; the loss of astrocytes correlates with the severity of cognitive impairments.[141,142] Astrocytes can also be a target for tau pathololgy, and specific expression of tau protein in astroglial cells can trigger age-dependent neurodegeneration.[143,144] Impairment of astroglia is also involved in pathogenesis of Wernicke encephalopathy, which is associated with a very substantial reduction in expression of astroglial glutamate transporters; this results in compromised clearance of glutamate with subsequent neuronal death through excitotoxicity.[145,146]

Astroglia in Alzheimer's disease

The pathological modification of astrocytes in the demented brains were initially observed by Alois Alzheimer,[112] who had found glial cells abundantly populating neuritic plaques. The reactive astrogliosis has been subsequently confirmed to be an archetypical morphological feature of plaque-infested Alzheimer's disease (AD) brains at the late stages of the disease (FIG. 1); this astrogliosis was observed in both human tissues and in the brains isolated from AD animal models.[147–150]

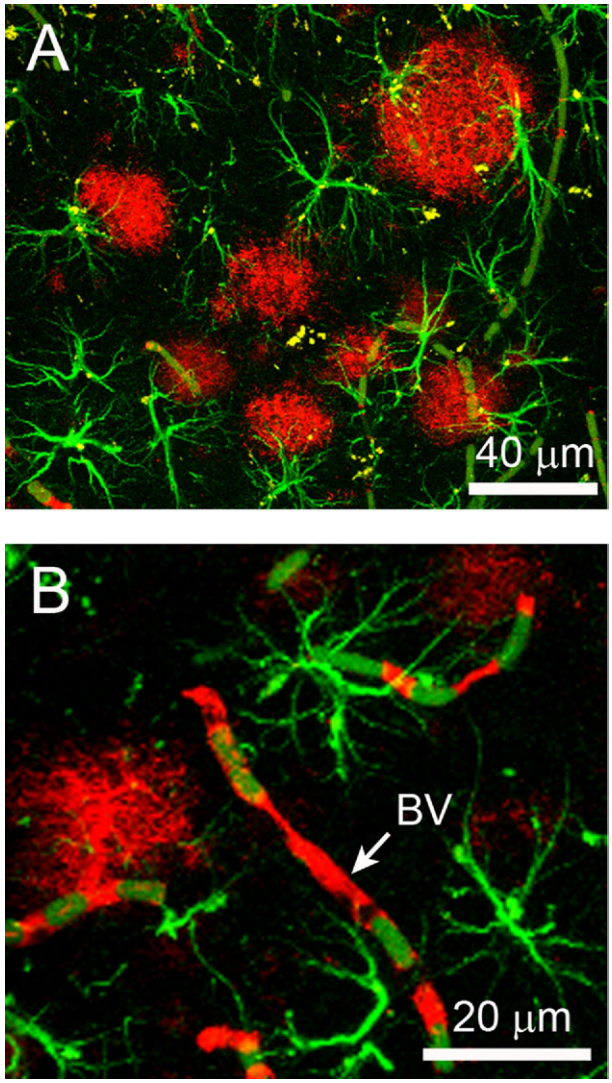

FIG. 1. Confocal images of hippocampal preparations dually labeled by GFAP and by anti-β amyloid monoclonal antibodies (GFAP in green and Aβ in red) illustrating differential changes in GFAP profiles in astrocytes associated with, and/or close to Aβ plaques (A, astrogliosis), as well as with vascular Aβ deposits (B). BV = blood vessel.

Morphology and numbers

Knowledge about the role of neuroglia in the progression of AD remains fragmentary, at best. Generalized astrogliosis, manifested by cellular hypertrophy and by an increase in expression of GFAP and astroglial S100B protein, was routinely observed in postmortem tissues from AD patients.[148,151–156] More detailed analysis of astrogliosis in the brains obtained from old patients (with and without confirmed AD) have demonstrated a correlation between the degree of astrogliosis and cognitive decline; however, the same analysis failed to reveal a direct correlation between astrogliotic changes and senile plaques.[157] The morphological data showed reactive astrocytes associated with some, but not with all Aβ plaques; astrogliotic fields were also found in areas without Aβ depositions in both AD and non-AD brains.[157] Moreover, there is no significant difference in GFAP expression in demented versus nondemented brains.[158]

The Aβ-independent astrogliosis may accompany normal brain aging, although the age-dependent changes of astroglia are in urgent need of proper investigation. The data describing astroglia in aged brains are scarce and controversial. For example, in rat retinal preparations, aging was associated with a decrease in the total number of astrocytes and with an increase in the proportion of cells with gliotic morphology.[159,160] Conversely, a rather significant (by one third) increase in the number of astrocytes was observed in hippocampus of female B57 mice[161]; similar age-dependent increase in astrocytes quantity was found in the CA1 hippocampal area and in the frontal cortex of male Sprague-Dawley rats; this was accompanied with hypertrophic remodeling that was more prominent in the cortex.[162] An increase (by ~20%) in the number of astrocytes was detected in parietal cortex and the dentate gyrus of old Wistar rats.[163,164] No change in the number of astroglial profiles was found in the primary visual cortex of old rhesus monkeys[165]; similarly, the quantity of astrocytes in the human neocortex did not change with age.[166] Significant increase in GFAP expression and astroglial hypertrophy was detected in the white matter of the brains of senescent monkeys, hinting for specific age-dependent alterations in axonal connectivity in the CNS.[167] Overall, not much in known regarding astroglia in the aged human brain; the generally accepted notion of an increased astrogliosis and increased astroglial numbers in the senescent brain[168] has

to be corroborated by further meticulous morphological analysis.

Astrogliosis and astroglial degeneration in AD

Reactive astrogliosis in AD can be initiated by several factors, which include signaling from damaged neurons/neuroglia, as well as extracellular deposition of the β-amyloid peptide (Aβ); the latter was shown to trigger astrogliosis *in vitro*.[169] Extracellular Aβ also affects physiological status of astroglial cells. Exposure of cultured astrocytes to β-amyloid induces spontaneous $[Ca^{2+}]_i$ signals and $[Ca^{2+}]_i$ oscillations, which somewhat contribute to astroglial neurotoxicity.[170,171] The abnormal, spontaneous Ca^{2+} oscillations and Ca^{2+} waves were also observed *in vivo* in astrocytes associated with neuritic plaques.[172] Furthermore, astrocytes in Aβ overexpressing transgenic mice demonstrated increased coupling in neocortical regions and had elevated expression of AMPA/kainate glutamate receptors and glutamate transporters.[173] In contrast, Aβ was reported to decrease expression and capacity of glutamate-aspartate transporter and glutamate transporter-1 mediated glutamate uptake in cultured astrocytes.[174] The activated astrocytes are intimately involved in the neuro-inflammatory component of the AD through the release of cytokines, pro-inflammatory factors, and nitric oxide/reactive oxygen species neurotoxicity.[124]

At the early stages of the AD pathology in the triple-transgenic mice (3xTg-AD), harboring the mutant genes for amyloid precursor protein (APP_{Swe}), presenilin $1PS1_{M146V}$, and tau_{P301L}[175]), the reduction in the morphological presence of astrocytes, indicative of astroglial degeneration/atrophy, was discovered.[149,150] In these experiments, the GFAP-positive astrocytes were morphologically analyzed in hippocampi of the 3xTg-AD mice of different ages (range, 3 to 18 months). It must be noted that GFAP labeling differs profoundly between brain regions; in hippocampus ~80% of astrocytes are GFAP-positive.[176] There were no significant age-dependent changes in the density of astrocytes in both control and AD brains. Already from 6 months of age, the astrocytes in CA1 and dentate gyrus of 3xTg-AD animals showed atrophic signs (i.e., decrease in the volume of GFAP-staining, decreased size of somatas, and decrease in number of processes (FIGS. 1 and 2). These changes became fully significant at older ages (range, 9 to 18 months).[149,150] Importantly, the appearance of senile plaques, which in the 3xTg-AD model occurs at 12 months of age in the CA1 region and at 18 months of age in the dentate gyrus, triggered morphological astrogliosis, but only in astrocytes directly associated with Aβ deposits; the astroglial cells distant to plaques remained atrophic.[149,150] Interestingly, that astroglial atrophy (manifested by decreased complexity of processes) was observed in postmortem analysis of the neocortex of demented patients.[177]

