

Please cite this article in press as: Rodríguez-Arellano JJ et al. Astrocytes in physiological aging and Alzheimer's disease. *Neuroscience* (2015), <http://dx.doi.org/10.1016/j.neuroscience.2015.01.007>

Neuroscience xxx (2015) xxx–xxx

REVIEW

ASTROCYTES IN PHYSIOLOGICAL AGING AND ALZHEIMER'S DISEASE

J. J. RODRÍGUEZ-ARELLANO,^{a,b*} V. PARPURA,^{c,d}
R. ZOREC^{e,f} AND A. VERKHRATSKY^{a,b,e,f,g*}

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

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

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

^d Department of Biotechnology, University of Rijeka, Radmila Matejčić 2, 51000 Rijeka, Croatia

^e University of Ljubljana, Institute of Pathophysiology, Laboratory of Neuroendocrinology and Molecular Cell Physiology, Zaloska cesta 4, SI-1000 Ljubljana, Slovenia

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

^g Faculty of Life Sciences, The University of Manchester, Manchester, M13 9PT, UK

Abstract—Astrocytes are fundamental for homoeostasis, defence and regeneration of the central nervous system. Loss of astroglial function and astroglial reactivity contributes to the aging of the brain and to neurodegenerative diseases. Changes in astroglia in aging and neurodegeneration are highly heterogeneous and region-specific. In animal models of Alzheimer's disease (AD) astrocytes undergo degeneration and atrophy at the early stages of pathological progression, which possibly may alter the homeostatic reserve of the brain and contribute to early cognitive deficits. At later stages of AD reactive astrocytes are associated with neurite plaques, the feature commonly found in animal models and in human diseased tissue. In animal models of the AD reactive astrogliosis develops in some (e.g. in the hippocampus) but not in all regions of the brain. For

instance, in entorhinal and prefrontal cortices astrocytes do not mount gliotic response to emerging β -amyloid deposits. These deficits in reactivity coincide with higher vulnerability of these regions to AD-type pathology. Astroglial morphology and function can be regulated through environmental stimulation and/or medication suggesting that astrocytes can be regarded as a target for therapies aimed at the prevention and cure of neurodegenerative disorders.

This article is part of a Special Issue entitled: Astrocyte-Neuron Interact. © 2015 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: neuroglia, astrocyte, aging, Alzheimer's disease, neurodegeneration.

Contents

| | |
|--|----|
| Physiological and pathological brain aging | 00 |
| Neuroglia: the housekeeper and the protector | 00 |
| Astroglial pathology: the general concept | 00 |
| Astrocytes in physiological aging | 00 |
| Astroglia in Alzheimer's disease (AD) | 00 |
| Astrogliosis | 00 |
| Astrodegeneration | 00 |
| Astroglial Ca^{2+} signaling in AD | 00 |
| Neuroglia as a potential therapeutic target | 00 |
| Acknowledgments | 00 |
| References | 00 |

PHYSIOLOGICAL AND PATHOLOGICAL BRAIN AGING

"There is no reason to suppose that protoplasm, the living material of organisms, has a necessarily limited duration of life, provided that the conditions proper to it are maintained, and it has been argued that since every living organism comes into existence as a piece of the protoplasm of the pre-existing living organism, protoplasm is potentially immortal" (Encyclopedia Britannica, 1911, 11th edition, v. 16, p. 974). Notwithstanding this optimistic assertion, multicellular organisms are mortal and their death often concludes a more or less protracted period generally referred to as aging. When aging begins, why it commences and how it proceeds is a topic for numerous scientific discussions, and many (hundreds are in existence (Medvedev, 1990)) theories of aging have been developed, none of them hitherto acquiring universal acknowledgment.

*Correspondence to: J. J. Rodríguez-Arellano, Achucarro Center for Neuroscience, IKERBASQUE, Basque Foundation for Science, 48011 Bilbao, Spain, A. Verkhratsky, Faculty of Life Sciences, The University of Manchester, Manchester M13 9PT, UK.

E-mail addresses: j.rodriguez-arellano@ikerbasque.org (J. J. Rodríguez-Arellano), Alexej.Verkhratsky@manchester.ac.uk (A. Verkhratsky).

Abbreviations: 3xTg-AD, triple transgenic mice model of Alzheimer's disease; AD, Alzheimer's disease; APP, amyloid precursor protein; ATP, adenosine triphosphate; CA1, Cornu Ammonis 1; CNS, Central nervous system; DG, dentate gyrus; GABA, γ -aminobutyric acid; GFAP, glial fibrillary acidic protein; GS, glutamine synthetase; iNOS, inducible nitric oxide synthetase; InsP₃, inositol 1,4,5-trisphosphate; mGluR5, metabotropic glutamate receptor 5; NFAT, nuclear factor of activated T-cells; PS1, presenilin 1; s100 β , protein s100 β ; TRPC1, transient receptor potential canonical (channel) 1.

