Chapter 17
Neuroglia: Functional Paralysis and Reactivity in Alzheimer's Disease and Other Neurodegenerative Pathologies

Alexei Verkhratsky, Robert Zorec, J.J. Rodriguez, and Vladimir Parpura

Abstract The most notable finding in neurodegenerative diseases is the progressive death of neurones cells. Yet, neuroglial changes can precede and facilitate neuronal loss. This is perhaps expected because astroglial cells maintain the brain homoeostasis, and are responsible for defence and regeneration, so that their malfunction manifested as degeneration or asthenia together with reactivity contribute to pathophysiology. Neuroglia may represent a novel target for therapeutic intervention, be that prevention, slowing progression of or possibly curing neurodegenerative diseases.

A. Verkhratsky, M.D., Ph.D. (✉)
Faculty of Life Sciences, The University of Manchester,
Oxford Road, Manchester M13 9PT, UK

Achucarro Center for Neuroscience, IKERBASQUE, Basque Foundation for Science,
48011 Bilbao, Spain

Department of Neurosciences, University of the Basque Country UPV/EHU and CIBERNED,
Leioa, Spain

University of Nizhny Novgorod, Nizhny Novgorod 603022, Russia

Laboratory of Neuroendocrinology and Molecular Cell Physiology, Institute of
Pathophysiology, University of Ljubljana, Zaloska cesta 4, 1000 Ljubljana, Slovenia
e-mail: Alexej.Verkhratsky@manchester.ac.uk

R. Zorec
Laboratory of Neuroendocrinology and Molecular Cell Physiology, Institute of
Pathophysiology, University of Ljubljana, Zaloska cesta 4, 1000 Ljubljana, Slovenia

Celica, BIOMEDICAL, Technology Park 24, 1000 Ljubljana, Slovenia

J.J. Rodriguez, Ph.D.
Achucarro Center for Neuroscience, IKERBASQUE, Basque Foundation for Science,
48011 Bilbao, Spain

Department of Neurosciences, University of the Basque Country UPV/EHU and CIBERNED,
Leioa, Spain

V. Parpura, M.D., Ph.D.
Department of Neurobiology, Civitan International Research Center and Center for Glial
Biology in Medicine, Evelyn F. McKnight Brain Institute, Atomic Force Microscopy
& Nanotechnology Laboratories, University of Alabama,
1719 6th Avenue South, CIRC 429, Birmingham, AL 35294-0021, USA

P. Beart et al. (eds.), *Neurodegenerative Diseases*, Advances in Neurobiology 15,
DOI 10.1007/978-3-319-57193-5_17

Keywords Neurology • Neuroglia • Astroglia • Neurodegenerative diseases • Alzheimer's disease • Huntington disease • Amyotrophic lateral sclerosis

Abbreviations

3xTg-AD	Triple transgenic mouse model of Alzheimer's disease, overexpressing mutant genes for amyloid precursor protein (APP_{Swe}), presenilin 1 ($PS1_{M146V}$) and microtubule-associated protein Tau (Tau_{P301L})
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
APP	Amyloid precursor protein
CNS	Central nervous system
CPA	Cyclopiazonic acid
DAT1	Dopamine transporter1
EAAT2	Excitable amino acid transporter 2
ER	Endoplasmic reticulum
HD	Huntington disease
GRP78	78 kDa glucose-regulated protein
IDE	Insulin degrading enzyme $InsP_3$: inositol 1,4,5-trisphosphate
JAK/STAT3	Janus kinase/signal transducers and activators of transcription
L-DOPA	L-3,4-Dihydroxyphenylalanine
MHC-II	Major histocompatibility complex II
mRNA	Messenger RNA
NFAT	Nuclear factor of activated T-cells
PD	Parkinson disease
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PS1	Presenilin 1
SOCE	Store-operated Ca^{2+} entry
SOD1	Superoxide dismutase 1
TLR2, TLR4	Toll receptors 2, 4
UPR	Unfolded protein response
VGLUT1	Vesicular L-glutamate transporter

17.1 Neuroglia as a Central Element of Neuropathology

Understanding the healthy human brain and principles of its pathological remodelling represents the major challenge ever faced by biomedical sciences. The complexity of the brain networking is exceptional, as indeed >200 billions of cells connected with tens of trillions of synapses (chemical and electrical) create the organ with unparalleled computing power and memory capacity in excess of a petabyte [1]. The brain as an organ is exceptionally resilient to the extracellular environmental pressure; despite exceedingly high metabolism (massively generating highly toxic reactive oxygen species), the brain withstands ageing better than any

other organ and system in the human body. Verily, human intellectual capacity remains high in advanced age, whereas the physical abilities start to decline already in the second or third decade of life.

The nervous system evolved through cell diversification and cell specialisation; the brain contains hundreds of distinct types of neurones, which are highly specialised for fast signalling through propagating action potentials coupled to the synaptic machinery that represents the primary element of integration and information processing. Neurones, however, account only for a half of all cells in the brain; the other half being represented by neuroglia. Neuroglial cells, generally classified into astroglia, oligodendroglia, NG2 cells and microglia [2, 3], are no less heterogeneous than neurones. The neuroglial cells are the homeostatic and defensive arm of the nervous system, which ensures proper organ internal environment associated with brain function.

Prominent neurologists of the nineteenth century, including Virchow, Andriezen and Alzheimer, considered glia as the main site of morbid changes. Nonetheless, the neurono-centric doctrine, that associated disease with neurones, prevailed over the last century; neurogliopathology, however, started to be acknowledged rapidly in the most recent decade [4–9]. Conceptually, neurological diseases can be defined as homeostatic failure, often associated with the inability of neuroglia to provide the full homeostatic and neuroprotective support. Furthermore, the responses of neural cells to the insult are fundamentally different: neurones become stressed and lose their primary function of information transfer and information processing, whereas glial cells actively respond by mounting an evolutionary conserved defensive response, collectively known as reactive gliosis [10, 11]. Both astroglial reactivity and astroglial asthenia contribute to neuropathology.

17.2 Neuroglia in Neurodegeneration

Neurodegenerative diseases, which affect almost exclusively humans, are chronic neurological disorders that lead to a progressive loss of function, structure and number of neural cells, ultimately resulting in the atrophy of the brain and profound cognitive deficits. Underlying mechanisms remain largely unknown although neurodegeneration is often associated with aberrant protein synthesis with an accumulation of pathological proteins (such as β-amyloid or α-synuclein) either inside the cells or in the brain parenchyma. Extracellular protein aggregates form disease-specific histopathological lesions represented, for example, by senile plaques, Lewy bodies or Rosenthal fibres.

Neuroglial alterations in neurodegeneration are complex and include both gliodegeneration with a loss of glial function and glial reactivity (Table 17.1). In many neurodegenerative processes, asthenic and degenerative changes in astroglia precede astrogliosis, the latter being activated, most likely, by specific lesions and the appearance of damaged or dying neurones. In amyotrophic lateral sclerosis (ALS) for example, astrodegeneration and astroglial atrophy occur before clinical symptoms and neuronal death. In the animal model of ALS expressing human mutant

Table 17.1 Neuroglia in major neurodegenerative diseases: an overview

Pathology	Neuroglial changes	References
Amyotrophic lateral sclerosis	*Astroglia* 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. The pathologically remodelled ALS astrocytes (expressing hSOD1) were specifically sensitive to L-glutamate, and contrary to healthy astrocytes displayed L-glutamate excitotoxicity. Silencing of ALS-related mutant SOD1 gene in astrocytes delayed the appearance of clinical symptoms in mice model of ALS	[12–15]
	Oligodendroglia/NG2 glia Post-mortem analysis reveals oligodendroglia degeneration and NG2 reactivity in humans with ALS. Spinal cord oligodendrocytes in mice models show pathological morphology and signs of apoptosis. Grey matter oligodendrocytes show significant signs of degeneration in the pre-symptomatic stage in SOD(G93A) mice. The NG2 glia in the same mice show abnormal enhanced proliferation; this is paralleled with failure in their remyelinating capacity	[16–18]
	Microglia Microglia undergoes activation in both early and late stages of ALS; in the early stages microglia can be neuroprotective and secrete numerous trophic factors. In the late stages microglia may contribute to neurotoxicity; at the same time degenerated forms of microglial cells are also observed	[19–22]
Parkinson disease	*Astroglia* Substantia nigra contains the lowest density of astrocytes; in vitro astrocytes protect dopaminergic neurones, and are instrumental for L-DOPA beneficial effects. In PD post-mortem tissue the levels of GFAP expression were reduced possibly indicating suppressed astrogliotic response and decreased neuroprotection	[23–25]
	Oligodendroglia The contributions of oligodendrocytes to PD are not yet analysed in depth; however, in the multiple system atrophy (MSA), which is a member of α-synucleopathies (to which PD also belongs), oligodendrocytes have abnormal morphology and cytosolic inclusion; selected expression of α-synuclein in oligodendrocytes replicates MSA pathology	[26, 27]
	Microglia Microglial density in the striatal structures is higher than in the rest of the brain. Microglia undergoes activation in PD associated with high secretion of cytokines; activated microglia is generally believed to produce neurotoxicity and contribute to the demise of dopaminergic neurones. Activated microglial cells are often clustered around degenerating dopaminergic neurones	[28–31]
Wernicke encephalopathy	Major (~70–80%) decrease in expression of astroglial plasmalemmal L-glutamate transporters represents the key pathological step in neurotoxicity and neuronal atrophy	[32, 33]

(continued)

Table 17.1 (continued)