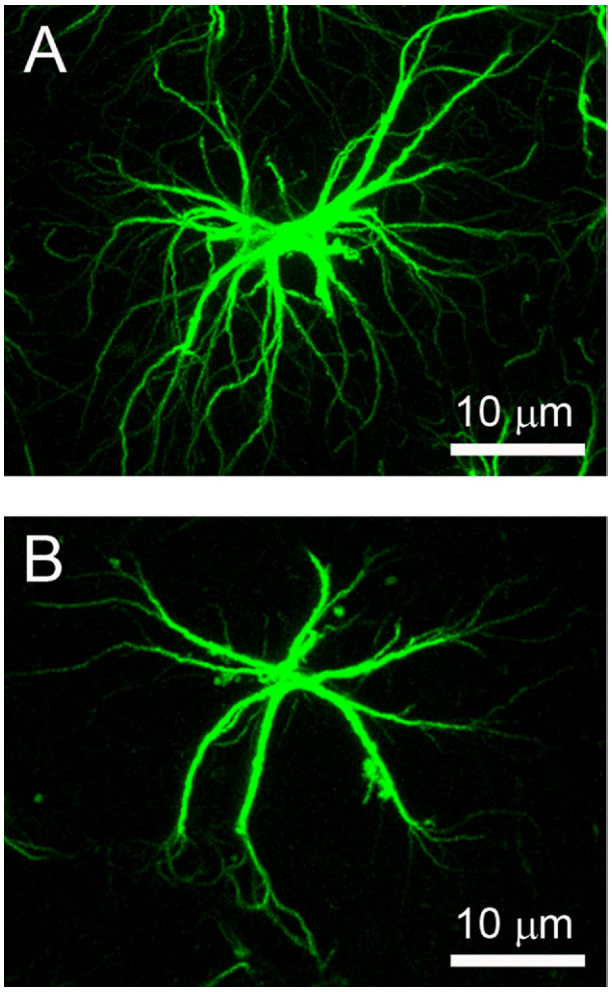

FIG. 2. Confocal micrographs of hippocampal astrocytes non-associated with Aβ plaques in transgenic mice model (3xTg-AD) of Alzheimer's disease. Note the evident astrocytic atrophy in the 3xTg-AD mice (B) when compared to the control animals (A).

Astroglia and β-amyloid

The role of astroglia in Aβ processing and metabolism represents another controversial matter. The reactive astrocytes in AD were suggested to participate in the clearance and degradation of β-amyloid (for review see References[178–180]). Indeed, activated astrocytes located in the close vicinity to Aβ plaques formed in the brains of transgenic APP mice were found to express neprilysin, the amyloid-degrading enzyme.[181] Accumulation of Aβ was observed in astrocytes from entorhinal cortex of AD patients,[147] although it was rarely found in astrocytes from 3xTG-AD mice.[149,150] Functional experiments also demonstrated the ability of astrocytes to phagocyte and degrade β-amyloid deposits in an *in vitro* system.[182] However, these experiments also demonstrated β-amyloid sequestration can be done only by astrocytes isolated

from healthy brains; the astroglial cells obtained from APP transgenic mice were ineffective.[182]

At the same time, the AD conditions may affect astroglia, turning them into Aβ producers. Production of Aβ requires the endoprotease known as β-site APP-cleaving enzyme 1 ([BACE 1] also referred to as β-secretase). In the healthy brain expression of BACE 1 seems to be exclusively confined to neurons. In conditions of AD-like pathology or even under chronic stress, astrocytes start to express BACE 1, thus acquiring Aβ−producing ability.[178] Astroglial BACE 1 was detected in activated astrocytes surrounding Aβ plaques in several transgenic AD mice models, such as Tg2576[183] and double mutated K670N-M671L APP.[184,185] Various brain insults that triggered astrogliosis (e.g., immunolesion of cholinergic septohippocampal afferents or occlusion of middle cerebral artery) also triggered astrocytic expression of BACE 1.[178] Similarly, increased APP production was detected in the rat model of chronic neocortical astrogliosis, induced by grafting foetal cortical tissue in the midbrain of neonatal animals; chronically activated astrocytes were immunostained for APP as well as for another AD-related marker apolipoprotein E.[186]

The neurovascular unit in AD: role for astrocytes

Vascular impairments represent an important factor in the pathology of AD. Numerous imaging studies of humans have found that significant reduction in blood flow in the brains of patients with AD and AD-like status indicated the role for vascular defects at the early stages of the disease (see References[187–189] for comprehensive review). Morphological analysis also found pronounced vascular pathology in AD brains.[190]

The elementary component of brain microcirculation is represented by a neurovascular unit, in which astrocytes integrate neurons, brain endothelium, pericytes, and vascular smooth muscle cells into a functionally independent entity.[23,188,189] In this structure, astrocytes assume the role of coordinating elements that establish the link between neuronal activity and local blood flow through several signaling cascades controlling vasoconstriction and vasodilatation.[23,24,26] Furthermore, astroglial endfeet, which plaster brain capillaries, regulate formation of tight junctions (i.e., controlling the blood-brain barrier) and have a central role in the transport of water and electrolytes, as well as in the utilization of glucose and providing neurons with energy substrates.[28,31,189,191] In AD, the neurovascular unit is specifically targeted because Aβ plaques often encompass brain capillaries (FIG. 1), thus affecting microcirculation and vascular Aβ clearance.[187] At the same time, the primary vascular pathology induces overproduction of Aβ through yet poorly characterized mechanisms.[188]

Control of local cerebral circulation and functional hyperemia accomplished by astrocytes is of fundamental importance for functional activity of neural networks. Pathological remodeling of the neurovascular unit that occurs in AD is likely to be associated with specific damage to astroglia, which may occur at the early stages of the disease and contribute to cognitive abnormalities. Presently, the mechanisms of AD-specific astroglia damage remain unknown, although atrophy of astrocytes may be also linked to neurovascular unit dysfunction.

Metabolic remodeling of astroglia in AD

Metabolic stress represents one of the early symptoms of AD-like pathology. Numerous functional imaging studies demonstrated significant and progressive decrease in glucose use from the very early stages of AD in humans.[192] The Aβ remodels astroglial metabolic phenotype *in vitro* by affecting glucose metabolism and increasing reactive oxygen species production in cultured astrocytes. The data on actual mechanisms of Aβ-dependent changes in glucose metabolic pathways are controversial. Several groups have found that Aβ decreased the astroglial use of glucose.[193–195] In contrast, treatment with Aβ significantly increased glucose use in cultured astroglial cells by enhancing the activity of all major glucose metabolism pathways and glycogenesis.[196] Furthermore, co-culturing neurons with astrocytes pre-treated with Aβ significantly decreased neuronal survival as compared with co-culturing with naive astrocytes.[196] Analysis of the activity of metabolic enzymes similarly yielded controversial results: both decrease[197,198] and increase[195,199] in the activity of enzymes associated with glucose metabolism have been reported in AD brain preparations. These discrepancies may reflect opposite cell-specific changes in glucose metabolism developing at different stages of AD.[196]

Astrodegeneration and failed synaptic connectivity: astroglia drive early cognitive decline in AD?