Despite uncertainties in fundamental mechanisms manifestations of aging are clear, and can be easily perceived from a decline of functional capacities of various organs and systems. The nervous tissue is not an exception, and cognitive decline accompanies brain senescence. Surprisingly, however, the brain appears as one of the most age-resilient systems of the human body. Indeed, the 40-year-old athlete cannot compete in the sprint with youngsters, whereas the 60-year-old academic has, as a rule, a much higher intellectual output than his/her 20-year-old student. This may reflect a remarkable plasticity and prolonged development of the central nervous system (CNS) in higher primates and especially in humans (in the *Homo sapiens*, for example myelination in the CNS proceeds well into the 3rd decade of life (Walhovd et al., 2014)); furthermore, the human brain is optimized for learning and the longer a person lives the more information he/she accumulates, which recompenses for age-dependent alterations. Different components of cognition are distinctly affected in an advanced age. For example, the real-time processing and formation of new memories suffer the most, whereas long-term memories, semantic knowledge and analyzing capabilities are impaired much less (Erickson and Barnes, 2003; Hedden and Gabrieli, 2004). In recalling a list of words, for instance, a group of young adults were significantly better than a group of elders, but this difference was dramatically reduced, under the level of statistical significance, when other cognitive strategies, which involved more elaborate processing activities were used (Logan et al., 2002). To all probabilities the senescent brain undergoes functional remodeling, while maintaining a reserve of plasticity that allows, for long periods of time, levels of functional activity for day-to-day tasks that are well within (and often are above) the normal range.

Unbridled cognitive aging is, however, not everybody's fortune and senile dementia, which reflects the neurodegenerative pathology, affects a substantial, and continuously growing part of the world population. Aging, in essence, is the main risk factor for neurodegenerative diseases and for a long time these diseases were considered as natural outcomes of the brain aging. This was supported by morphometric studies that claimed a substantial and progressive age-dependent loss of neurones leading to the brain atrophy; these are precisely the same changes that are observed in neurodegeneration (albeit these latter being faster and more prominent). The dogma of continuous age-dependent neuronal death, which represents a direct link between senescence and neurodegeneration being exceedingly simple and hence self-explanatory, still dominates contemporary culture, both popular and medical. In reality, healthy brain aging is not associated with a notable loss in neurones, the number of which does not change significantly during physiological aging in human, monkey and rodent nervous systems (West, 1993; Calhoun et al., 1998; Dickstein et al., 2006; Fabricius et al., 2013). Similarly, neither the total number nor the density of synapses (at least in the hippocampus) is affected with aging (Smith et al., 2000; Geinisman et al., 2004). However, a certain reduction in the size of the syn-

apses has been noted (Nicholson et al., 2004). In neurodegenerative diseases, in contrast, a massive loss of neurones leading to a remarkable shrinkage of the brain is a common feature (Braak et al., 1998a,b).

NEUROGLIA: THE HOUSEKEEPER AND THE PROTECTOR

The maintenance of homeostasis of the CNS as well as its defence are the fundamental functions of neuroglia (Reichenbach and Bringmann, 2010; Kettenmann and Ransom, 2013; Verkhratsky and Butt, 2013). The neuroglia is represented by highly heterogeneous cell types optimized for numerous homeostatic functions. The oligodendrocytes, for example, enwrap axons with the myelin sheath and provide them with a local metabolic, structural and homeostatic support (Walhovd et al., 2014), whereas the NG-2 glia most likely maintain a pool of myelinating cells throughout life (Frohlich et al., 2011). The microglial cells, which invade the brain as fetal erythromyeloid precursors contribute to a surprisingly wide array of functions in the CNS development (for example, in shaping synaptic connections and removing redundant neurones (Kettenmann et al., 2013; Xavier et al., in press)) and in CNS plasticity (through secreting factors that affect synaptic transmission (Pascual et al., 2012)). Astrocytes are arguably the most diverse type of neuroglia responsible for every conceivable homeostatic task in the CNS (Parpura et al., 2012; Parpura and Verkhratsky, 2012; Verkhratsky and Butt, 2013). Astroglial cells in the gray matter parcellate the neural tissue into relatively independent astroglial-vascular units delineated by the arborization of a single protoplasmic astrocyte and connected to the vasculature through the astroglial perivascular process terminating in the endfoot (Nedergaard et al., 2003). Within these territorial domains astroglial membranes cover the majority of synaptic contacts and neurones; these astroglial membranes bear numerous pumps and transporters that are responsible for ion, metabolite, reactive oxygen species and neurotransmitter homeostasis of the interstitial fluid (Verkhratsky and Nedergaard, in press). The endfeet of astrocytes plaster blood vessels and create a "glymphatic" system that promotes the removal of metabolites from the CNS parenchyma (Iliff and Nedergaard, 2013). Astrocytes also act as secretory cells of the CNS releasing neurotransmitters (e.g. ATP, glutamate or GABA), neuromodulators (such as D-serine or kynurenic acid) and trophic factors, also known as tissue hormones (such as growth factors or tumor necrosis factor- α , TNF- α), which all affect various aspects of plasticity and information processing in the CNS (Malarkey and Parpura, 2008; Parpura et al., 2011; Martineau et al., 2014). Astroglial secretion employs several mechanisms, which include exocytotic vesicular release, diffusion through plasmalemmal channels or solute carrier transporters (Malarkey and Parpura, 2008; Parpura et al., 2011). Astrocytes are important elements of systemic homeostasis being a part of CNS chemosensing, for example, for pH, CO₂ or sodium ion (Gourine and Kasparov, 2011).