Pathology	Neuroglial changes	References
Alzheimer disease	*Astrocytes* Astroglia undergo both degeneration and reactivity in a time- and region-specific manner. In entorhinal and prefrontal cortices astroglia become atrophic and fails to mount astrogliotic response which may define higher vulnerability of these regions to the AD pathology	[34–38]
	Oligodendroglia In AD, prominent degeneration of white matter and oligodendroglial death are observed. In familial forms of AD, expression of mutant PS1 gene increases astroglial sensitivity to L-glutamate and β-amyloid. The NG2 glia also show signs of morphological degeneration in human post-mortem tissues and in animal AD models	[39–43]
	Microglia Both prominent microglial reactivity and degeneration along with the loss of function are considered to contribute to AD pathology. Abnormal proliferation of microglia has been detected in early stages of AD in animal models	[44–47]

superoxide dismutase 1 (Tg(SOD1*G93A)1Gur mice), the occurrence of atrophic astrocytes seems to be the earliest pathological signature [12, 14]. The ability of these atrophic astrocytes to remove L-glutamate is compromised, which is thought to result in the accumulation of extracellular L-glutamate with ensuing excitotoxicity [48]. At the later stages of ALS, astrocytes also become activated, albeit atrophic forms also remain. The importance of astrocytes in ALS pathogenesis is further corroborated by the observation that silencing of ALS-related mutant SOD1 gene in astrocytes delayed the appearance of clinical symptoms in the transgenic mouse model [15]. Astroglial degeneration and loss of function also assumes a pathological proportion in Wernicke encephalopathy, a thalamo-cortical neurodegeneration, which represents the substrate for Korsakoff syndrome; significant down-regulation of astroglial L-glutamate transporters results in prominent excitotoxicity [32, 33]. Similarly, in Huntington disease (HD), a decreased astroglial L-glutamate uptake as well as an aberrant release of L-glutamate from astrocytes contributes to neurotoxicity. Astroglial reactivity also contributes to HD. Suppression of astrogliotic response by inhibition of JAK/STAT3 signalling cascade increases the number of huntingtin aggregates [49], thus exacerbating pathological progression. In the context of Parkinson disease (PD), astrocytes are supposed to play a neuroprotective role [23, 24]. Astrocytes contribute to metabolism of dopamine, a transmitter being transported to astroglial cells by neutral amino acids transporter *SLC7A5* as well as *by* dopamine transporter DAT1/SLC6A3. Astrocytes were also shown to convert L-DOPA to dopamine [50]. In the striatum, astrocytes act as a reservoir for L-DOPA, which they release to be subsequently transported to neurones [51]. The level of glial fibrillary acidic protein (GFAP) expression was decreased in astrocytes in PD human tissue [25], indicating astroglial atrophy and reduced astrogliotic response, which may reflect compromised astroglial neuroprotection.

Neurodegenerative diseases are almost invariably accompanied by chronic neuroinflammation with activation of microglia. Again, the precise contribution of microglia in the neurodegenerative process remains controversial, because both microglial activation with generation of neuroinflammatory cellular phenotypes and microglial paralysis are considered [44, 46, 52, 53]. This dichotomy in particular may reflect the differences between human and laboratory animal brains; there is little evidence for microglial activation in AD human tissue [52, 54]. Furthermore, human ageing (in contrast to laboratory animals) is associated with significant atrophy of microglial cells, which may be a key factor for creating environment, permissive for neurodegenerative alterations [54]. Finally, oligodendrocytes also undergo degenerative changes in the context of neurodegeneration. The progression of AD, for example, is accompanied by substantial shrinkage of the white matter. Degenerative changes are also observed in oligodendroglial precursors/NG2 glial cells that may reflect a reduction in their remyelinating capacity [42].

17.3 Astrocytes in AD

The pathological potential of astroglia in the context of dementia was realised by Alois Alzheimer, who often observed activated glial cells in close contacts with pathologically altered neurones, and who also described glia as a cellular component of the senile plaque [55]. Subsequent studies frequently mentioned astroglial reactivity in the context of AD, although detailed analysis of astroglial pathology started to be investigated only very recently [38, 45, 56].

17.3.1 Astrodegeneration

Astrocytes undergo complex morphological changes in animal AD models (Figs. 17.1 and 17.2). For example, in the triple transgenic 3xTg-AD mouse, which harbours mutant genes for amyloid precursor protein (APP_{Swe}), presenilin 1 ($PS1_{M146V}$) and microtubule-associated protein Tau (Tau_{P301L}), at the early presymptomatic stages (i.e. before considerable accumulation of extracellular β-amyloid and formation of senile plaques) astrocytes in hippocampus, prefrontal and entorhinal cortices demonstrate signs of atrophy and astrodegeneration [34, 57, 59]. The above changes are manifested by a decrease in the GFAP-positive astroglial profiles (both in area and volume measurements), decreased somata volume as well as a decrease in the number and branching of cell processes (Fig. 17.1). The atrophic changes in astrocytes developed in a particular spatio-temporal pattern. Hence, the earliest signs of atrophy were observed in the entorhinal cortex (at 1 months of age), in the prefrontal cortex morphological atrophy developed from 3 months of age, and in the hippocampus from 9 to 12 months of age [34, 57, 59]. Atrophic astrocytes also showed some signs of loss of homeostatic function, i.e., in

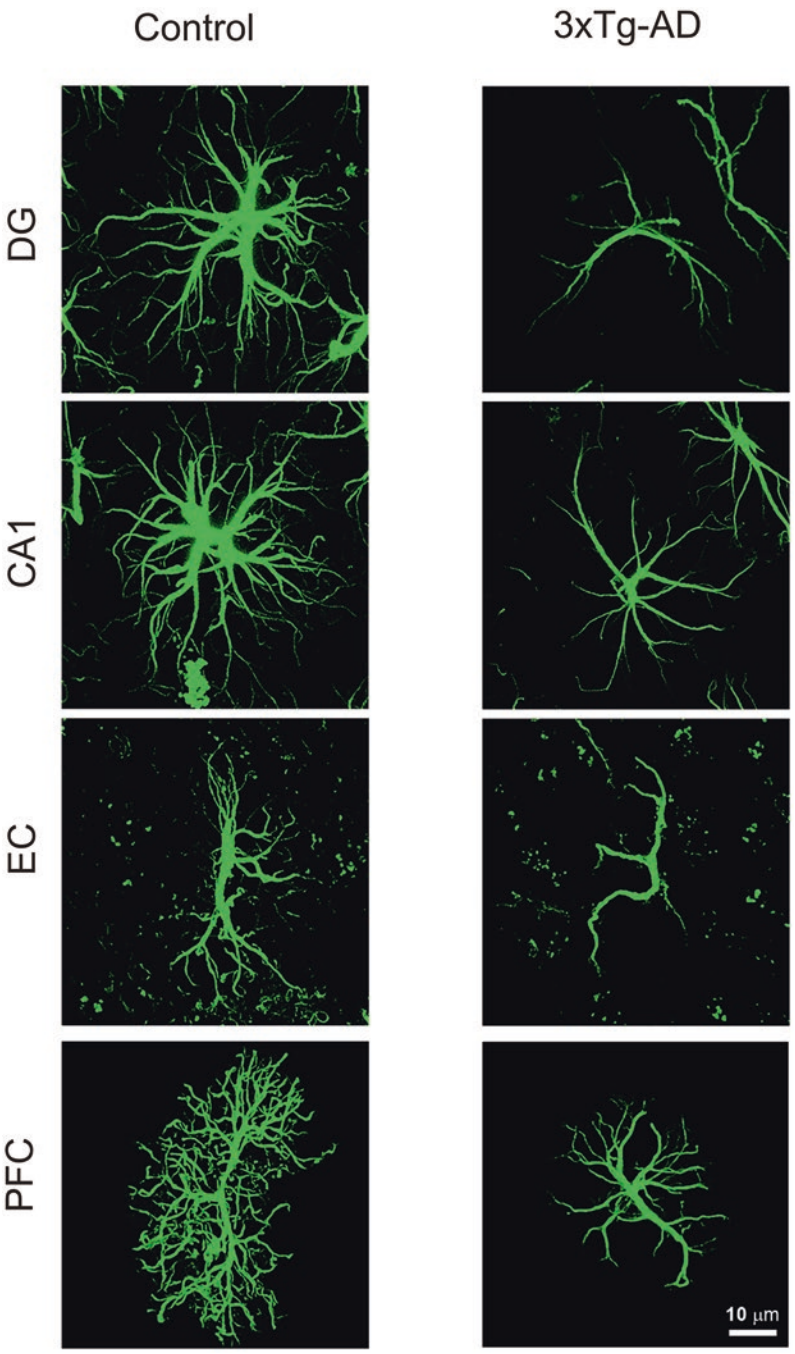

Fig. 17.1 Confocal micrographs illustrating atrophy of GFAP-positive astroglial profiles in the hippocampal dentate gyrus (DG), hippocampal cornu ammonis 1 (CA1) area, entorhinal cortex (EC) and prefrontal cortex (PFC) in 3xTg-AD mice compared with control animals. Modified from [35, 57–59]

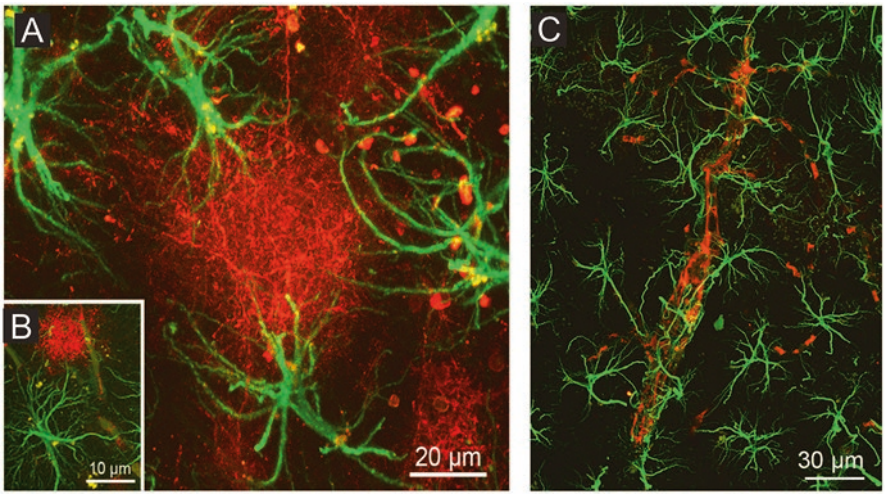

Fig. 17.2 Confocal images of hippocampal preparations labelled by anti-GFAP (*green*) and anti-β amyloid (*red*) monoclonal antibodies illustrating accumulation of reactive astrocytes around senile plaques (**a**, **b**) and vascular β-amyloid deposits (**c**) Modified from [34]

hippocampus a decrease in L-glutamine synthase (an enzyme central for L-glutamate turnover and L-glutamate-glutamine shuttle) have been detected [60]. There are, however, no significant changes in the overall number of GFAP-positive astrocytes either in the hippocampus or in the cortex.