Cognitive deficits are the first signs of AD, which occur well before the development of disease-specific histopathology manifested by the appearance of senile plaques and neurofibrillary tangles.[200,201] This observation indicates disruptions in neural connectivity. These disruptions occur at the early stages of the disease and are responsible for the decline of the brain function. Numerous studies have demonstrated that synaptic weakness and synaptic loss are the earliest morphological correlates of the AD.[127,200] Moreover, clinical studies confirmed a strong correlation between the degree of dementia and the extent of synaptic loss.[202–204] In contrast, there is a rather poor correlation between the level of Aβ load and tangles expression and cognitive function.

Mechanisms of early synaptic failure in dementia and AD are obscure. Of course, synaptic loss may reflect neurodegenerative process solely associated with mal-

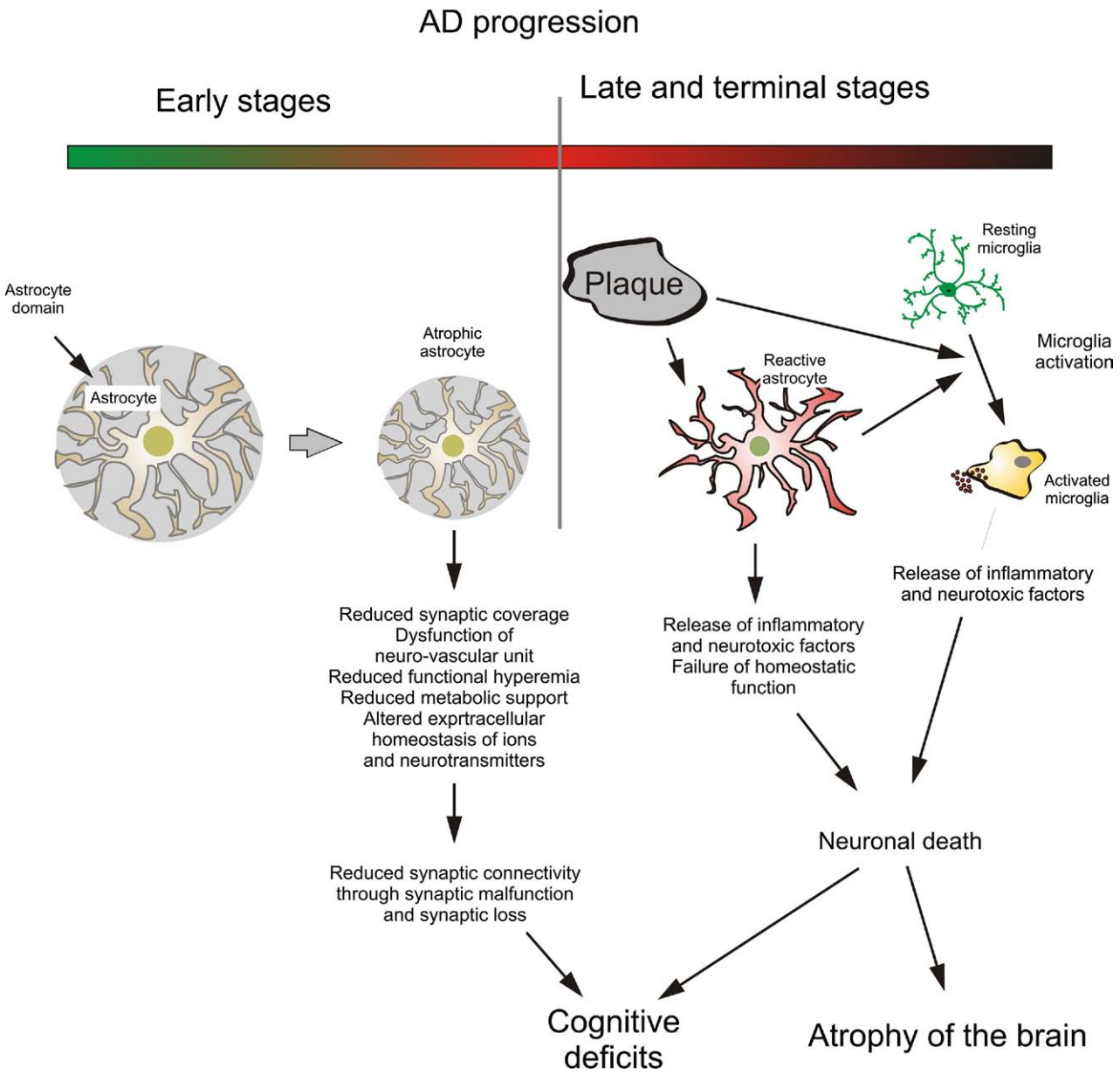

FIG. 3. Astroglial hypothesis of Alzheimer's disease (AD). The initial impairments of brain connectivity and synaptic transmission observed in AD (and possibly in other neurodegenerative diseases) can result from generalized atrophy of astrocytes. Atrophy of astroglia may cause reduced synaptic coverage, affect homeostasis of ions and neurotransmitters, alter neurovascular unit performance, and reduce metabolic support to neurons. These factors can contribute to synaptic malfunction and synaptic loss, thus causing early cognitive deficits. At the later stages of AD, appearance of senile plaques presents a strong pro-gliotic signal, which triggers activation of both astrocytes and microglia. Reactive astrocytes further reduce synaptic support and may exacerbate microglial activation. Reactive glia release inflammatory and neurotoxic factors, which induce neuronal death and brain atrophy, thus causing severe dementia. (Drawings of astrocytes were kindly provided by Prof. M. Nedergaard.)

function of neurons; yet the central role of astrocytes in brain homeostasis may justify alternative, astrocentric hypothesis (FIG. 3). Indeed, astrocytes are fundamentally important for synaptogenesis and synaptic maintenance. Astroglial transporters control the composition of the extra-synaptic environment and prevent local toxicity of glutamate or local depolarizations by excessive accumulation of K^+ ions. The glutamate-glutamine shuttle, expressed in astroglia, sustains neuronal glutamate levels, thus maintaining glutamatergic transmission. Finally, astrocytes provide local metabolic support, which, assuming excessive energy demands of synaptic compartment,[205,206] is critically important for neurotransmission. Therefore, we may suggest that atrophy of astroglia, which occurs at the early stages of AD and is likely to accompany early stages of other neurodegenerative diseases, determines synaptic malfunction, synaptic loss, and cognitive deficits.

Therapeutic implications

At present, there is no cure for AD or other neurodegenerative diseases; existing therapy is purely symptomatic. Numerous attempts to target β-amyloid depositions, although successful in reducing Aβ load, did not improve either cognitive status or disease progression.[207] Can the discovery of pathological relevance of astroglia lead to a cell-specific therapy/prevention of AD? Several strategies can be suggested.

First, the astroglia-specific molecules can be specifically targeted. The obvious candidate is GFAP, which is increased in reactive astrocytes. Reduction of GFAP expression affects synaptic plasticity,[208,209] whereas increase in GFAP expression induces various forms of encephalopathy and alters synaptic activity.[168] Conceptually, levels of GFAP expression can be affected by steroid hormones[168] and even by caloric restriction.[210] However, this strategy can be effective at the later stages of the AD characterized by prominent astrogliosis.

Second, molecules can be designed to affect astrocyte-specific homeostatic cascades (e.g., astroglial glutamate uptake). The neuroprotective drug Riluzole,[211] which inhibits neuronal glutamate release was also reported to enhance astroglial glutamate uptake.[212] Incidentally, the beta-lactam antibiotics, (e.g., represented by penicillin and ceftriaxone) increase astroglial expression of glutamate transporter-1 through gene activation.[213] Both compounds are considered potential drugs for the treatment of motor neuron diseases associated with glutamate excitotoxicity, resulting from astroglial deficiency.[214] Similar strategies may be adapted to the treatment of AD by reducing excitotoxic neuronal death and improving synaptic function.