Neuroglial “homeostatic” role is tightly linked to their protective and defensive capabilities, as indeed neuroglial cells respond to every kind of pathological insult by mounting neuroprotection (e.g. supplying neurones with metabolic substrates in conditions of oxygen deprivation) and by initiating the complex program of activation, generally known as gliosis (Verkhratsky et al., 2013a; Burda and Sofroniew, 2014; Pekny et al., 2014). The gliotic response (astrogliosis, activation of microglia and NG2 cells) is an evolutionary conserved reaction, operative already in invertebrates, which results in the generation of numerous glial phenotypes contributing to neuroprotection (all glial cells), physical fencing of the damaged areas (astroglial scar) or a removal of pathogens through phagocytosis (activated microglia).

ASTROGLIOPATHOLOGY: THE GENERAL CONCEPT

Dysfunction of neuroglia ultimately results in the failure of CNS homeostasis and compromises defensive capabilities of neural tissue. Hence, the gliopathology is an essential component of the most, and probably all, neurological diseases. The resolution of neuropathology is similarly directly linked to the ability of glia to limit the lesion, remove the pathogens and post-lesion cellular debris and permit and facilitate regeneration.

The concept of astroglial pathology is still in the nascent state (Verkhratsky et al., 2012); general belief firmly rooted in the conscience of neuropathologists connects astrocytes with astrogliosis, which is considered a purely pathological, detrimental reaction linked to a vaguely defined process known as “neuroinflammation”. Recent decade, however, brought forward new experimental data and new concepts indicating much wider and more complex developments in astroglia in various forms of neuropathology (see e.g. (Sofroniew, 2009; Verkhratsky et al., 2013a, in press; Burda and Sofroniew, 2014; Pekny et al., 2014) for details and references).

First, the perception of reactive astrogliosis as a purely pathological remodeling with a negative outcome has been replaced by a concept of reactivity as a defensive process that represents a wide spectrum of cellular changes specific for distinct pathological contexts (Sofroniew, 2009; Pekny et al., 2014). Astrogliotic response hence results in various cellular phenotypes optimized for neuroprotection against particular insults. Reactive astrocytes increase neuroprotection and trophic support of stressed neurones, contribute to the formation of a glial scar that isolates the area of damage from the rest of the CNS tissue, provide for reconstruction of the compromised blood–brain barrier and are indispensable for post-lesion regeneration. The adaptive result of reactive astrogliosis is usually beneficial, and suppression of astroglial reactivity increases neuronal vulnerability, exacerbates pathological development and alters regeneration (Burda and Sofroniew, 2014; Pekny et al., 2014). In certain circumstances however gliotic changes may assume

a deleterious proportion and may contribute to neurotoxicity (Heneka et al., 2010a; Wyss-Coray and Rogers, 2012).

Second, numerous forms of neuropathology are associated with acute or chronic astroglial pathology, manifested in a loss or remodeling of basic astroglial functions. Astroglial asthenia reflected by morphological atrophy and functional weakness has been described in many different types of neurological diseases from psychiatric pathology to neurodegeneration (Rajkowska and Stockmeier, 2013; Verkhratsky et al., 2013b, in press). A loss of astroglial function, such as, for example, a failure in regulating glutamate concentration in the interstitium, takes a leading role in disorders associated with the excitotoxic neuronal death such as, for example, Wernicke encephalopathy (Hazzell, 2009) or amyotrophic lateral sclerosis (Rossi et al., 2008; Valori et al., 2014). As troglic insufficiency in maintaining glutamine–glutamate metabolism in conditions of hyperammonemia underlies the evolution of hepatic encephalopathy (Butterworth, 2010; Rose et al., 2013), whereas an impairment of the astroglia-dependent water transport affects the paravascular glymphatic system and contributes to the development of the brain edema (Thrane et al., in press). Pathological remodeling of astrocytes represents a key pathogenic step in genetic disorders, such as Alexander disease (Messing et al., 2012), and contributes to epilepsy (Crunelli et al., in press).