Astrodegenerative changes may contribute to the development of the early AD pathology. Astroglial atrophy is likely to result in a reduced synaptic coverage, which in turn may affect neurotransmission and connectivity of neuronal networks. In addition, atrophic astrocytes fail to provide an adequate homeostatic support, thus further exacerbating neuronal function. These changes may, therefore, account for the early cognitive impairment, which results from loss and weakening of synapses, rather than from neuronal death. Incidentally, morphological atrophy of astrocytes in transgenic AD animals can be restored by an exposure to enriched environment or physical activity environmental stimulation, which arguably may coincide with certain cognitive improvement [61–63].

17.3.2 Astroglial Reactivity

Emergence of parenchymal depositions of β-amyloid triggers astroglial reactivity, with reactive astrocytes mainly being associated with senile plaques and β-amyloid "infested" blood vessels [34, 36, 38] (Fig. 17.2). Astroglial reactivity is observed both in human post-mortem tissues and in the brains of AD animal models [37]. In the 3xTg-AD mice, for example, the reactivity in the hippocampus is characterised

by a significant (up to 70%) increase in GFAP-positive astroglial profiles [34, 36]. The reactive astroglial response differs between brain regions. In the 3xTg-AD mice prominent reactivity is observed in the hippocampus whereas it is absent in entorhinal and prefrontal cortices [34, 57, 59]. Astroglial reactivity in the context of AD has been shown both in vitro [64] and in situ [65], and is triggered by the exposure to extracellular β-amyloid. Intracellular signalling cascades responsible for astroglial reactivity involve Ca^{2+} signalling, which is also affected in AD.

17.3.3 Aberrant Astroglial Ca^{2+} Signalling

Exposure of astrocytes to β-amyloid has complex effects on $[Ca^{2+}]_i$ regulation and Ca^{2+} signalling [66, 67]. Treatment of astroglial cultures with oligomeric β-amyloid$_{1–42}$ for several hours increased resting $[Ca^{2+}]_i$ several fold [68, 69], which, however, was not confirmed in experiments with longer treatments and other amyloid peptides; 48 h exposure neither to 1–20 μM β-amyloid$_{1–40}$ [70] nor to 200 nM β-amyloid$_{25–35}$ [71] had any significant effect on $[Ca^{2+}]_i$. Treatment of astrocytes in dissociated or organotypic cultures with β-amyloid induced acute $[Ca^{2+}]_i$ elevations or even $[Ca^{2+}]_i$ oscillations [65, 72–75], although this finding was not confirmed by others [69–71]. These differences may reflect, for example, different concentrations of β-amyloid (higher concentration tend to induce $[Ca^{2+}]_i$ elevations more reliably) or different species of β-amyloid that were used in various experiments. Of note, picomolar (200–300 pM) concentrations of β-amyloid were found to activate α7 nicotinic receptors that can mediate $[Ca^{2+}]_i$ transients [76, 77]. Treatment with β-amyloid was also found to increase intracellular Ca^{2+} release and store-operated Ca^{2+} entry, SOCE [78, 79]. Overall it seems that β-amyloid generally increases astroglial Ca^{2+} excitability [66, 67].

An increased astroglial Ca^{2+} signalling has been observed in several AD animal models. For example, an increase in the resting $[Ca^{2+}]_i$ as well as $[Ca^{2+}]_i$ hyperactivity with aberrant long-spreading intercellular Ca^{2+} waves were detected in astrocytes surrounding senile plaques in APP/PS1 mice [80], expressing mutant human β-amyloid precursor protein (APP_{swe}) and mutant PS1 (PS1ΔE9). High-frequency aberrant Ca^{2+} waves were also observed in astrocytes in APP_{Swe} mice at the preplaque stages [81]. There is evidence that increased Ca^{2+} excitability of astrocytes from AD mice may result from an elevated release of ATP from reactive astrocytes, with subsequent over-activation of $P2Y_1$ purinoceptors [82].

Aberrant Ca^{2+} signalling observed in transgenic AD animals can be also associated with an expression of pathologically mutated gene for PS1, rather than solely reflect effects of extracellular β-amyloid. Indeed, abnormal Ca^{2+} signalling was observed in primary cultures of astrocytes isolated from neonatal 3xTg-AD mice [69, 79]. Challenge with ATP triggered larger $[Ca^{2+}]_i$ in cultured hippocampal astrocytes from 3xTg-AD mice [83] when compared to their respective controls. Likewise, the SOCE was also increased in cultured transgenic astrocytes [79]. Expression of mutant PS1 affected not only Ca^{2+} dynamics but also impaired

vesicular trafficking and secretion of glio-signalling molecules [84], again suggesting that the M146V PS1 mutation perturbs several fundamental cellular functions. These abnormal functions result in early pathological remodelling of astroglia, which might contribute to evolution of pathology. Expression of APP also had complex effects on astroglial Ca^{2+} dynamics. The overexpression of APP did not affect $[Ca^{2+}]_i$ transients and SOCE in primary cultured astroglial cells from Tg5469 AD mice. On the contrary, deletion of APP inhibited astroglial SOCE, possibly through down-regulation of expression of TRPC1 and Orai 1 Ca^{2+} channels [85]. In astrocytes obtained from Trisomy 16 mice (a mouse model of Down syndrome that shares several key features with AD), resting Ca^{2+} levels were elevated two-fold when compared to wild-type controls [86]. Challenge of these astrocytes with cyclopiazonic acid (CPA, a SERCA inhibitor which initiates unopposed leakage of Ca^{2+} from the endoplasmic reticulum, ER) triggered large $[Ca^{2+}]_i$ elevations, which reflected higher ER Ca^{2+} content with a positive correlation between resting $[Ca^{2+}]_i$ and the amplitude of CPA-induced $[Ca^{2+}]_i$ transients [86].

The chronic exposure to β-amyloid also affects astroglial Ca^{2+} dynamics by modifying the calcium signalling toolkit, through changing expression of ionotropic and metabotropic receptors, intracellular Ca^{2+} channels, SOCE and Ca^{2+}-dependent enzymes. These modifications have been observed both in vitro and in situ. Chronic (24–72 h) exposure of cultured astrocytes to oligomeric β-amyloid (100 nM to 20 μM) up-regulated expression of astroglial metabotropic L-glutamate receptors mGluR5 [69, 70, 83]. A rather similar up-regulation of mGluR5 expression was also found in the brains of patients with Down's syndrome [87], as well as in cortical astrocytes associated with senile plaques in the APP_{swe}/PS1ΔE9 mice [88], in post-mortem hippocampi of Braak V-VI stage AD patients [69] and in post-mortem hippocampi from late-stage sporadic AD cases [70]. Chronic treatment of astroglial cultures with low concentrations (0.1–100 nM) of β-$amyloid_{1–42}$ [89] also increased expression of several nicotinic cholinoreceptors including highly Ca^{2+} permeable α7nAChRs. These latter receptors were found to be selectively expressed in astrocytes in the post-mortem brains of sporadic AD patients as well as in patients carrying the Swedish APP (KM670/671NL) mutation [90]. Another possible mechanism for aberrant Ca^{2+} signalling may be associated with direct activation of metabotropic Ca^{2+} sensing receptors by β-amyloid [91, 92].

Exposure of astrocytes to β-amyloid also affected expression of intracellular Ca^{2+} release channels. At mRNA and protein levels both inositol 1,4,5-trisphosphate receptors types 1 and 2 ($InsP_3R1$ and $InsP_3R2$) were up-regulated following 48 h treatment of rat hippocampal astrocytes with 100 nM oligomeric β-$amyloid_{1–42}$ [69, 83]. This increase, however, was not observed in all regions of the brain; treatment with β-amyloid failed to increase expression of $InsP_3R1$ protein in astrocytes isolated from the entorhinal cortex [83]. Even more interestingly, β-amyloid increased expression of $InsP_3R1$ only in hippocampal astrocytes isolated from wild-type healthy mice, but not that in cells isolated from 3xTg-AD animals, suggesting that exogenous β-amyloid and genetic modifications of astroglia share common

molecular mechanism in deregulating Ca^{2+} homeostasis [83]. Several reports showed overall decrease in $InsP_3Rs$ expression in post-mortem brains of AD patients in frontal, parietal and entorhinal cortices and hippocampus [93–95]; apparent contradiction to the in vitro*/culture* experiments need further clarifications. Expression of several other genes related to Ca^{2+} signalling was found to be modified in astrocytes in vitro or in AD animal models; these genes includes calsenilin [96], calpain-10 [97], NFAT [98], NF-kB [78], calcineurin [78, 99] as well as some subunits of L-type calcium channels [100]. Signalling through calcineurin (which is, a Ca^{2+}/calmodulin-dependent protein phosphatase) may link $[Ca^{2+}]_i$ fluctuations to astroglial reactivity or dysfunction. Expression of calcineurin is increased in β-amyloid treated astrocytes in vitro [78] and in vivo in APP/PS1AD model mice [101] and human AD hippocampus [69].