However, the most promising strategy seems to be aimed at long-term modulation of astroglial function by promoting endogenous cell proliferation and differentiation. As astrocytes have some stem cell properties and can (at least in principle) re-enter the cell cycle, manipulation with these abilities can develop the true cell-specific therapy, which can be used for arresting AD progression at the very early stages.

CONCLUSIONS

Astrocytes are the central element of brain homeostatic system, which through their multiple functions provide for maintenance and defence of neural networks. Astroglial cells are specifically involved in various neurological diseases, determining their pathogenesis and outcome. Astrocytes are involved in all types of neurodegenerative processes, and display prominent remodelling in the AD; early dystrophic changes in astroglia can represent an important step in initiation and progression of Alzheimer's disease. Targeting of astroglia may provide a new principle for treatment of AD at the early stages of the disease.

Acknowledgments: The authors' research was supported by the Alzheimer's Research Trust (UK) Program Grant No. ART/PG2004A/1 to AV and JJR, the Grant Agency of the Czech Republic Grant No. GACR 309/09/1696) to JJR, and the Grant Agency of the Czech Republic Grant No. GACR 305/08/1381 and Grant No. GACR 305/08/1384 to AV.

REFERENCES

1. Case RM, Eisner D, Gurney A, Jones O, Muallem S, Verkhratsky A. Evolution of calcium homeostasis: from birth of the first cell to an omnipresent signalling system. Cell Calcium 2007;42:345–350.
2. Durell SR, Guy HR. A putative prokaryote voltage-gated Ca^{2+} channel with only one 6TM motif per subunit. Biochem Biophys Res Commun 2001;281:741–746.
3. Matsushita T, Hirata H, Kusaka I. Calcium channels in bacteria. Purification and characterization. Ann N Y Acad Sci 1989;560:426–429.
4. Shemarova IV, Nesterov VP. Evolution of mechanisms of calcium signaling: the role of calcium ions in signal transduction in prokaryotes. Zh Evol Biokhim Fiziol 2005;41:12–17.
5. Tisa LS, Sekelsky JJ, Adler J. Effects of organic antagonists of Ca^{2+}, Na^{+}, and K^{+} on chemotaxis and motility of *Escherichia coli*. J Bacteriol 2000;182:4856–4861.
6. Eckert R, Brehm P. Ionic mechanisms of excitation in Paramecium. Annu Rev Biophys Bioeng 1979;8:353–383.
7. Franciolini F, Petris A. Evolution of ionic channels of biological membranes. Mol Biol Evol 1989;6:503–513.
8. Koishi R, Xu H, Ren D, et al. A superfamily of voltage-gated sodium channels in bacteria. J Biol Chem 2004;279:9532–9538.
9. Shemarova IV, Nesterov VP. Evolution of Ca^{2+} signaling mechanisms. Role of calcium ions in signal transduction in lower eukaryotes. Zh Evol Biokhim Fiziol 2005;41:303–313.
10. Ladenburger EM, Sehring IM, Korn I, Plattner H. Novel types of Ca^{2+} release channels participate in the secretory cycle of Paramecium cells. Mol Cell Biol 2009;29:3605–3622.
11. Matt H, Plattner H, Reichel K, Lefort-Tran M, Beisson J. Genetic dissection of the final exocytosis steps in Paramecium tetraurelia cells: trigger analyses. J Cell Sci 1980;46:41–60.
12. Plattner H, Reichel K, Matt H, Beisson J, Lefort-Tran M, Pouphile M. Genetic dissection of the final exocytosis steps in Paramecium tetraurelia cells: cytochemical determination of Ca2+-ATPase activity over performed exocytosis sites. J Cell Sci 1980;46:17–40.
13. Burnstock G, Verkhratsky A. Evolutionary origins of the purinergic signalling system. Acta Physiol (Oxf) 2009;195:415–447.
14. Sakaguchi M, Mizusina A, Kobayakawa Y. Structure, development, and maintenance of the nerve net of the body column in Hydra. J Comp Neurol 1996;373:41–54.
15. Bacaj T, Tevlin M, Lu Y, Shaham S. Glia are essential for sensory organ function in *C. elegans*. Science 2008;322:744–747.
16. Reichenbach A, Pannicke T. Neuroscience. A new glance at glia. Science 2008;322:693–694.
17. Verkhratsky A, Butt A. Glial neurobiology. A textbook. Chichester: John Wiley & Sons, 2007.
18. Oberheim NA, Takano T, Han X, et al. Uniquely hominid features of adult human astrocytes. J Neurosci 2009;29:3276–3287.
19. Oberheim NA, Wang X, Goldman S, Nedergaard M. Astrocytic complexity distinguishes the human brain. Trends Neurosci 2006;29:547–553.
20. Bushong EA, Martone ME, Ellisman MH. Maturation of astrocyte morphology and the establishment of astrocyte domains during postnatal hippocampal development. Int J Dev Neurosci 2004;22:73–86.
21. Bushong EA, Martone ME, Jones YZ, Ellisman MH. Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. J Neurosci 2002;22:183–192.