In conclusion, glial reactivity, as well as glial atrophy and pathological remodeling alone or in combination, are the necessary components of every neurological disease, and hence further insights into astroglial pathology, may be critical for advancing new therapeutic strategies.

ASTROCYTES IN PHYSIOLOGICAL AGING

A coherent concept of neuroglial aging has not yet emerged. Morphometric studies, however, have demonstrated that two types of glia, the oligodendrocytes and the microglia, are most affected by physiological aging. The white matter seems to be the most affected in aging with ~11% reduction in the volume (compared with only 3% reduction in the cortical volume (Haug and Eggers, 1991)). Cell counts in the human brain showed a very significant (~30% or even more) decline in the number of oligodendrocytes with age (Pelvig et al., 2008; Fabricius et al., 2013). Experiments on old monkeys, however, revealed the opposite trend: the total number of oligodendrocytes was found to increase very substantially; in the visual cortex of old monkeys, for instance, oligodendrocytes were ~50% more numerous when compared to the adult animals (Peters and Sethares, 2004). These numerous oligodendrocytes, however, showed an altered morphology and were considered to be functionally impaired, which defined a decreased myelinization in CNS structures. Similarly, aging (in rodents) was found to increase the number of microglial cells, which coincided with signs of a morphological atrophy such as deramification, a

decrease in volume and fragmentation of processes, less spherical somata, etc. (Tremblay et al., 2012; Streit and Xue, 2013). These changes apparently indicate dwindling of microglial neuroprotective and defensive capabilities (Streit et al., 2009).

The number of astrocytes, at least in human brains, for which the relevant counts were performed (Pelvig et al., 2008; Fabricius et al., 2013), does not change with age, remaining unaffected even in centenarians (Fabricius et al., 2013). Although the concept of the age-dependent increase in astrogliosis reactivity is quite widespread (Unger, 1998; Lynch et al., 2010) and is used to corroborate the ideas of the so called “inflammaging” (Franceschi, 2007), which regards brain senescence as a chronic neuroinflammation; the experimental data on this matter are, however, controversial. In aged animals a decrease (e.g. Cerbai et al., 2012) and an increase (Diniz et al., 2010) in numbers of glial fibrillary acidic protein (GFAP)-positive astrocytes, as well as astrogliosis atrophy and hypertrophy were observed. In the hippocampus of 21-month-old rats, the volume of astrogliosis domains, defined from the morphometric analysis of cells staining with anti-glutamine synthetase (GS) antibodies, was about 2 times larger when compared with 5-month-old animals (Grosche et al., 2013). The total number of hippocampal astrocytes, however, did not change with age, which stipulated a substantial increase in the overlap of astrogliosis domains (Grosche et al., 2013). In depth morphometric analysis of astrocytes labeled with three distinct markers, antibodies against GFAP, s100 β protein and GS revealed complex and region-specific changes in the astrogliosis appearance (Fig. 1 and (Rodríguez et al., 2014)). The GFAP-positive profiles were substantially larger in the Cornu Ammonis 1 (CA1) region and dentate gyrus (DG) of the hippocampus, and significantly smaller in the entorhinal cortex (EC). The EC astrocytes

in aging display less main processes with very little number of secondary branches, the process which starts already at 9-month-old animals (see Rodriguez et al., 2014) and develops with age. At the same time the s100 β profiles significantly increased in the aged DG and EC but not in the CA1 region, whereas GS-positive profiles were smaller in the old CA1 and DG, while no changes in the EC were identified. GS is a central enzyme for glutamine–glutamate/GABA shuttle and for ammonium detoxification (Rose et al., 2013), and hence an age-dependent decrease in the GS expression may affect both excitatory and inhibitory neurotransmission. There are also some indications for metabolic remodeling of aged astrocytes; oxidative metabolism in astrogliosis cells seems to increase with age, which may limit their ability to supply neurones with metabolic substrates (Jiang and Cadena, in press).

Age-dependent increase in GFAP expression and hypertrophy of astrocytes can, however, reflect upon astrogliosis adaptive plasticity. For example, exposure of aged mice and rats to physical activity or an enriched environment increased GFAP expression in hippocampal regions and led to astrocitic hypertrophy with an increased morphological complexity (Rodríguez et al., 2013; Sampedro-Piquero et al., 2014); these changes in astrogliosis coincided with cognitive improvement (Sampedro-Piquero et al., 2014).