17.3.4 Calcium Signalling and Astroglial Reactivity

Regulation of astroglial reactivity is one of key components of defensive response of the CNS to all types of neuropathology. Although molecular cascades involved in the initiation of astrogliosis are far from being completely understood, there is growing evidence of the critical importance of cytosolic Ca^{2+} signalling and particularly that of Ca^{2+} release from the ER. Indeed, genetic deletion of $InsP_3R$ type II, an ER Ca^{2+} release channel, in astrocytes greatly diminishes astroglial reactive response to various lesions [102]. In the context of AD, the ER Ca^{2+} release is directly linked to initiation of reactive astrogliosis and inhibition of ER Ca^{2+} release channels (ryanodine receptors or $InsP_3Rs$) suppressed astrogliotic response [65]. Exposure of astrocytes to β-amyloid not only triggers ER Ca^{2+} release and initiates astrogliotic remodelling but also induces ER stress (as indicated by substantial phosphorylation of eIF2α and an increased expression of the GRP78 ER-resident chaperones, both being the hallmark of a specific type of ER stress known as unfolded protein response (UPR). Inhibition of Ca^{2+} release channels in astrocytes effectively suppressed the UPR [65]. Incidentally, the ER stress may be instrumental in the initiation of reactive astrogliosis; depletion of the ER from releasable Ca^{2+} instigates the UPR, which in turn triggers biochemical remodelling that underlie reactive response of astroglia.

As has been mentioned before, astroglial reactivity in the AD animals differs between brain regions with strong reactive response in the hippocampus and negligible reactive remodelling in the entorhinal and prefrontal cortices. This difference also correlated with different sensitivity of Ca^{2+} signalling toolkits in astrocytes from these regions to β-amyloid; Ca^{2+} signalling components were up-regulated in the hippocampus but not in the entorhinal cortex [83]. This specific property of the entorhinal astroglia may possibly account for their inability to mount astrogliotic response to accumulating β-amyloid. This "functional paralysis" of astrocytes may be directly related to differences in vulnerability of brain regions to AD. Indeed,

senile plaques appear in the entorhinal cortex much earlier than in the hippocampus. In this way Ca^{2+} signalling toolkits and their remodelling in the pathological context may be linked to the disease progression [66].

17.3.5 Vesicular Trafficking and Secretion in Astrocytes Are Altered in AD

Pathological changes in astroglia (for example, signs of astrogliotic activation) have been observed at the pre-symptomatic phase of AD before the formation of β-amyloid deposits [103] and hence changes in astroglial signalling may also occur early in the disease. Gliosignalling molecules, stored in membrane bound vesicles, are secreted by astrocytes through the stimulation-secretion coupling that involves exocytotic release [104]. In addition to molecules stored in vesicle lumen, ion channels, membrane receptors and transporters, such as major histocompatibility complex II (MHC-II [105]) and excitable amino acid transporter 2 (EAAT2 [106]) are delivered to the plasma membrane by vesicle traffic [107]. This brings vesicles from the Golgi complex, deep in the cytoplasm, to the cell surface. This traffic is maintained by an elaborated system regulated by increases in $[Ca^{2+}]_i$ [107, 108]. The complexity of vesicle traffic regulation in astrocytes is characterised by two typical, yet opposing, properties of vesicles that contain peptides, such as atrial natriuretic peptide, and those that carry amino acid transmitters and are labelled by the vesicular L-glutamate transporter VGLUT1 [108, 109]. Namely, glutamatergic vesicle motility is accelerated by an increase in $[Ca^{2+}]_i$ [110], whereas the same increase in $[Ca^{2+}]_i$ slows down peptidergic vesicles and endolysosomes [111]. Similar regulation also applies to recycling peptidergic vesicles, which have merged with the plasma membrane and subsequently entered back the cytoplasm. The mobility of recycling peptidergic vesicles was studied in cultured astrocytes [112] and those residing in the intact brain slices [113]. At rest, peptidergic vesicles moved faster and more directionally, linked to cytoskeletal elements, than after the exposure of astrocytes to a calcium ionophore to increase $[Ca^{2+}]_i$ [112]. The effect of increased $[Ca^{2+}]_i$ was remarkable; the movement of vesicles was almost halted, with only a jitter remaining (that was associated with random diffusional movement). At least some of the peptidergic vesicles carry ATP and a similar attenuation was observed in their mobility when astrocytes were stimulated [114]. Proteolytic enzymes stored in endolysosomes may contribute to the development of AD. One of these proteases is represented by the insulin degrading enzyme (IDE), which, when secreted to the extracellular space, may degrade β-amyloid. While IDE is secreted from neurones [115], the main cell type secreting IDE in AD appears to be astrocytes [116, 117]. It has been proposed that in AD the capacity of secreting IDE is reduced, leading to an increase in β-amyloid, which involves a reduction in autophagy-based lysosomal secretion of IDE [117]. Why this reduction occurs is not clear, but it may relate to a general vesicle traffic impairment that has been observed in AD.

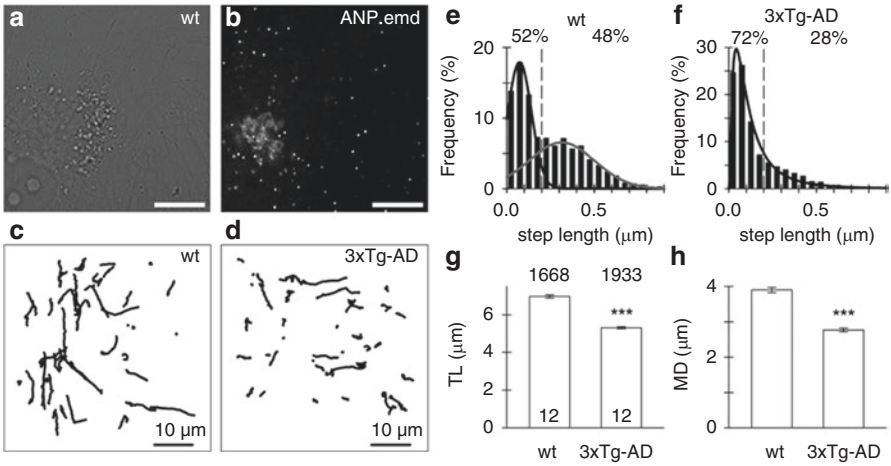

Fig. 17.3 Decreased spontaneous mobility of peptidergic vesicles in 3xTg-AD astrocytes. (**a**) Live cultured wild-type (wt) astrocyte under DIC optics and (**b**) the confocal image of the same cell expressing fluorescent peptide atrial natriuretic peptide-emerald green (ANP.emd), stored in individual vesicles, observed as bright fluorescent puncta; scale bars, 10 μm. (**c**) Vesicle tracks ($N = 50$) obtained in a 15-s epoch of imaging representative control (wt) and (**d**) 3xTg-AD astrocytes expressing ANP.emd, respectively. Note less elongated vesicle tracks in the 3xTg-AD astrocyte. (**e**, **f**) Frequency histogram of the step length in spontaneously moving vesicles in wt ($N = 5025$, **e**) and 3xTg-AD ($N = 5072$, **f**) astrocytes. The data were fitted with the function $f = a \times \exp(-0.5 \times (x/\mathrm{x0})/b)2/x$, where $a = 17.88 \pm 0.00$, $b = 0.07 \pm 0.00$ μm − 0.5, $\mathrm{x0} = 0.07 \pm 0.00$ μm (*black curve*) and $a = 6.53 \pm 0.13$, $b = 0.19 \pm 0.01$ μm − 0.5, $\mathrm{x0} = 0.31 \pm 0.01$ μm (*grey curve*) in wt astrocyte, and with the function $f = a \times \exp(-0.5 \times (\ln x/\mathrm{x0})/\mathrm{b})2/x$, where $a = 1.96 \pm 0.04$, $b = 0.92 \pm 0.02$ μm − 0.5, $\mathrm{x0} = 0.10 \pm 0.00$ μm (*black curve*) in 3xTg-AD astrocyte. The *vertical dashed line* indicates the step length of 0.2 μm obtained close to the intersection of distributions (*black* and *grey curve*) in wt astrocytes to discriminate small (<0.2 μm) from large (≥0.2 μm) steps. Note the higher proportion (%) of smaller steps lengths in the 3xTg-AD astrocyte indicated by the absence of the second mode distribution seen in wt astrocytes. (**g**) Track length (TL), (**h**) maximal displacement (MD), Note substantially diminished TL, MD in 3xTg-AD astrocytes. The *numbers* above the *top* of the bars (mean ± SEM) indicate the number of vesicles analysed; the *numbers* at the *bottom* of the bars indicate the number of cells analysed; "***"—indicates P values <0.001. Modified with permission from [84]

Astrocytes from 3xTg-AD mice isolated in the pre-symptomatic phase of the disease exhibit alterations in vesicle traffic (Fig. 17.3). Spontaneous mobility of peptidergic and endolysosomal vesicles as well as the ATP-evoked, Ca^{2+}-dependent, vesicle mobility were all diminished in diseased astrocytes. Similar impairment of peptidergic vesicle trafficking was observed in healthy rat astrocytes transfected with familial AD-associated mutated presenilin 1 (PS1M146V). The stimulation-dependent peptide discharge from single vesicles was less efficient in 3xTg-AD and PS1M146V-expressing astrocytes than in respective controls. The impaired vesicle dynamics and reduced evoked secretion of the signalling peptides both may contribute to the development of AD [84]. Although in this study atrial natriuretic peptide-containing vesicles were examined, it is likely that all peptidergic vesicles exhibit similar changes.