22. Nedergaard M, Ransom B, Goldman SA. New roles for astrocytes: redefining the functional architecture of the brain. Trends Neurosci 2003;26:523–530.
23. Iadecola C, Nedergaard M. Glial regulation of the cerebral microvasculature. Nat Neurosci 2007;10:1369–1376.
24. Mulligan SJ, MacVicar BA. Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. Nature 2004;431:195–199.
25. Takano T, Tian GF, Peng W, et al. Astrocyte-mediated control of cerebral blood flow. Nat Neurosci 2006;9:260–267.
26. Zonta M, Angulo MC, Gobbo S, et al. Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. Nat Neurosci 2003;6:43–50.
27. Magistretti PJ. Neuron-glia metabolic coupling and plasticity. J Exp Biol 2006;209:2304–2311.
28. Magistretti PJ. Role of glutamate in neuron-glia metabolic coupling. Am J Clin Nutr 2009;90:875S–880S.
29. Kofuji P, Newman EA. Potassium buffering in the central nervous system. Neuroscience 2004;129:1045–1056.
30. Newman EA, Frambach DA, Odette LL. Control of extracellular potassium levels by retinal glial cell K^+ siphoning. Science 1984;225:1174–1175.
31. Simard M, Nedergaard M. The neurobiology of glia in the context of water and ion homeostasis. Neuroscience 2004;129:877–896.
32. Swanson RA. Astrocyte neurotransmitter uptake. In: Neuroglia. Kettenmann H, Ransom B, eds. Oxford: Oxford University Press, 2005:346–354.
33. Verkhratsky A, Kirchhoff F. Glutamate-mediated neuronal-glial transmission. J Anat 2007;210:651–660.
34. Danbolt NC. Glutamate uptake. Progr Neurobiol 2001;65:1–105.
35. Zerangue N, Kavanaugh MP. Flux coupling in a neuronal glutamate transporter. Nature 1996;383:634–637.
36. Kirischuk S, Kettenmann H, Verkhratsky A. Membrane currents and cytoplasmic sodium transients generated by glutamate transport in Bergmann glial cells. Pflugers Arch 2007;454:245–252.
37. Kirischuk S, Kettenmann H, Verkhratsky A. Na^+/Ca^{2+} exchanger modulates kainate-triggered Ca^{2+} signaling in Bergmann glial cells in situ. Faseb J 1997;11:566–572.
38. Minelli A, Castaldo P, Gobbi P, Salucci S, Magi S, Amoroso S. Cellular and subcellular localization of Na^+-Ca^{2+} exchanger protein isoforms, NCX1, NCX2, and NCX3 in cerebral cortex and hippocampus of adult rat. Cell Calcium 2007;41:221–234.
39. Martinez-Hernandez A, Bell KP, Norenberg MD. Glutamine synthetase: glial localization in brain. Science 1977;195:1356–1358.
40. Bowman CL, Kimelberg HK. Excitatory amino acids directly depolarize rat brain astrocytes in primary culture. Nature 1984;311:656–659.
41. Kettenmann H, Backus KH, Schachner M. Aspartate, glutamate and gamma-aminobutyric acid depolarize cultured astrocytes. Neurosci Lett 1984;52:25–29.
42. Kettenmann H, Gilbert P, Schachner M. Depolarization of cultured oligodendrocytes by glutamate and GABA. Neurosci Lett 1984;47:271–276.
43. Bevan S, Chiu SY, Gray PT, Ritchie JM. The presence of voltage-gated sodium, potassium and chloride channels in rat cultured astrocytes. Proc R Soc Lond B Biol Sci 1985;225:299–313.
44. Blankenfeld GV, Verkhratsky AN, Kettenmann H. Ca^{2+} channel expression in the oligodendrocyte lineage. Eur J Neurosci 1992;4:1035–1048.
45. Dave V, Gordon GW, McCarthy KD. Cerebral type 2 astroglia are heterogeneous with respect to their ability to respond to neuroligands linked to calcium mobilization. Glia 1991;4:440–447.
46. Enkvist MO, Holopainen I, Akerman KE. Glutamate receptor-linked changes in membrane potential and intracellular Ca^{2+} in primary rat astrocytes. Glia 1989;2:397–402.
47. Glaum SR, Holzwarth JA, Miller RJ. Glutamate receptors activate Ca^{2+} mobilization and Ca^{2+} influx into astrocytes. Proc Natl Acad Sci U S A 1990;87:3454–3458.
48. McCarthy KD, Salm AK. Pharmacologically-distinct subsets of astroglia can be identified by their calcium response to neuroligands. Neuroscience 1991;41:325–333.
49. Pearce B, Murphy S, Jeremy J, Morrow C, Dandona P. ATP-evoked Ca^{2+} mobilisation and prostanoid release from astrocytes: P2-purinergic receptors linked to phosphoinositide hydrolysis. J Neurochem 1989;52:971–977.
50. Sontheimer H, Ransom BR, Cornell Bell AH, Black JA, Waxman SG. Na^+-current expression in rat hippocampal astrocytes in vitro: alterations during development. J Neurophysiol 1991;65:3–19.
51. Verkhratsky AN, Trotter J, Kettenmann H. Cultured glial precursor cells from mouse cortex express two types of calcium currents. Neurosci Lett 1990;112:194–198.
52. Verkhratsky A, Shmigol A. Calcium-induced calcium release in neurones. Cell Calcium 1996;19:1–14.
53. Kirischuk S, Scherer J, Moller T, Verkhratsky A, Kettenmann H. Subcellular heterogeneity of voltage-gated Ca^{2+} channels in cells of the oligodendrocyte lineage. Glia 1995;13:1–12.
54. Verkhratsky A, Steinhauser C. Ion channels in glial cells. Brain Res Brain Res Rev 2000;32:380–412.
55. Rodriguez JJ, Mackie K, Pickel VM. Ultrastructural localization of the CB1 cannabinoid receptor in mu-opioid receptor patches of the rat Caudate putamen nucleus. J Neurosci 2001;21:823–833.
56. Kirischuk S, Moller T, Voitenko N, Kettenmann H, Verkhratsky A. ATP-induced cytoplasmic calcium mobilization in Bergmann glial cells. J Neurosci 1995;15:7861–7871.
57. Kirischuk S, Tuschick S, Verkhratsky A, Kettenmann H. Calcium signalling in mouse Bergmann glial cells mediated by a_1-adrenoreceptors and H_1 histamine receptors. Eur J Neurosci 1996;8:1198–1208.
58. Kirischuk S, Matiash V, Kulik A, Voitenko N, Kostyuk P, Verkhratsky A. Activation of P2-purino-, a_1-adreno and H_1-histamine receptors triggers cytoplasmic calcium signalling in cerebellar Purkinje neurons. Neuroscience 1996;73:643–647.
59. Verkhratsky A, Orkand RK, Kettenmann H. Glial calcium: homeostasis and signaling function. Physiol Rev 1998;78:99–141.
60. Kirischuk S, Kirchhoff F, Matyash V, Kettenmann H, Verkhratsky A. Glutamate-triggered calcium signalling in mouse Bergmann glial cells in situ: role of inositol-1,4,5-trisphosphate-mediated intracellular calcium release. Neuroscience 1999;92:1051–1059.
61. Verkhratsky A, Krishtal OA, Burnstock G. Purinoceptors on neuroglia. Mol Neurobiol 2009;39:190–208.
62. Verkhratsky A, Kirchhoff F. NMDA Receptors in Glia. Neuroscientist 2007;13:28–37.
63. Karadottir R, Cavelier P, Bergersen LH, Attwell D. NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. Nature 2005;438:1162–1166.
64. Lalo U, Pankratov Y, Kirchhoff F, North RA, Verkhratsky A. NMDA receptors mediate neuron-to-glia signaling in mouse cortical astrocytes. J Neurosci 2006;26:2673–2683.
65. Lipton SA. NMDA receptors, glial cells, and clinical medicine. Neuron 2006;50:9–11.
66. Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci 1999;22:208–215.
67. Halassa MM, Fellin T, Haydon PG. The tripartite synapse: roles for gliotransmission in health and disease. Trends Mol Med 2007;13:54–63.
68. Giaume C, Venance L. Intercellular calcium signaling and gap junctional communication in astrocytes. Glia 1998;24:50–64.
69. Giaume C, Maravall M, Welker E, Bonvento G. The barrel cortex as a model to study dynamic neuroglial interaction. Neuroscientist 2009;15:351–366.
70. Houades V, Koulakoff A, Ezan P, Seif I, Giaume C. Gap junction-mediated astrocytic networks in the mouse barrel cortex. J Neurosci 2008;28:5207–5217.
71. Berridge MJ. The endoplasmic reticulum: a multifunctional signaling organelle. Cell Calcium 2002;32:235–249.
72. Berridge MJ, Irvine RF. Inositol phosphates and cell signalling. Nature 1989;341:197–205.
73. Verkhratsky A, Petersen OH. The endoplasmic reticulum as an integrating signalling organelle: from neuronal signalling to neuronal death. Eur J Pharmacol 2002;447:141–154.