How aging affects astrogliosis physiology remains generally unknown. Resting membrane potential measured from astrocytes, voltage-clamped in slices isolated from mice, was about -80 mV for all ages between 1 and 21 months. Age, however, affected the functional expression of ionotropic glutamate and purinergic receptors; their density increased several times between 1 month and 3–6 months and then rapidly declined, so that in 21-month-old animals, it was similar

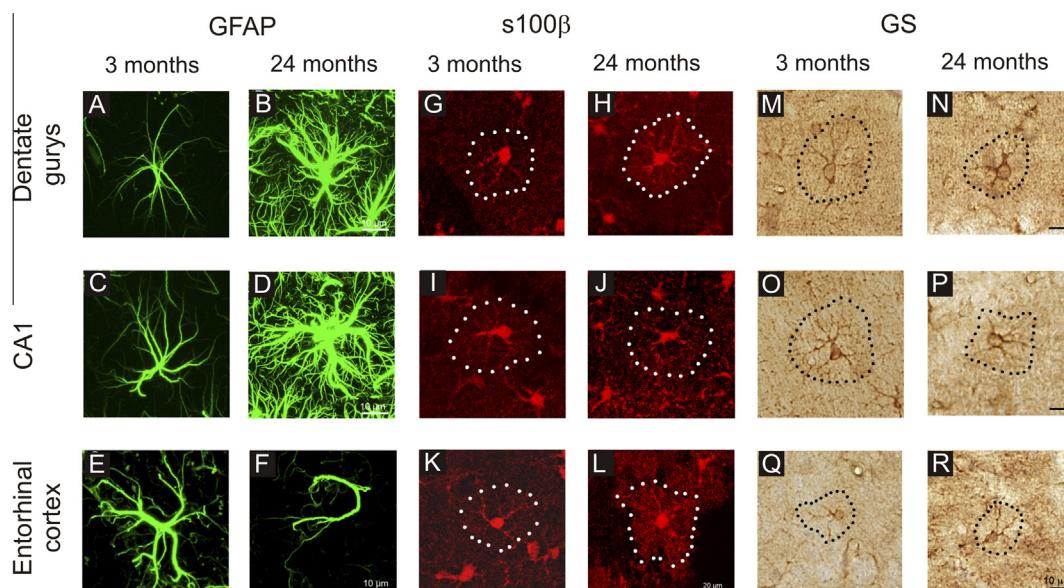


Fig. 1. Age-dependent remodeling of astrogliosis profiles in different brain areas. Confocal images showing GFAP (A–F), s100 β (G–L) and GS (M–R) immunolabeled astrocytes in the dentate gyrus and CA1 hippocampal areas as well as in the entorhinal cortex of mice at 3 and 24 months. Modified from (Rodríguez et al., 2014).

to that recorded in 1-month-old mice (Lalo et al., 2011). In parallel, the neurotransmitter-induced Ca^{2+} signaling was reduced in aged astrocytes (Palygin et al., 2010), which in turn may affect the astroglial release of neuroactive substances (Lalo et al., 2014). There are some indications that old age may decrease gap junctional coupling between astrocytes cells (Peters et al., 2009), although no changes in astroglial coupling were found in brain slices of 8–14-month-old mice (Cruz et al., 2010). Old age also reduced expression of water channels (aquaporins 4) in astroglial perivascular processes and markedly diminished clearance of the brain parenchyma through the glymphatic pathway (Kress et al., in press). Furthermore, vesicle traffic, which regulates the density of aquaporin 4 at the astrocytic plasma membrane (Potokar et al., 2013a) may be a key process contributing to aging and a number of neurologic diseases (Potokar et al., 2013b). Vesicle traffic may be involved in astrocyte morphology alterations, which can dynamically change in a rather short time (Vardjan et al., 2014), contributing to the diurnal variation of the flux of cerebrospinal fluid clearance (Xie et al., 2013).

ASTROGLIA IN ALZHEIMER'S DISEASE (AD)

Astrogliosis

The pathological potential of neuroglia in the context of AD has been realized already by Alois Alzheimer, who observed glial cells in close association with damaged neurones; he also found that glial cells were obligatory components of senile plaques (Alzheimer, 1910); similarly, reactive astrocytes are present in the brains of AD animal models. In the AD both reactivity and atrophic changes in astrocytes occur (Fig. 2), and, moreover, glial changes often precede formation of specific histopathology, i.e. plaques and tangles (for details and extensive reference lists see (Fuller et al., 2009; Verkhratsky et al., 2010; Verkhratsky and Rodriguez, 2011).

et al., 2010, in press; Orre et al., in press)). Local groups of reactive astrocytes, for example, have been identified in the brain parenchyma of transgenic mice over-expressing the London mutant of amyloid precursor protein, APP [V717I] long before any detectable β -amyloid depositions. These reactive astrocytes produced pro-inflammatory factors and up-regulated expression of inducible nitric oxide synthetase (iNOS), and arguably prepared the ground for future senile plaque evolution (Heneka et al., 2005). At the same time, in transgenic AD models such as 3xTg-AD (the triple transgenic mouse model of AD that harbors three mutant genes for the APPSwe, the PS1M146V and the tauP301L (Oddo et al. 2003a,b)), and in PDAPP-J20 mice (expressing mutant APP) morphological atrophy of astroglial cells also occurred early (see below), preceding an emergence of β -amyloid deposits and senile plaques (Olabarria et al., 2010; Yeh et al., 2011; Beauquis et al., 2013). At a more advanced age, reactive GFAP-positive astrocytes mainly associated with senile plaques and perivascular β -amyloid deposits have been also observed in these transgenic animals, as well as in other animal models of the AD (Verkhratsky et al., 2010; Rodriguez and Verkhratsky, 2011).