17.4 Oligodendroglia in Alzheimer's Disease

In the human brain white matter accounts for more than 50% of the total volume and is critical for assembling the CNS connectome [118]. The central myelination is provided by oligodendrocytes which are present in both white and grey matter; they are likely to be as numerous as astrocytes. Degeneration and death of oligodendrocytes with a subsequent decrease in CNS myelination and the shrinkage of the white matter are observed in the most (if not all) diseases of the brain and of the spinal cord including stroke, perinatal ischemia, multiple sclerosis, psychiatric disorders, traumatic injury and AD [119, 120]. The loss of myelin is a characteristic feature of the ageing CNS; in particular decreased myelination and oligodendroglial demise has been identified in the cerebral cortex, in areas related to cognition and memory including the frontal lobes [121]. In the course of human life the myelination of the CNS profile steadily increases during postnatal development, peaks at around 45 years and subsequently decreases in centenarians to levels comparable to those observed in infancy [121]. In the primary visual cortices of rhesus monkey, the age-dependent myelin deterioration has been characterised, and it appeared that the length of paranodes is decreased in ageing indicating some shortcomings in demyelisation [122]. These changes in the myelin developed in parallel with the decrease in the self-renewal capacity of oligodendroglial precursors/NG2 cells [123].

Oligodendroglial cell death and myelin shortages are associated with aberrant Ca^{2+} homeostasis and signalling, which is caused by either extracellular (neurotransmitter dyshomeostasis) or cellular (alterations in the Ca^{2+} homeostatic cascades such as channels, transporters and pumps) factors. Oligodendrocytes express several types of ionotropic receptors, including L-glutamate and P2X receptors, which are permeable to Ca^{2+}. Prolonged or excessive activation of these receptors induces cytosolic Ca^{2+} overload, accumulation of Ca^{2+} within mitochondria, increased production of reactive oxygen species, and release of pro-apoptotic factors, which all, acting in concert, trigger oligodendrocyte death and myelin destruction [120, 124]. Excitotoxicity mediated by L-glutamate and ATP may also contribute to oligodendroglia death in the context of AD.

In AD, the white matter degenerates and the number of oligodendrocytes is decreased [39]. Experiments in vitro have demonstrated that the exposure to β-amyloid damages oligodendrocytes [43] possibly because the expression of mutant PS1 increases their sensitivity to L-glutamate toxicity [41]. Similar effect has been observed in vivo when injection of β-amyloid$_{1–42}$ into white matter induced axon disruption and damaged myelin and triggered the death of oligodendrocytes [125]. The role for mutant PS1 has also been confirmed; exposure of PS1 mutant mice to the demyelinating agent cuprizone resulted in extended white matter damage and learning and memory deficits, which was not the case for healthy wild-type animals [41]. In the 3xTg-AD mice, gross deterioration of axonal morphology and compromised myelin integrity together with decreased expression of oligodendroglial markers was observed in the CA1 area of the hippocampus and in the layers IV/V of the entorhinal cortex at the early stages of the disease, much before the

emergence of β-amyloid depositions [126]. Hence, myelin and oligodendrocyte defects in AD occur before the onset of symptoms and may be considered as early markers. White matter lesions are also quite prominent in the early-stage AD in periventricular and deep white matter [127]. In 3xTg-AD mice marked morphological atrophy and decreased numbers of NG2 glia were detected at the early stages [42]; at the later phase, the NG2 glia associate themselves with senile plaques and infiltrate the latter with processes [42]. Similar decrease in the NG2-positive profiles was observed in human AD post-mortem tissue [40]. All these alterations in myelin, oligodendroglia as well as degenerative changes in oligodendroglial precursors/NG2 cells may contribute to pathological remodelling of the connectome and hence to cognitive deficiency.

17.5 Microglia in Alzheimer's Disease

Microglial changes, both reactive and degenerative, are now considered to be an important part of AD progression [44]. Activated microglial cells (together with astrocytes) are closely associated with senile plaques; they secrete numerous pro-inflammatory factors that may contribute to neuronal damage [44, 128]. At the same time the loss of microglial function has also been observed. In APP/PS1 mice, appearance of senile plaques coincided with the loss of microglial phagocytotic function (which, arguably, reduced β-amyloid clearance and facilitated plaque formation [46]). In the ageing human brain, degeneration of microglia can define neural tissue vulnerability to the AD pathology [54].

Activation of microglia can be triggered by β-amyloid [129], either soluble or oligomeric [45]. In vivo imaging of B6C3-YFP transgenic mice (harbouring APP_{swe} and PS1d9x-yellow fluorescent protein genes) demonstrated that microglia are activated and recruited to Aβ plaques only after the plaques had been formed [130]. In another AD mice model (APP V717I transgenic mice), activation of microglia, however, occurred much earlier (at 3 month) than the formation of senile plaques (10–12) months [131]. Activation of microglia in AD context may also involve purinergic signalling. In particular, $P2X_7$ receptors were found to be necessary for activation of microglia in response to β-amyloid injection [132]. Microglial activation in AD may also be regulated by TLR4 and TLR2 Toll receptors, which are up-regulated in AD animal models and in post-mortem AD brains [133, 134]. Of note, a spontaneous loss-of-function mutation in the TLR4 gene markedly decreased microglial activation induced by Aβ [135].

Microglial status does change in the progression of AD. In 3xTg-AD mice, a substantial increase in the density of resting microglia at both the early (i.e. pre-plaque) and late stages of the disease was identified [136]. At 9 month of age (before the emergence of senile plaques), the density of resting microglia in the hippocampus of 3xTg-AD was twice (by 105%) larger than in control mice. This increased density of resting microglia remained at older ages (54% higher at 12 months and 131% higher at 18 month when compared to the controls), when senile plaques

became evident. Emergence of the microglial activation seen as a significant increase in the density of activated microglia in the CA1 hippocampal area of 3xTg-AD mice was detected at 12 and 18 months, a period that correlates with the appearance and development of Aβ plaques [136]. The early increase in the density of resting microglia may represent the generalised response of the brain defence system to the developing AD pathology. Exposure of 3xTg-AD mice to running and enriched environment prevented this increase in the density of both resting and activated microglia [47]. This finding indicates that environmental stress may affect microglial response to the AD pathology.

17.6 Conclusions

Pathological changes in neuroglia, including but not restricted to astrocytes, oligodendrocytes, NG2 cells and microglia, are omnipresent in neurodegenerative diseases. These neuroglial changes include cellular degeneration and asthenic responses at earlier stages of a disease, and, as disease progresses, evident by occurring neuronal damages, neuroglia turns to reactive phenotypes. The specific morpho-functional changes in glia not only occur during distinct temporal domains, but also are region-specific. Since the changes in neuroglia precede those in neurones, it is likely that the neuroglial cells failure to maintain CNS homeostasis is a malefactor causative to neuronal death. Neuroglia therefore may represent an opportunistic target for therapeutic intervention directed towards prevention and conceivably curing neurodegenerative diseases.

Acknowledgements Authors research was supported by Alzheimer's Research Trust (UK) Programme Grant (ART/PG2004A/1) to A.V. and J.J.R.; by National Institutes of Health (The Eunice Kennedy Shriver National Institute of Child Health and Human Development award HD078678) to V.P.; by the grants P3 310, J3 4051, J3 3632, J3 6790, J3 7605 and J3 4146 from the Slovenian Research Agency (ARRS) and the EduGlia ITN EU grant to R.Z. and A.V.; by Plan Nacional de I+D+I 2008–2011 and ISCIII-Subdirección General de Evaluación y Fomento de la investigación co-financed by FEDER (grant PI10/02738 to J.J.R. and A.V.); by the Government of the Basque Country grants AE-2010-1-28, AEGV10/16 and GV-2011111020 to J.J.R. A.V. was also supported by the Federal Target Program "Research and development in priority areas of the development of the scientific and technological complex of Russia for 2014–2020" of the Ministry of Education and Science of Russia, contract 14.581.21.0016 (Project ID RFMEFI58115X0016).

References

1. Bartol TM, Bromer C, Kinney J, Chirillo MA, Bourne JN, Harris KM, Sejnowski TJ (2015) Nanoconnectomic upper bound on the variability of synaptic plasticity. eLife 4:e10778
2. Kettenmann H, Ransom BR (eds) (2013) Neuroglia. Oxford University Press, Oxford, p 864
3. Verkhratsky A, Butt AM (2013) Glial physiology and pathophysiology. Wiley-Blackwell, Chichester