74. Verkhratsky A. Physiology and pathophysiology of the calcium store in the endoplasmic reticulum of neurons. Physiol Rev 2005; 85:201–279.
75. Kostyuk P, Verkhratsky A. Calcium stores in neurons and glia. Neuroscience 1994;63:381–404.
76. Petersen OH, Michalak M, Verkhratsky A. Calcium signalling: Past, present and future. Cell Calcium 2005;38:161–169.
77. Verkhratsky A. Calcium ions and integration in neural circuits. Acta Physiol (Oxf) 2006;187:357–369.
78. Burdakov D, Petersen OH, Verkhratsky A. Intraluminal calcium as a primary regulator of endoplasmic reticulum function. Cell Calcium 2005;38:303–310.
79. Verkhratsky A. The endoplasmic reticulum and neuronal calcium signalling. Cell Calcium 2002;32:393–404.
80. Wuytack F, Raeymaekers L, Missiaen L. Molecular physiology of the SERCA and SPCA pumps. Cell Calcium 2002;32:279–305.
81. Solovyova N, Verkhratsky A. Monitoring of free calcium in the neuronal endoplasmic reticulum: an overview of modern approaches. J Neurosci Methods 2002;122:1–12.
82. Solovyova N, Veselovsky N, Toescu EC, Verkhratsky A. Ca^{2+} dynamics in the lumen of the endoplasmic reticulum in sensory neurons: direct visualization of Ca^{2+}-induced Ca^{2+} release triggered by physiological Ca^{2+} entry. Embo J 2002;21:622–630.
83. Petersen OH, Tepikin A, Park MK. The endoplasmic reticulum: one continuous or several separate Ca^{2+} stores? Trends Neurosci 2001;24:27127–6.
84. Solovyova N, Verkhratsky A. Neuronal endoplasmic reticulum acts as a single functional Ca^{2+} store shared by ryanodine and inositol-1,4,5-trisphosphate receptors as revealed by intra-ER [Ca^{2+}] recordings in single rat sensory neurones. Pflugers Arch 2003;446:447–454.
85. Petersen OH, Verkhratsky A. Endoplasmic reticulum calcium tunnels integrate signalling in polarised cells. Cell Calcium 2007; 42:373–378.
86. Bezprozvanny I. The inositol 1,4,5-trisphosphate receptors. Cell Calcium 2005;38:261–272.
87. Hamilton SL. Ryanodine receptors. Cell Calcium 2005;38:253–260.
88. Deitmer JW, Verkhratsky AJ, Lohr C. Calcium signalling in glial cells. Cell Calcium 1998;24:405–416.
89. Grosche J, Matyash V, Moller T, Verkhratsky A, Reichenbach A, Kettenmann H. Microdomains for neuron-glia interaction: parallel fiber signaling to Bergmann glial cells. Nat Neurosci 1999;2: 139–143.
90. Cornell Bell AH, Finkbeiner SM, Cooper MS, Smith SJ. Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. Science 1990;247:470–473.
91. Dani JW, Chernjavsky A, Smith SJ. Neuronal activity triggers calcium waves in hippocampal astrocyte networks. Neuron 1992; 8:429–440.
92. Scemes E, Giaume C. Astrocyte calcium waves: what they are and what they do. Glia 2006;54:716–725.
93. Anderson CM, Bergher JP, Swanson RA. ATP-induced ATP release from astrocytes. J Neurochem 2004;88:246–256.
94. Arcuino G, Lin JH, Takano T, Liu C, Jiang L, Gao Q, et al. Intercellular calcium signaling mediated by point-source burst release of ATP. Proc Natl Acad Sci U S A 2002;99:9840–9845.
95. Bennett MR, Farnell L, Gibson WG. A quantitative model of purinergic junctional transmission of calcium waves in astrocyte networks. Biophys J 2005;89:2235–2250.
96. Suadicani SO, Brosnan CF, Scemes E. $P2X_7$ receptors mediate ATP release and amplification of astrocytic intercellular Ca^{2+} signaling. J Neurosci 2006;26:1378–1385.
97. Angulo MC, Le Meur K, Kozlov AS, Charpak S, Audinat E. GABA, a forgotten gliotransmitter. Prog Neurobiol 2008;86:297–303.
98. Bezzi P, Carmignoto G, Pasti L, et al. Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. Nature 1998; 391:281–285.
99. Bezzi P, Gundersen V, Galbete JL, et al. Astrocytes contain a vesicular compartment that is competent for regulated exocytosis of glutamate. Nat Neurosci 2004;7:613–620.
100. Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G. Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. Neuron 2004;43: 729–743.
101. Jourdain P, Bergersen LH, Bhaukaurally K, et al. Glutamate exocytosis from astrocytes controls synaptic strength. Nat Neurosci 2007;10:331–339.
102. Oliet SH, Mothet JP. Regulation of N-methyl-D-aspartate receptors by astrocytic D-serine. Neuroscience 2009;158:275–283.
103. Volterra A, Meldolesi J. Astrocytes, from brain glue to communication elements: the revolution continues. Nat Rev Neurosci 2005;6:626–640.
104. Montana V, Malarkey EB, Verderio C, Matteoli M, Parpura V. Vesicular transmitter release from astrocytes. Glia 2006;54:700–715.
105. Parpura V, Mohideen U. Molecular form follows function: (un-)snaring the SNAREs. Trends Neurosci 2008;31:435–443.
106. Reyes RC, Parpura V. The trinity of Ca^{2+} sources for the exocytotic glutamate release from astrocytes. Neurochem Int 2009; 55:2–8.
107. Cotrina ML, Lin JH, Alves-Rodrigues A, et al. Connexins regulate calcium signaling by controlling ATP release. Proc Natl Acad Sci U S A 1998;95:15735–15740.
108. Duan S, Anderson CM, Keung EC, Chen Y, Swanson RA. $P2X_7$ receptor-mediated release of excitatory amino acids from astrocytes. J Neurosci 2003;23:1320–1328.
109. Kang J, Kang N, Lovatt D, et al. Connexin 43 hemichannels are permeable to ATP. J Neurosci 2008;28:4702–4711.
110. Pankratov Y, Lalo U, Verkhratsky A, North RA. Vesicular release of ATP at central synapses. Pflugers Arch 2006;452:589–597.
111. Perea G, Navarrete M, Araque A. Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci 2009; 32:421–431.
112. Alzheimer A. Beiträge zur Kenntnis der pathologischen Neuroglia und ihrer Beziehungen zu den Abbauvorgängen im Nervengewebe. In: Nissl F, Alzheimer A, eds. Histologische und Histopathologische Arbeiten über die Grosshirnrinde mit besonderer Berücksichtigung der pathologischen Anatomie der Geisteskrankheiten Jena: Verlag von Gustav Fischer 1910:401–562.
113. Frommann C. Untersuchungen über die Gewebsveränderungen bei der Multiplen Sklerose des Gehirns und Rückenmarks. Jena: Verlag von Gustav Fischer, 1878.
114. Nissl F. Ueber einige Beziehungen zwischen Nervenzellerkrankungen und gliösen Erscheinungen bei verschiedenen Psychosen. Arch. Psychiat 1899;32:1–21.
115. Giaume C, Kirchhoff F, Matute C, Reichenbach A, Verkhratsky A. Glia: the fulcrum of brain diseases. Cell Death Differ 2007; 14:1324–1335.
116. Nedergaard M, Rodriguez JJ, Verkhratsky A. Glial calcium and diseases of the nervous system. Cell Calcium 2010;47:140–149.
117. Nedergaard M, Dirnagl U. Role of glial cells in cerebral ischemia. Glia 2005;50:281–286.
118. Li L, Lundkvist A, Andersson D, et al. Protective role of reactive astrocytes in brain ischemia. J Cereb Blood Flow Metab 2008; 28:468–481.
119. Pekny M, Nilsson M. Astrocyte activation and reactive gliosis. Glia 2005;50:427–434.
120. Rossi D, Volterra A. Astrocytic dysfunction: Insights on the role in neurodegeneration. Brain Res Bull 2009;80:224–232.
121. Jabs R, Seifert G, Steinhauser C. Astrocytic function and its alteration in the epileptic brain. Epilepsia 2008;49(suppl 2):3–12.
122. Seifert G, Schilling K, Steinhauser C. Astrocyte dysfunction in neurological disorders: a molecular perspective. Nat Rev Neurosci 2006;7:194–206.
123. Tsai G, Coyle JT. Glutamatergic mechanisms in schizophrenia. Annu Rev Pharmacol Toxicol 2002;42:165–179.
124. Heneka MT, Rodriguez JJ, Verkhratsky A. Neuroglia in neurodegeneration. Brain Res Rev 2010;63:189–211.
125. Kano M, Hashimoto K. Synapse elimination in the central nervous system. Curr Opin Neurobiol 2009;19:154–161.
126. Scheff SW, Price DA, Schmitt FA, DeKosky ST, Mufson EJ.

Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. Neurology 2007;68:1501–1508.
127. Terry RD. Cell death or synaptic loss in Alzheimer disease. J Neuropathol Exp Neurol 2000;59:1118–1119.
128. Charcot JM. Amyotrophic lateral sclerosis: symptomatology. In: Lectures on diseases of the nervous system London: New Sydenham Society, 1881:192–204.
129. Charcot JM, Joffroy A. Deux cas d'atrophie musculaire progressive avec lesions de la substance grise et de faisceaux anterolateraux de la moelle epiniere. Arch Physiol Norm Pathol 1869;1: 354–367.
130. Turner BJ, Talbot K. Transgenics, toxicity and therapeutics in rodent models of mutant SOD1-mediated familial ALS. Prog Neurobiol 2008;85:94–134.
131. Rossi D, Brambilla L, Valori CF, et al. Focal degeneration of astrocytes in amyotrophic lateral sclerosis. Cell Death Differ 2008;15:1691–1700.
132. Yamanaka K, Chun SJ, Boillee S, et al. Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. Nat Neurosci 2008;11:251–253.
133. Johansson A, Engler H, Blomquist G, et al. Evidence for astrocytosis in ALS demonstrated by [11C](L)-deprenyl-D2 PET. J Neurol Sci 2007;255:17–22.
134. McGeer PL, McGeer EG. Inflammatory processes in amyotrophic lateral sclerosis. Muscle Nerve 2002;26:459–470.
135. Parkinson J. An Essay on the Shaking Palsy. London: Sherwood, Neely, and Jones, 1817.
136. McGeer PL, McGeer EG. Glial reactions in Parkinson's disease. Mov Disord 2008;23:474–483.
137. Mena MA, Garcia de Yebenes J. Glial cells as players in parkinsonism: the "good," the "bad," and the "mysterious" glia. Neuroscientist 2008;14:544–560.
138. Broe M, Kril J, Halliday GM. Astrocytic degeneration relates to the severity of disease in frontotemporal dementia. Brain 2004; 127:2214–2220.
139. Kersaitis C, Halliday GM, Kril JJ. Regional and cellular pathology in frontotemporal dementia: relationship to stage of disease in cases with and without Pick bodies. Acta Neuropathol 2004;108: 515–523.
140. Potts R, Leech RW. Thalamic dementia: an example of primary astroglial dystrophy of Seitelberger. Clin Neuropathol 2005;24: 271–275.
141. Thompson KA, McArthur JC, Wesselingh SL. Correlation between neurological progression and astrocyte apoptosis in HIV-associated dementia. Ann Neurol 2001;49:745–752.
142. Vanzani MC, Iacono RF, Caccuri RL, Troncoso AR, Berria MI. Regional differences in astrocyte activation in HIV-associated dementia. Medicina (B Aires) 2006;66:108–112.
143. Dabir DV, Trojanowski JQ, Richter-Landsberg C, Lee VM, Forman MS. Expression of the small heat-shock protein aB-crystallin in tauopathies with glial pathology. Am J Pathol 2004;164:155–166.
144. Forman MS, Lal D, Zhang B, et al. Transgenic mouse model of tau pathology in astrocytes leading to nervous system degeneration. J Neurosci 2005;25:3539–3550.
145. Hazell AS. Astrocytes are a major target in thiamine deficiency and Wernicke's encephalopathy. Neurochem Int 2009;55:129–135.
146. Hazell AS, Sheedy D, Oanea R, et al. Loss of astrocytic glutamate transporters in Wernicke encephalopathy. Glia 2009;58:148–156.
147. Nagele RG, D'Andrea MR, Lee H, Venkataraman V, Wang HY. Astrocytes accumulate A b 42 and give rise to astrocytic amyloid plaques in Alzheimer disease brains. Brain Res 2003;971:197–209.
148. Nagele RG, Wegiel J, Venkataraman V, Imaki H, Wang KC. Contribution of glial cells to the development of amyloid plaques in Alzheimer's disease. Neurobiol Aging 2004;25:663–674.
149. Rodriguez JJ, Olabarria M, Chvatal A, Verkhratsky A. Astroglia in dementia and Alzheimer's disease. Cell Death Differ 2009;16: 378–385.
150. Olabarria M, Noristani HN, Verkhratsky A, Rodriguez JJ. Concomitant astroglial atrophy and astrogliosis in a triple transgenic animal model of Alzheimer's disease. Glia 2010;58:831–838.
151. Beach TG, McGeer EG. Lamina-specific arrangement of astrocytic gliosis and senile plaques in Alzheimer's disease visual cortex. Brain Res 1988;463:357–361.
152. Griffin WS, Stanley LC, Ling C, et al. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proc Natl Acad Sci U S A 1989;86:7611–7615.
153. Kashon ML, Ross GW, O'Callaghan JP, et al. Associations of cortical astrogliosis with cognitive performance and dementia status. J Alzheimers Dis 2004;6:595–604.
154. Mrak RE, Griffin WS. Glia and their cytokines in progression of neurodegeneration. Neurobiol Aging 2005;26:349–354.
155. Sheng JG, Mrak RE, Rovnaghi CR, Kozlowska E, Van Eldik LJ, Griffin WS. Human brain S100 beta and S100 beta mRNA expression increases with age: pathogenic implications for Alzheimer's disease. Neurobiol Aging 1996;17:359–363.
156. Meda L, Baron P, Scarlato G. Glial activation in Alzheimer's disease: the role of Abeta and its associated proteins. Neurobiol Aging 2001;22:885–893.
157. Simpson JE, Ince PG, Lace G, et al. Astrocyte phenotype in relation to Alzheimer-type pathology in the ageing brain. Neurobiol Aging 2010;31:578–590.
158. Wharton SB, O'Callaghan JP, Savva GM, et al. Population variation in glial fibrillary acidic protein levels in brain aging: relationship to Alzheimer-type pathology and dementia. Dement Geriatr Cogn Disord 2009;27:465–473.
159. Mansour H, Chamberlain CG, Weible MW 2nd, Hughes S, Chu Y, Chan-Ling T. Aging-related changes in astrocytes in the rat retina: imbalance between cell proliferation and cell death reduces astrocyte availability. Aging Cell 2008;7:526–540.
160. Ramirez JM, Ramirez AI, Salazar JJ, de Hoz R, Trivino A. Changes of astrocytes in retinal ageing and age-related macular degeneration. Exp Eye Res 2001;73:601–615.
161. Mouton PR, Long JM, Lei DL, et al. Age and gender effects on microglia and astrocyte numbers in brains of mice. Brain Res 2002;956:30–35.
162. Amenta F, Bronzetti E, Sabbatini M, Vega JA. Astrocyte changes in aging cerebral cortex and hippocampus: a quantitative immunohistochemical study. Microsc Res Tech 1998;43:29–33.
163. Peinado MA, Quesada A, Pedrosa JA, et al. Quantitative and ultrastructural changes in glia and pericytes in the parietal cortex of the aging rat. Microsc Res Tech 1998;43:34–42.
164. Pilegaard K, Ladefoged O. Total number of astrocytes in the molecular layer of the dentate gyrus of rats at different ages. Anal Quant Cytol Histol 1996;18:279–285.
165. Peters A, Verderosa A, Sethares C. The neuroglial population in the primary visual cortex of the aging rhesus monkey. Glia 2008; 56:1151–1161.
166. Pakkenberg B, Pelvig D, Marner L, et al. Aging and the human neocortex. Exp Gerontol 2003;38:95–99.
167. Hinman JD, Abraham CR. What's behind the decline? The role of white matter in brain aging. Neurochem Res 2007;32:2023–2031.
168. Cotrina ML, Nedergaard M. Astrocytes in the aging brain. J Neurosci Res 2002;67:1–10.
169. DeWitt DA, Perry G, Cohen M, Doller C, Silver J. Astrocytes regulate microglial phagocytosis of senile plaque cores of Alzheimer's disease. Exp Neurol 1998;149:329–340.
170. Abramov AY, Canevari L, Duchen MR. Changes in intracellular calcium and glutathione in astrocytes as the primary mechanism of amyloid neurotoxicity. J Neurosci 2003;23:5088–5095.
171. Abramov AY, Canevari L, Duchen MR. b-Amyloid peptides induce mitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase. J Neurosci 2004;24:565–575.
172. Kuchibhotla KV, Lattarulo CR, Hyman BT, Bacskai BJ. Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice. Science 2009;323:1211–1215.
173. Peters O, Schipke CG, Philipps A, et al. Astrocyte function is modified by Alzheimer's disease-like pathology in aged mice. J Alzheimers Dis 2009;18:177–189.
174. Matos M, Augusto E, Oliveira CR, Agostinho P. Amyloid-b peptide decreases glutamate uptake in cultured astrocytes: in-