At late stages of AD astroglial reactivity (as judged by an increased expression of GFAP and s100 β) has been often described in post-mortem tissues from AD patients (Beach and McGeer, 1988; Griffin et al., 1989; Meda et al., 2001). No correlation, however between an increased GFAP expression and β -amyloid load has been revealed, although sporadically some degree of correlation between the GFAP expression and the Braak stage of AD has been suggested (Simpson et al., 2010). In another study, no differences in the GFAP expression were found between demented and non-demented brains (Wharton et al., 2009). Hypertrophic, reactive astrocytes in post-mortem tissues have been located both around senile plaques and in plaque-free parts of

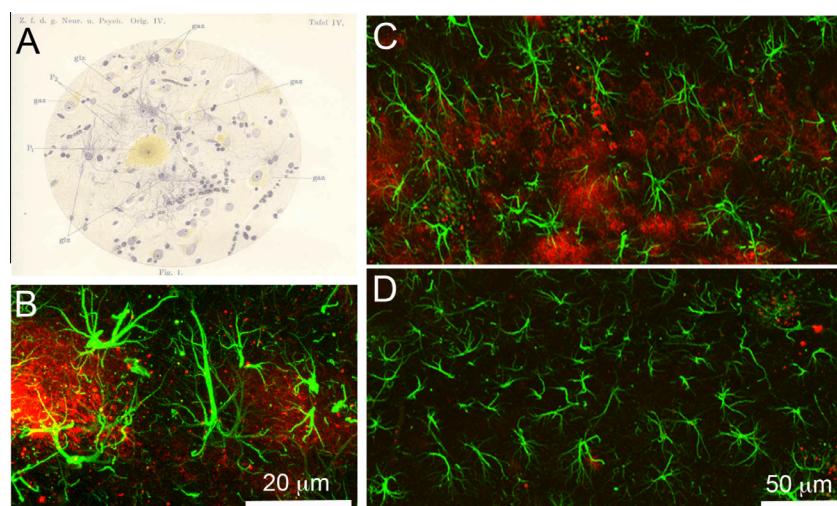


Fig. 2. Reactive astrogliosis in association with senile plaques. A. Neuritic A β plaques (P in A) as seen and drawn by Alzheimer (1910); the plaque core (P_1) is surrounded by activated glial cells (glz). (B, C) Confocal images of β -amyloid (red) and reactive astrocytes (green) associated with senile plaques (B) and diffuse amyloid deposits (C) in the hippocampus of 18-month-old 3xTg-AD mice. D. From the same preparation as shown in (C) confocal images of astrocytes in β -amyloid-free area show cells that are much smaller than reactive astrocytes.

the parenchyma (Simpson et al., 2010). Reactive astrogliosis in the AD is represented by the “isomorphic” or mild astrogliosis, in which astrocytes retain their territorial domains and are arguably, of a more neuroprotective phenotype (Sofroniew, 2009); there are no indications of formation of an astroglial scar around amyloid deposits.

Astrogliotic response is not homogeneous throughout the AD-affected brains. In the transgenic model, in 3xTG-AD mice profound astrogliotic response is observed in the hippocampus, where it is linked with an emergence of perivascular β -amyloid deposits and senile plaques, with which reactive astrocytes are associated (Olabaria et al., 2010, 2011). A very different situation has been, however, observed in the entorhinal and prefrontal cortex of the same transgenic animals. In neither of these locations extracellular accumulation of β -amyloid (in a form of diffuse deposits, plaques or perivascular accumulation) induced astroglial reactivity (Fig. 3, (Yeh et al., 2011; Kulijewicz-Nawrot et al., 2012)). This compromised astrogliotic response may indicate defensive failure. Indeed both entorhinal and prefrontal cortices are believed to be more vulnerable to AD pathology. Such a conjecture is corroborated by the fact that experimental attenuation of astrogliotic response using transgenic technologies (suppression of GFAP and vimentin expression) shown to facilitate plaque formations in APP/PS1 AD mouse model (Kraft et al., 2013).