4. Burda JE, Sofroniew MV (2014) Reactive gliosis and the multicellular response to CNS damage and disease. Neuron 81:229–248
5. De Keyser J, Mostert JP, Koch MW (2008) Dysfunctional astrocytes as key players in the pathogenesis of central nervous system disorders. J Neurol Sci 267:3–16
6. Parpura V, Heneka MT, Montana V, Oliet SH, Schousboe A, Haydon PG, Stout RF Jr, Spray DC, Reichenbach A, Pannicke T, Pekny M, Pekna M, Zorec R, Verkhratsky A (2012) Glial cells in (patho)physiology. J Neurochem 121:4–27
7. Pekny M, Pekna M, Messing A, Steinhauser C, Lee JM, Parpura V, Hol EM, Sofroniew MV, Verkhratsky A (2016) Astrocytes: a central element in neurological diseases. Acta Neuropathol 131:323–345
8. Verkhratsky A, Rodriguez JJ, Parpura V (2013) Astroglia in neurological diseases. Future Neurol 8:149–158
9. Verkhratsky A, Sofroniew MV, Messing A, deLanerolle NC, Rempe D, Rodriguez JJ, Nedergaard M (2012) Neurological diseases as primary gliopathies: a reassessment of neurocentrism. ASN Neuro 4:e00082
10. Pekny M, Wilhelmsson U, Pekna M (2014) The dual role of astrocyte activation and reactive gliosis. Neurosci Lett 565:30–38
11. Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119:7–35
12. Rossi D, Brambilla L, Valori CF, Roncoroni C, Crugnola A, Yokota T, Bredesen DE, Volterra A (2008) Focal degeneration of astrocytes in amyotrophic lateral sclerosis. Cell Death Differ 15:1691–1700
13. Rossi D, Volterra A (2009) Astrocytic dysfunction: insights on the role in neurodegeneration. Brain Res Bull 80:224–232
14. Valori CF, Brambilla L, Martorana F, Rossi D (2014) The multifaceted role of glial cells in amyotrophic lateral sclerosis. Cell Mol Life Sci 71:287–297
15. Yamanaka K, Chun SJ, Boillee S, Fujimori-Tonou N, Yamashita H, Gutmann DH, Takahashi R, Misawa H, Cleveland DW (2008) Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. Nat Neurosci 11:251–253
16. Kang SH, Li Y, Fukaya M, Lorenzini I, Cleveland DW, Ostrow LW, Rothstein JD, Bergles DE (2013) Degeneration and impaired regeneration of gray matter oligodendrocytes in amyotrophic lateral sclerosis. Nat Neurosci 16:571–579
17. Nonneman A, Robberecht W, Van Den Bosch L (2014) The role of oligodendroglial dysfunction in amyotrophic lateral sclerosis. Neurodegener Dis Manag 4:223–239
18. Philips T, Bento-Abreu A, Nonneman A, Haeck W, Staats K, Geelen V, Hersmus N, Kusters B, Van Den Bosch L, Van Damme P, Richardson WD, Robberecht W (2013) Oligodendrocyte dysfunction in the pathogenesis of amyotrophic lateral sclerosis. Brain 136:471–482
19. Brites D, Vaz AR (2014) Microglia centered pathogenesis in ALS: insights in cell interconnectivity. Front Cell Neurosci 8:117
20. Lasiene J, Yamanaka K (2011) Glial cells in amyotrophic lateral sclerosis. Neurol Res Int 2011:718987
21. Luo XG, Chen SD (2012) The changing phenotype of microglia from homeostasis to disease. Transl Neurodegener 1:9
22. Zhao W, Beers DR, Appel SH (2013) Immune-mediated mechanisms in the pathoprogression of amyotrophic lateral sclerosis. J Neuroimmune Pharmacol 8:888–899
23. Mena MA, de Bernardo S, Casarejos MJ, Canals S, Rodriguez-Martin E (2002) The role of astroglia on the survival of dopamine neurons. Mol Neurobiol 25:245–263
24. Mena MA, Garcia de Yebenes J (2008) Glial cells as players in parkinsonism: the "good," the "bad," and the "mysterious" glia. Neuroscientist 14:544–560
25. Tong J, Ang LC, Williams B, Furukawa Y, Fitzmaurice P, Guttman M, Boileau I, Hornykiewicz O, Kish SJ (2015) Low levels of astroglial markers in Parkinson's disease: relationship to alpha-synuclein accumulation. Neurobiol Dis 82:243–253
26. Liu Y, Zhou J (2013) Oligodendrocytes in neurodegenerative diseases. Front Biol 8:127–133

27. Stefanova N, Reindl M, Neumann M, Kahle PJ, Poewe W, Wenning GK (2007) Microglial activation mediates neurodegeneration related to oligodendroglial alpha-synucleinopathy: implications for multiple system atrophy. Mov Disord 22:2196–2203
28. Long-Smith CM, Sullivan AM, Nolan YM (2009) The influence of microglia on the pathogenesis of Parkinson's disease. Prog Neurobiol 89:277–287
29. Perry VH (2012) Innate inflammation in Parkinson's disease. Cold Spring Harb Perspect Med 2:a009373
30. Qian L, Flood PM (2008) Microglial cells and Parkinson's disease. Immunol Res 41:155–164
31. Rogers J, Mastroeni D, Leonard B, Joyce J, Grover A (2007) Neuroinflammation in Alzheimer's disease and Parkinson's disease: are microglia pathogenic in either disorder? Int Rev Neurobiol 82:235–246
32. Hazell AS (2009) Astrocytes are a major target in thiamine deficiency and Wernicke's encephalopathy. Neurochem Int 55:129–135
33. Hazell AS, Sheedy D, Oanea R, Aghourian M, Sun S, Jung JY, Wang D, Wang C (2009) Loss of astrocytic glutamate transporters in Wernicke encephalopathy. Glia 58:148–156
34. Olabarria M, Noristani HN, Verkhratsky A, Rodriguez JJ (2010) Concomitant astroglial atrophy and astrogliosis in a triple transgenic animal model of Alzheimer's disease. Glia 58:831–838
35. Rodriguez-Arellano JJ, Parpura V, Zorec R, Verkhratsky A (2016) Astrocytes in physiological aging and Alzheimer's disease. Neuroscience 323:170–182
36. Rodriguez JJ, Olabarria M, Chvatal A, Verkhratsky A (2009) Astroglia in dementia and Alzheimer's disease. Cell Death Differ 16:378–385
37. Verkhratsky A, Marutle A, Rodríguez-Arellano JJ, Nordberg A (2015) Glial asthenia and functional paralysis: a new perspective on neurodegeneration and Alzheimer's disease. Neuroscientist 21:552–568
38. Verkhratsky A, Olabarria M, Noristani HN, Yeh CY, Rodriguez JJ (2010) Astrocytes in Alzheimer's disease. Neurotherapeutics 7(4):399–412. in press
39. Brown WR, Moody DM, Thore CR, Challa VR (2000) Cerebrovascular pathology in Alzheimer's disease and leukoaraiosis. Ann N Y Acad Sci 903:39–45
40. Nielsen HM, Ek D, Avdic U, Orbjorn C, Hansson O, Netherlands Brain B, Veerhuis R, Rozemuller AJ, Brun A, Minthon L, Wennstrom M (2013) NG2 cells, a new trail for Alzheimer's disease mechanisms? Acta Neuropathol Commun 1:7
41. Pak K, Chan SL, Mattson MP (2003) Presenilin-1 mutation sensitizes oligodendrocytes to glutamate and amyloid toxicities, and exacerbates white matter damage and memory impairment in mice. NeuroMolecular Med 3:53–64
42. Rivera A, Vanzuli I, Arellano JJ, Butt A (2016) Decreased regenerative capacity of oligodendrocyte progenitor cells (NG2-glia) in the ageing brain: a vicious cycle of synaptic dysfunction, myelin loss and neuronal disruption? Curr Alzheimer Res 13:413–418
43. Xu J, Chen S, Ahmed SH, Chen H, Ku G, Goldberg MP, Hsu CY (2001) Amyloid-β peptides are cytotoxic to oligodendrocytes. J Neurosci 21:RC118
44. Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM, Herrup K, Frautschy SA, Finsen B, Brown GC, Verkhratsky A, Yamanaka K, Koistinaho J, Latz E, Halle A, Petzold GC, Town T, Morgan D, Shinohara ML, Perry VH, Holmes C, Bazan NG, Brooks DJ, Hunot S, Joseph B, Deigendesch N, Garaschuk O, Boddeke E, Dinarello CA, Breitner JC, Cole GM, Golenbock DT, Kummer MP (2015) Neuroinflammation in Alzheimer's disease. Lancet Neurol 14:388–405
45. Heneka MT, Rodriguez JJ, Verkhratsky A (2010) Neuroglia in neurodegeneration. Brain Res Rev 63(1–2):189–211
46. Krabbe G, Halle A, Matyash V, Rinnenthal JL, Eom GD, Bernhardt U, Miller KR, Prokop S, Kettenmann H, Heppner FL (2013) Functional impairment of microglia coincides with β-amyloid deposition in mice with Alzheimer-like pathology. PLoS One 8:e60921

47. Rodriguez JJ, Noristani HN, Verkhratsky A (2015) Microglial response to Alzheimer's disease is differentially modulated by voluntary wheel running and enriched environments. Brain Struct Funct 220:941–953
48. Rossi D (2015) Astrocyte physiopathology: at the crossroads of intercellular networking, inflammation and cell death. Prog Neurobiol 130:86–120
49. Ben Haim L, Ceyzeriat K, Carrillo-de Sauvage MA, Aubry F, Auregan G, Guillermier M, Ruiz M, Petit F, Houitte D, Faivre E, Vandesquille M, Aron-Badin R, Dhenain M, Deglon N, Hantraye P, Brouillet E, Bonvento G, Escartin C (2015) The JAK/STAT3 pathway is a common inducer of astrocyte reactivity in Alzheimer's and Huntington's diseases. J Neurosci 35:2817–2829
50. Mena MA, Casarejos MJ, Carazo A, Paino CL, Garcia de Yebenes J (1996) Glia conditioned medium protects fetal rat midbrain neurones in culture from L-DOPA toxicity. Neuroreport 7:441–445
51. Asanuma M, Miyazaki I, Murakami S, Diaz-Corrales FJ, Ogawa N (2014) Striatal astrocytes act as a reservoir for L-DOPA. PLoS One 9:e106362
52. Streit WJ, Xue QS (2012) Alzheimer's disease, neuroprotection, and CNS immunosenescence. Front Pharmacol 3:138
53. Verkhratsky A, Marutle A, Rodriguez-Arellano JJ, Nordberg A (2014) Glial asthenia and functional paralysis: a new perspective on Neurodegeneration and Alzheimer's disease. Neuroscientist 21(5):552–568
54. Streit WJ, Braak H, Xue QS, Bechmann I (2009) Dystrophic (senescent) rather than activated microglial cells are associated with tau pathology and likely precede neurodegeneration in Alzheimer's disease. Acta Neuropathol 118:475–485
55. Alzheimer A (1910) 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, vol 1–3. Gustav Fischer, Jena, pp 401–562
56. Nagele RG, Wegiel J, Venkataraman V, Imaki H, Wang KC (2004) Contribution of glial cells to the development of amyloid plaques in Alzheimer's disease. Neurobiol Aging 25:663–674
57. Kulijewicz-Nawrot M, Verkhratsky A, Chvatal A, Sykova E, Rodriguez JJ (2012) Astrocytic cytoskeletal atrophy in the medial prefrontal cortex of a triple transgenic mouse model of Alzheimer's disease. J Anat 221:252–262
58. Verkhratsky A, Zorec R, Rodriguez JJ, Parpura V (2016b) Astroglia dynamics in ageing and Alzheimer's disease. Curr Opin Pharmacol 26:74–79
59. Yeh CY, Vadhwana B, Verkhratsky A, Rodriguez JJ (2011) Early astrocytic atrophy in the entorhinal cortex of a triple transgenic animal model of Alzheimer's disease. ASN Neuro 3:271–279
60. Olabarria M, Noristani HN, Verkhratsky A, Rodriguez JJ (2011) Age-dependent decrease in glutamine synthetase expression in the hippocampal astroglia of the triple transgenic Alzheimer's disease mouse model: mechanism for deficient glutamatergic transmission? Mol Neurodegener 6:55
61. Beauquis J, Pavia P, Pomilio C, Vinuesa A, Podlutskaya N, Galvan V, Saravia F (2013) Environmental enrichment prevents astroglial pathological changes in the hippocampus of APP transgenic mice, model of Alzheimer's disease. Exp Neurol 239:28–37
62. Rodriguez JJ, Terzieva S, Olabarria M, Lanza RG, Verkhratsky A (2013) Enriched environment and physical activity reverse astrogliodegeneration in the hippocampus of AD transgenic mice. Cell Death Dis 4:e678
63. Ryan SM, Kelly AM (2016) Exercise as a pro-cognitive, pro-neurogenic and anti-inflammatory intervention in transgenic mouse models of Alzheimer's disease. Ageing Res Rev 27:77–92. in press
64. DeWitt DA, Perry G, Cohen M, Doller C, Silver J (1998) Astrocytes regulate microglial phagocytosis of senile plaque cores of Alzheimer's disease. Exp Neurol 149:329–340