volvement of oxidative stress and mitogen-activated protein kinase cascades. Neuroscience 2008;156:898–810.
175. Oddo S, Caccamo A, Shepherd JD, et al. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Ab and synaptic dysfunction. Neuron 2003;39:409–421.
176. Kimelberg HK. The problem of astrocyte identity. Neurochem Int 2004;45:191–202.
177. Senitz D, Reichenbach A, Smith TG Jr. Surface complexity of human neocortical astrocytic cells: changes with development, aging, and dementia. J Hirnforsch 1995;36:531–537.
178. Rossner S, Lange-Dohna C, Zeitschel U, Perez-Polo JR. Alzheimer's disease beta-secretase BACE1 is not a neuron-specific enzyme. J Neurochem 2005;92:226–234.
179. Guenette SY. Astrocytes: a cellular player in Abeta clearance and degradation. Trends Mol Med 2003;9:279–280.
180. Nicoll JA, Weller RO. A new role for astrocytes: beta-amyloid homeostasis and degradation. Trends Mol Med 2003;9:281–282.
181. Apelt J, Ach K, Schliebs R. Aging-related down-regulation of neprilysin, a putative beta-amyloid-degrading enzyme, in transgenic Tg2576 Alzheimer-like mouse brain is accompanied by an astroglial upregulation in the vicinity of beta-amyloid plaques. Neurosci Lett 2003;339:183–186.
182. Wyss-Coray T, Loike JD, Brionne TC, et al. Adult mouse astrocytes degrade amyloid-b *in vitro* and *in situ*. Nat Med 2003;9:453–457.
183. Rossner S, Apelt J, Schliebs R, Perez-Polo JR, Bigl V. Neuronal and glial beta-secretase (BACE) protein expression in transgenic Tg2576 mice with amyloid plaque pathology. J Neurosci Res 2001;64:437–446.
184. Hartlage-Rubsamen M, Zeitschel U, Apelt J, et al. Astrocytic expression of the Alzheimer's disease b-secretase (BACE1) is stimulus-dependent. Glia 2003;41:169–179.
185. Heneka MT, Sastre M, Dumitrescu-Ozimek L, et al. Focal glial activation coincides with increased BACE1 activation and precedes amyloid plaque deposition in APP[V717I] transgenic mice. J Neuroinflam 2005;2:22.
186. Martins RN, Taddei K, Kendall C, Evin G, Bates KA, Harvey AR. Altered expression of apolipoprotein E, amyloid precursor protein and presenilin-1 is associated with chronic reactive gliosis in rat cortical tissue. Neuroscience 2001;106:557–569.
187. Bell RD, Zlokovic BV. Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. Acta Neuropathol 2009;118:103–113.
188. Iadecola C. Neurovascular regulation in the normal brain and in Alzheimer's disease. Nat Rev Neurosci 2004;5:347–360.
189. Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron 2008;57:178–201.
190. Farkas E, Luiten PG. Cerebral microvascular pathology in aging and Alzheimer's disease. Prog Neurobiol 2001;64:575–611.
191. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci 2006;7:41–53.
192. Mosconi L, Pupi A, De Leon MJ. Brain glucose hypometabolism and oxidative stress in preclinical Alzheimer's disease. Ann N Y Acad Sci 2008;1147:180–195.
193. Parpura-Gill A, Beitz D, Uemura E. The inhibitory effects of beta-amyloid on glutamate and glucose uptakes by cultured astrocytes. Brain Res 1997;754:65–71.
194. Schubert D, Soucek T, Blouw B. The induction of HIF-1 reduces astrocyte activation by amyloid beta peptide. Eur J Neurosci 2009;29:1323–1334.
195. Soucek T, Cumming R, Dargusch R, Maher P, Schubert D. The regulation of glucose metabolism by HIF-1 mediates a neuroprotective response to amyloid beta peptide. Neuron 2003;39:43–56.
196. Allaman I, Gavillet M, Belanger M, Laroche T, Viertl D, Lashuel HA, et al. Amyloid-b aggregates cause alterations of astrocytic metabolic phenotype: impact on neuronal viability. J Neurosci 2010;30:3326–3338.
197. Blass JP, Sheu RK, Gibson GE. Inherent abnormalities in energy metabolism in Alzheimer disease. Interaction with cerebrovascular compromise. Ann N Y Acad Sci 2000;903:204–221.
198. Liang WS, Reiman EM, Valla J, et al. Alzheimer's disease is associated with reduced expression of energy metabolism genes in posterior cingulate neurons. Proc Natl Acad Sci U S A 2008;105:4441–4446.
199. Bigl M, Bruckner MK, Arendt T, Bigl V, Eschrich K. Activities of key glycolytic enzymes in the brains of patients with Alzheimer's disease. J Neural Transm 1999;106:499–511.
200. Coleman P, Federoff H, Kurlan R. A focus on the synapse for neuroprotection in Alzheimer disease and other dementias. Neurology 2004;63:1155–1162.
201. Elias MF, Beiser A, Wolf PA, Au R, White RF, D'Agostino RB. The preclinical phase of alzheimer disease: A 22-year prospective study of the Framingham Cohort. Arch Neurol 2000;57:808–813.
202. DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann Neurol 1990;27:457–464.
203. Samuel W, Masliah E, Hill LR, Butters N, Terry R. Hippocampal connectivity and Alzheimer's dementia: effects of synapse loss and tangle frequency in a two-component model. Neurology 1994;44:2081–2088.
204. Terry RD, Masliah E, Salmon DP, et al. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 1991;30:572–580.
205. Alle H, Roth A, Geiger JR. Energy-efficient action potentials in hippocampal mossy fibers. Science 2009;325:1405–1408.
206. Magistretti PJ. Neuroscience. Low-cost travel in neurons. Science 2009;325:1349–1351.
207. Biran Y, Masters CL, Barnham KJ, Bush AI, Adlard PA. Pharmacotherapeutic targets in Alzheimer's disease. J Cell Mol Med 2009;13:61–86.
208. McCall MA, Gregg RG, Behringer RR, et al. Targeted deletion in astrocyte intermediate filament (Gfap) alters neuronal physiology. Proc Natl Acad Sci U S A 1996;93:6361–6366.
209. Shibuki K, Gomi H, Chen L, et al. Deficient cerebellar long-term depression, impaired eyeblink conditioning, and normal motor coordination in GFAP mutant mice. Neuron 1996;16:587–599.
210. Guarente L, Kenyon C. Genetic pathways that regulate ageing in model organisms. Nature 2000;408:255–262.
211. Doble A. The pharmacology and mechanism of action of riluzole. Neurology 1996;47:S233–S241.
212. Frizzo ME, Dall'Onder LP, Dalcin KB, Souza DO. Riluzole enhances glutamate uptake in rat astrocyte cultures. Cell Mol Neurobiol 2004;24:123–128.
213. Ji HF, Shen L, Zhang HY. Beta-lactam antibiotics are multipotent agents to combat neurological diseases. Biochem Biophys Res Commun 2005;333:661–663.
214. Blackburn D, Sargsyan S, Monk PN, Shaw PJ. Astrocyte function and role in motor neuron disease: a future therapeutic target? Glia 2009;57:1251–1264.