Astrodegeneration

Reduction in astroglial volume, surface area and in their morphological complexity has been observed in two distinct (3xTG-AD and PDAPP-J20) AD transgenic mice models (Olabaria et al., 2010; Yeh et al., 2011; Kulijewicz-Nawrot et al., 2012; Beauquis et al., 2013). In recent studies of the authors of this paper (Rodríguez and Verkhratsky unpublished data) atrophic astrocytes have been also observed in family AD human post-mortem tissues. Reduced morphological appearance of astrocytes in animal models was identified by analyzing astroglial profiles labeled with antibodies against GFAP (which mostly outlines primary and possibly secondary processes; Figs. 4 and 5) as well as with antibodies against GS and s100 β (which reveal much of astroglial arborization including the finest processes, because both GS and s100 β are cytosolic proteins whereas GFAP an intermediate filament, i.e. is a component of a cytoskeleton). The total number of astrocytes stained with these markers did not change with AD pathology progression over age (Olabaria et al., 2010; Yeh et al., 2011; Kulijewicz-Nawrot et al., 2012). Changes in the expression of GS in the AD progression differ between brain regions: in the hippocampus and prefrontal cortex, for example, expression of GS as well as number of GFAP-positive astrocytes expressing GS are significantly reduced, whereas GS levels in the entorhinal cortex remain unchanged (Fig. 5 and (Olabaria et al., 2011; Kulijewicz-Nawrot et al., 2013; Yeh et al., 2013)).

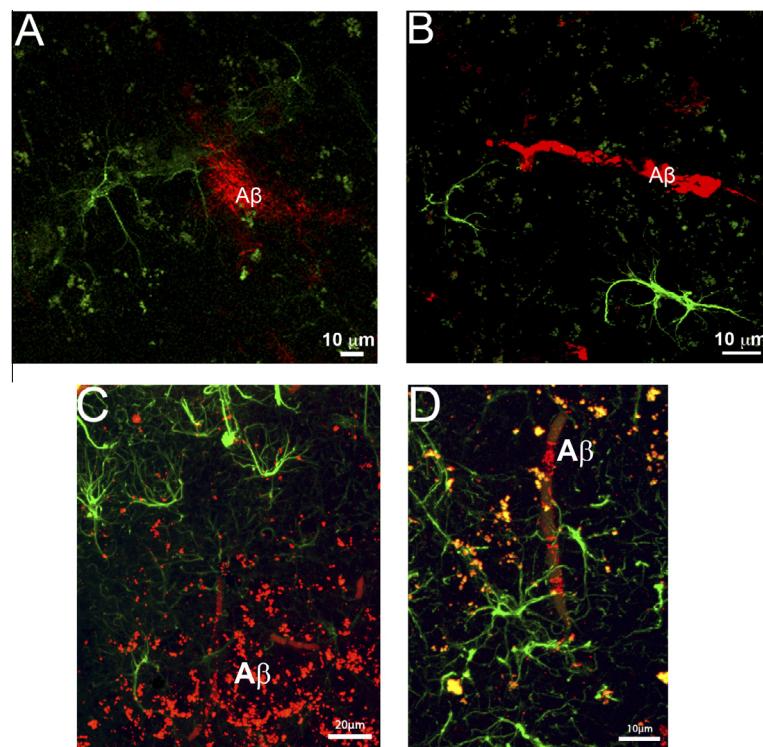


Fig. 3. Astroglial reactivity is absent in the entorhinal and prefrontal cortices of 18-month-old 3xTg-AD mice infested with β -amyloid. (A–D) Confocal images of entorhinal cortex (A, B) and prefrontal cortex (C, D) tissues labeled for GFAP (green) and β -amyloid (red) show the absence of astroglial hypertrophy and association of astrocytes with senile plaques (A, C) or β -amyloid deposits associated with blood vessels (B, D). Modified from (Yeh et al., 2011; Kulijewicz-Nawrot et al., 2012; Verkhratsky et al., in press).