65. Alberdi E, Wyssenbach A, Alberdi M, Sanchez-Gomez MV, Cavaliere F, Rodriguez JJ, Verkhratsky A, Matute C (2013) Ca^{2+}-dependent endoplasmic reticulum stress correlates with astrogliosis in oligomeric amyloid β-treated astrocytes and in a model of Alzheimer's disease. Aging Cell 12:292–302
66. Lim D, Rodriguez-Arellano JJ, Parpura V, Zorec R, Zeidan-Chulia F, Genazzani AA, Verkhratsky A (2016) Calcium signalling toolkits in astrocytes and spatio-temporal progression of Alzheimer's disease. Curr Alzheimer Res 13:359–369
67. Lim D, Ronco V, Grolla AA, Verkhratsky A, Genazzani AA (2014) Glial calcium signalling in Alzheimer's disease. Rev Physiol Biochem Pharmacol 167:45–65
68. Haughey NJ, Mattson MP (2003) Alzheimer's amyloid β-peptide enhances ATP/gap junction-mediated calcium-wave propagation in astrocytes. NeuroMolecular Med 3:173–180
69. Lim D, Iyer A, Ronco V, Grolla AA, Canonico PL, Aronica E, Genazzani AA (2013) Amyloid β deregulates astroglial mGluR5-mediated calcium signaling via calcineurin and Nf-κB. Glia 61:1134–1145
70. Casley CS, Lakics V, Lee HG, Broad LM, Day TA, Cluett T, Smith MA, O'Neill MJ, Kingston AE (2009) Up-regulation of astrocyte metabotropic glutamate receptor 5 by amyloid-β peptide. Brain Res 1260:65–75
71. Toivari E, Manninen T, Nahata AK, Jalonen TO, Linne ML (2011) Effects of transmitters and amyloid-beta peptide on calcium signals in rat cortical astrocytes: Fura-2AM measurements and stochastic model simulations. PLoS One 6:e17914
72. Abramov AY, Canevari L, Duchen MR (2003) Changes in intracellular calcium and glutathione in astrocytes as the primary mechanism of amyloid neurotoxicity. J Neurosci 23:5088–5095
73. Abramov AY, Canevari L, Duchen MR (2004) Calcium signals induced by amyloid β peptide and their consequences in neurons and astrocytes in culture. Biochim Biophys Acta 1742:81–87
74. Chow SK, Yu D, Macdonald CL, Buibas M, Silva GA (2010) Amyloid β-peptide directly induces spontaneous calcium transients, delayed intercellular calcium waves and gliosis in rat cortical astrocytes. ASN Neuro 2:e00026
75. Jalonen TO, Charniga CJ, Wielt DB (1997) β-Amyloid peptide-induced morphological changes coincide with increased K^+ and Cl^- channel activity in rat cortical astrocytes. Brain Res 746:85–97
76. Lee L, Kosuri P, Arancio O (2014) Picomolar amyloid-β peptides enhance spontaneous astrocyte calcium transients. J Alzheimers Dis 38:49–62
77. Pirttimaki TM, Codadu NK, Awni A, Pratik P, Nagel DA, Hill EJ, Dineley KT, Parri HR (2013) α7 nicotinic receptor-mediated astrocytic gliotransmitter release: Aβ effects in a preclinical Alzheimer's mouse model. PLoS One 8:e81828
78. Grolla AA, Fakhfouri G, Balzaretti G, Marcello E, Gardoni F, Canonico PL, DiLuca M, Genazzani AA, Lim D (2013a) Aβ leads to Ca^{2+} signaling alterations and transcriptional changes in glial cells. Neurobiol Aging 34:511–522
79. Ronco V, Grolla AA, Glasnov TN, Canonico PL, Verkhratsky A, Genazzani AA, Lim D (2014) Differential deregulation of astrocytic calcium signalling by amyloid-β, TNFα, IL-1β and LPS. Cell Calcium 55:219–229
80. Kuchibhotla KV, Lattarulo CR, Hyman BT, Bacskai BJ (2009) Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice. Science 323:1211–1215
81. Takano T, Han X, Deane R, Zlokovic B, Nedergaard M (2007) Two-photon imaging of astrocytic Ca^{2+} signaling and the microvasculature in experimental mice models of Alzheimer's disease. Ann N Y Acad Sci 1097:40–50
82. Delekate A, Fuchtemeier M, Schumacher T, Ulbrich C, Foddis M, Petzold GC (2014) Metabotropic P2Y1 receptor signalling mediates astrocytic hyperactivity *in vivo* in an Alzheimer's disease mouse model. Nat Commun 5:5422

83. Grolla AA, Sim JA, Lim D, Rodriguez JJ, Genazzani AA, Verkhratsky A (2013b) Amyloid-β and Alzheimer's disease type pathology differentially affects the calcium signalling toolkit in astrocytes from different brain regions. Cell Death Dis 4:e623
84. Stenovec M, Trkov S, Lasic E, Terzieva S, Kreft M, Rodriguez Arellano JJ, Parpura V, Verkhratsky A, Zorec R (2016) Expression of familial Alzheimer disease presenilin 1 gene attenuates vesicle traffic and reduces peptide secretion in cultured astrocytes devoid of pathologic tissue environment. Glia 64:317–329
85. Linde CI, Baryshnikov SG, Mazzocco-Spezzia A, Golovina VA (2011) Dysregulation of Ca^{2+} signaling in astrocytes from mice lacking amyloid precursor protein. Am J Physiol Cell Physiol 300:C1502–C1512
86. Bambrick LL, Golovina VA, Blaustein MP, Yarowsky PJ, Krueger BK (1997) Abnormal calcium homeostasis in astrocytes from the trisomy 16 mouse. Glia 19:352–358
87. Iyer AM, van Scheppingen J, Milenkovic I, Anink JJ, Lim D, Genazzani AA, Adle-Biassette H, Kovacs GG, Aronica E (2014) Metabotropic glutamate receptor 5 in Down's syndrome hippocampus during development: increased expression in astrocytes. Curr Alzheimer Res 11:694–705
88. Shrivastava AN, Kowalewski JM, Renner M, Bousset L, Koulakoff A, Melki R, Giaume C, Triller A (2013) β-Amyloid and ATP-induced diffusional trapping of astrocyte and neuronal metabotropic glutamate type-5 receptors. Glia 61:1673–1686
89. Xiu J, Nordberg A, Zhang JT, Guan ZZ (2005) Expression of nicotinic receptors on primary cultures of rat astrocytes and up-regulation of the α7, α4 and β2 subunits in response to nanomolar concentrations of the β-amyloid peptide$_{1-42}$. Neurochem Int 47:281–290
90. Yu WF, Guan ZZ, Bogdanovic N, Nordberg A (2005) High selective expression of α7 nicotinic receptors on astrocytes in the brains of patients with sporadic Alzheimer's disease and patients carrying Swedish APP 670/671 mutation: a possible association with neuritic plaques. Exp Neurol 192:215–225
91. Chiarini A, Gardenal E, Whitfield JF, Chakravarthy B, Armato U, Dal Pra I (2015) Preventing the spread of Alzheimer's disease neuropathology: a role for calcilytics? Curr Pharm Biotechnol 16:696–706
92. Dal Pra I, Chiarini A, Pacchiana R, Gardenal E, Chakravarthy B, Whitfield JF, Armato U (2014) Calcium-sensing receptors of human astrocyte-Neuron teams: amyloid-beta-driven mediators and therapeutic targets of Alzheimer's disease. Curr Neuropharmacol 12:353–364
93. Haug LS, Ostvold AC, Cowburn RF, Garlind A, Winblad B, Bogdanovich N, Walaas SI (1996) Decreased inositol (1,4,5)-trisphosphate receptor levels in Alzheimer's disease cerebral cortex: selectivity of changes and possible correlation to pathological severity. Neurodegeneration 5:169–176
94. Kurumatani T, Fastbom J, Bonkale WL, Bogdanovic N, Winblad B, Ohm TG, Cowburn RF (1998) Loss of inositol 1,4,5-trisphosphate receptor sites and decreased PKC levels correlate with staging of Alzheimer's disease neurofibrillary pathology. Brain Res 796:209–221
95. Young LT, Kish SJ, Li PP, Warsh JJ (1988) Decreased brain [^{3}H]inositol 1,4,5-trisphosphate binding in Alzheimer's disease. Neurosci Lett 94:198–202
96. Jin JK, Choi JK, Wasco W, Buxbaum JD, Kozlowski PB, Carp RI, Kim YS, Choi EK (2005) Expression of calsenilin in neurons and astrocytes in the Alzheimer's disease brain. Neuroreport 16:451–455
97. Garwood C, Faizullabhoy A, Wharton SB, Ince PG, Heath PR, Shaw PJ, Baxter L, Gelsthorpe C, Forster G, Matthews FE, Brayne C, Simpson JE (2013) Calcium dysregulation in relation to Alzheimer-type pathology in the ageing brain. Neuropathol Appl Neurobiol 39:788–799
98. Abdul HM, Sama MA, Furman JL, Mathis DM, Beckett TL, Weidner AM, Patel ES, Baig I, Murphy MP, LeVine H 3rd, Kraner SD, Norris CM (2009) Cognitive decline in Alzheimer's disease is associated with selective changes in calcineurin/NFAT signaling. J Neurosci 29:12957–12969

99. Norris CM, Kadish I, Blalock EM, Chen KC, Thibault V, Porter NM, Landfield PW, Kraner SD (2005) Calcineurin triggers reactive/inflammatory processes in astrocytes and is upregulated in aging and Alzheimer's models. J Neurosci 25:4649–4658
100. Daschil N, Geisler S, Obermair GJ, Humpel C (2014) Short- and long-term treatment of mouse cortical primary astrocytes with beta-amyloid differentially regulates the mRNA expression of L-type calcium channels. Pharmacology 93:24–31
101. Simpson JE, Ince PG, Shaw PJ, Heath PR, Raman R, Garwood CJ, Gelsthorpe C, Baxter L, Forster G, Matthews FE, Brayne C, Wharton SB (2011) Microarray analysis of the astrocyte transcriptome in the aging brain: relationship to Alzheimer's pathology and APOE genotype. Neurobiol Aging 32:1795–1807
102. Kanemaru K, Kubota J, Sekiya H, Hirose K, Okubo Y, Iino M (2013) Calcium-dependent N-cadherin up-regulation mediates reactive astrogliosis and neuroprotection after brain injury. Proc Natl Acad Sci U S A 110:11612–11617
103. Rodriguez-Vieitez E, Saint-Aubert L, Carter SF, Almkvist O, Farid K, Scholl M, Chiotis K, Thordardottir S, Graff C, Wall A, Langstrom B, Nordberg A (2016) Diverging longitudinal changes in astrocytosis and amyloid PET in autosomal dominant Alzheimer's disease. Brain 139:922–936
104. Verkhratsky A, Matteoli M, Parpura V, Mothet JP, Zorec R (2016a) Astrocytes as secretory cells of the central nervous system: idiosyncrasies of vesicular secretion. EMBO J 35:239–257
105. Vardjan N, Gabrijel M, Potokar M, Svajger U, Kreft M, Jeras M, de Pablo Y, Faiz M, Pekny M, Zorec R (2012) IFN-γ-induced increase in the mobility of MHC class II compartments in astrocytes depends on intermediate filaments. J Neuroinflammation 9:144
106. Stenovec M, Kreft M, Grilc S, Pangrsic T, Zorec R (2008) EAAT2 density at the astrocyte plasma membrane and Ca^{2+}-regulated exocytosis. Mol Membr Biol 25:203–215
107. Vardjan N, Verkhratsky A, Zorec R (2015) Pathologic potential of astrocytic vesicle traffic: new targets to treat neurologic diseases? Cell Transplant 24:599–612
108. Potokar M, Vardjan N, Stenovec M, Gabrijel M, Trkov S, Jorgacevski J, Kreft M, Zorec R (2013) Astrocytic vesicle mobility in health and disease. Int J Mol Sci 14:11238–11258
109. Potokar M, Kreft M, Pangrsic T, Zorec R (2005) Vesicle mobility studied in cultured astrocytes. Biochem Biophys Res Commun 329:678–683
110. Stenovec M, Kreft M, Grilc S, Potokar M, Kreft ME, Pangrsic T, Zorec R (2007) Ca^{2+}-dependent mobility of vesicles capturing anti-VGLUT1 antibodies. Exp Cell Res 313:3809–3818
111. Potokar M, Stenovec M, Gabrijel M, Li L, Kreft M, Grilc S, Pekny M, Zorec R (2010) Intermediate filaments attenuate stimulation-dependent mobility of endosomes/lysosomes in astrocytes. Glia 58:1208–1219
112. Potokar M, Stenovec M, Kreft M, Kreft ME, Zorec R (2008) Stimulation inhibits the mobility of recycling peptidergic vesicles in astrocytes. Glia 56:135–144
113. Potokar M, Kreft M, Lee SY, Takano H, Haydon PG, Zorec R (2009) Trafficking of astrocytic vesicles in hippocampal slices. Biochem Biophys Res Commun 390:1192–1196
114. 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 282:28749–28758
115. Vekrellis K, Ye Z, Qiu WQ, Walsh D, Hartley D, Chesneau V, Rosner MR, Selkoe DJ (2000) Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. J Neurosci 20:1657–1665
116. Dorfman VB, Pasquini L, Riudavets M, Lopez-Costa JJ, Villegas A, Troncoso JC, Lopera F, Castano EM, Morelli L (2010) Differential cerebral deposition of IDE and NEP in sporadic and familial Alzheimer's disease. Neurobiol Aging 31:1743–1757
117. Son SM, Cha MY, Choi H, Kang S, Choi H, Lee MS, Park SA, Mook-Jung I (2016) Insulin-degrading enzyme secretion from astrocytes is mediated by an autophagy-based unconventional secretory pathway in Alzheimer disease. Autophagy 12:784–800

118. Fields RD (2008) White matter in learning, cognition and psychiatric disorders. Trends Neurosci 31:361–370
119. Fern RF, Matute C, Stys PK (2014) White matter injury: ischemic and nonischemic. Glia 62:1780–1789
120. Matute C (2010) Calcium dyshomeostasis in white matter pathology. Cell Calcium 47:150–157
121. Bartzokis G (2011) Alzheimer's disease as homeostatic responses to age-related myelin breakdown. Neurobiol Aging 32:1341–1371
122. Peters A, Verderosa A, Sethares C (2008) The neuroglial population in the primary visual cortex of the aging rhesus monkey. Glia 56:1151–1161
123. Psachoulia K, Jamen F, Young KM, Richardson WD (2009) Cell cycle dynamics of NG2 cells in the postnatal and ageing brain. Neuron Glia Biol 5:57–67
124. Salter MG, Fern R (2005) NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. Nature 438:1167–1171
125. Jantaratnotai N, Ryu JK, Kim SU, McLarnon JG (2003) Amyloid β peptide-induced corpus callosum damage and glial activation *in vivo*. Neuroreport 14:1429–1433
126. Desai MK, Sudol KL, Janelsins MC, Mastrangelo MA, Frazer ME, Bowers WJ (2009) Triple-transgenic Alzheimer's disease mice exhibit region-specific abnormalities in brain myelination patterns prior to appearance of amyloid and tau pathology. Glia 57:54–65
127. Burns JM, Church JA, Johnson DK, Xiong C, Marcus D, Fotenos AF, Snyder AZ, Morris JC, Buckner RL (2005) White matter lesions are prevalent but differentially related with cognition in aging and early Alzheimer disease. Arch Neurol 62:1870–1876
128. Heneka MT, O'Banion MK (2007) Inflammatory processes in Alzheimer's disease. J Neuroimmunol 184:69–91
129. Schubert P, Morino T, Miyazaki H, Ogata T, Nakamura Y, Marchini C, Ferroni S (2000) Cascading glia reactions: a common pathomechanism and its differentiated control by cyclic nucleotide signaling. Ann N Y Acad Sci 903:24–33
130. Meyer-Luehmann M, Spires-Jones TL, Prada C, Garcia-Alloza M, de Calignon A, Rozkalne A, Koenigsknecht-Talboo J, Holtzman DM, Bacskai BJ, Hyman BT (2008) Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. Nature 451:720–724
131. Heneka MT, Sastre M, Dumitrescu-Ozimek L, Dewachter I, Walter J, Klockgether T, Van Leuven F (2005) Focal glial activation coincides with increased BACE1 activation and precedes amyloid plaque deposition in APP[V717I] transgenic mice. J Neuroinflammation 2:22
132. Sanz JM, Chiozzi P, Ferrari D, Colaianna M, Idzko M, Falzoni S, Fellin R, Trabace L, Di Virgilio F (2009) Activation of microglia by amyloid {beta} requires P2X7 receptor expression. J Immunol 182:4378–4385
133. Lotz M, Ebert S, Esselmann H, Iliev AI, Prinz M, Wiazewicz N, Wiltfang J, Gerber J, Nau R (2005) Amyloid β peptide 1-40 enhances the action of Toll-like receptor-2 and -4 agonists but antagonizes Toll-like receptor-9-induced inflammation in primary mouse microglial cell cultures. J Neurochem 94:289–298
134. Okun E, Griffioen KJ, Lathia JD, Tang SC, Mattson MP, Arumugam TV (2009) Toll-like receptors in neurodegeneration. Brain Res Rev 59:278–292
135. Walter S, Letiembre M, Liu Y, Heine H, Penke B, Hao W, Bode B, Manietta N, Walter J, Schulz-Schuffer W, Fassbender K (2007) Role of the toll-like receptor 4 in neuroinflammation in Alzheimer's disease. Cell Physiol Biochem 20:947–956
136. Rodriguez JJ, Witton J, Olabarria M, Noristani HN, Verkhratsky A (2010) Increase in the density of resting microglia precedes neuritic plaque formation and microglial activation in a transgenic model of Alzheimer's disease. Cell Death Dis 1:e1