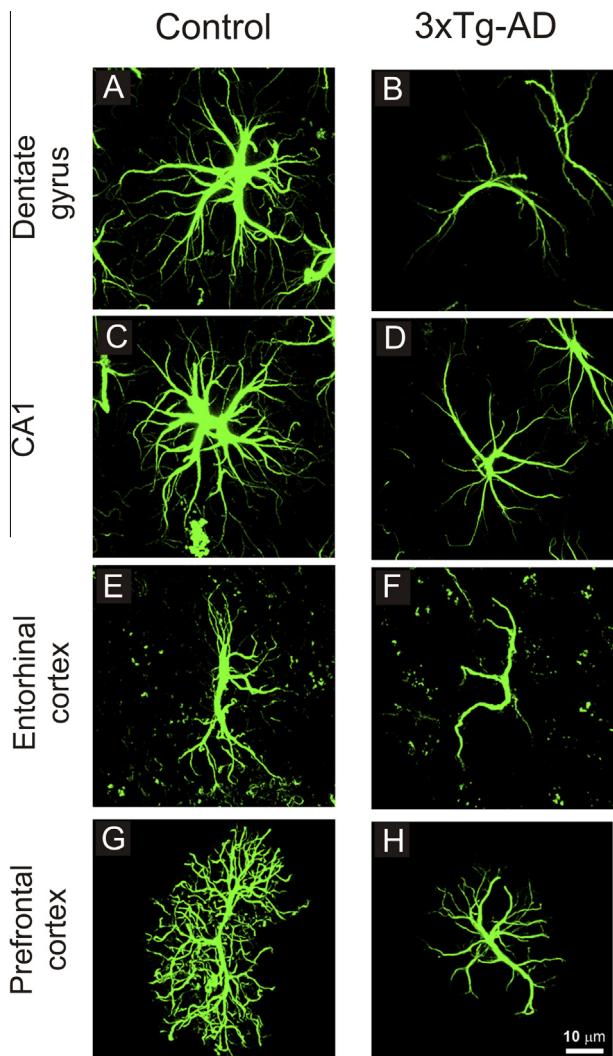


Fig. 4. Morphological atrophy of GFAP-positive astroglial profiles in astrocytes from different brain areas of transgenic AD mice. Confocal images showing GFAP-labeled astrocytes from dentate gyrus and CA1 hippocampal area as well as from entorhinal and prefrontal cortices in wild-type control mice (A, C, E, G) and in the transgenic 18-month-old 3xTg-AD mice. Reproduced with permission from (Verkhratsky et al., in press).

The age-dependent development of astroglial atrophy differed between regions of the brain. In the 3xTg-AD animals the reduction in astroglial profiles first occurred in the entorhinal cortex (at 1 month of age); in the prefrontal cortex the atrophic changes became significant at 3 months of age, whereas in the hippocampus atrophic astrocytes appeared much later at 9–12 months of age (Olabarria et al., 2010; Yeh et al., 2011; Kulijewicz-Nawrot et al., 2012). Apposite to note that atrophic astroglia emerged in all these brain regions before an appearance of obvious extracellular β -amyloid depositions.

Morphological atrophy of astroglial cells reflects a decrease in their territorial domains, and most likely indicates a reduction in the astroglial coverage of neurones and synapses located in these domains. Astroglial atrophy may be directly linked to a reduction

of astroglial homeostatic support, which may have dire consequences for performance and survival of neurones as well as may affect functional activity of synapses. All of this can result in trimming synaptic contacts, affecting transmission and weakening synaptic plasticity, which are precisely the early pathological events observed in AD (Terry, 2000; Coleman et al., 2004); a decrease in synaptic densities has been reported to correlate with the severity of dementia (DeKosky and Scheff, 1990; Samuel et al., 1994). Astrocytes support synaptic transmission through multiple mechanisms (Verkhratsky and Nedergaard, in press), these mechanisms include control over ions in the synaptic cleft, uptake of neurotransmitters and supplying neuronal terminals with glutamine, which is an obligatory precursor for glutamate and GABA. Decrease in synaptic coverage by astroglia arguably reduces this support causing functional synaptic weakness.

In addition, atrophy of astroglia may compromise the neuro-vascular unit and decrease endfeet coverage of brain vessels thus contributing to vascular deficits observed already in the early stages of AD (Zlokovic, 2008; Bell and Zlokovic, 2009). Similarly, in the early development of AD, a progressive loss of glucose utilization is frequently detected by functional brain imaging (Mosconi et al., 2008). Astrocytes are primary sites for glycogen processing in the brain and hence may contribute to this metabolic failure. Indeed the exposure of astrocytes to β -amyloid affects their metabolism (Allaman et al., 2010). Early stages of AD are also characterized by a remarkable decrease in noradrenergic innervations of the brain due to an early degeneration of the locus coeruleus from which noradrenergic projections originate (Chalermpalanupap et al., 2013). Astroglial calcium signaling, metabolism, and morphological plasticity, along with gap junctional connectivity of astroglial syncytia, are all subjects of noradrenergic regulation (Hertz et al., 2004; Ding et al., 2013); the failure of the latter may further exacerbate astrodegeneration in AD. Astrocytes may also contribute to the β -amyloid pathology through either failure of β -amyloid clearance or even through additional β -amyloid production, although data on an astroglial role in these processes remain somewhat controversial; the contribution of astroglia to β -amyloidogenesis was the subject of numerous papers and reviews (e.g. Apelt et al., 2003; Nagele et al., 2003; Wyss-Coray et al., 2003; Heneka et al., 2010b). Astrocytes also were reported to regulate microglial phagocytosis of β -amyloid, this regulation being dependent on astroglial apolipoprotein E and liver X receptor- α localized in astrocytes (Terwel et al., 2011).

Astroglial Ca^{2+} signaling in AD

Deregulation of Ca^{2+} signaling is widely regarded as an important component of neurodegenerative diseases in general (Nedergaard et al., 2010) and AD in particular; the latter has been even suggested to represent chronic “calciumopathy” (Stutzmann, 2007; Bezprozvanny and Mattson, 2008). Abnormal Ca^{2+} signaling was also observed in astroglia in the context of AD-type pathology (Lim et al., in press). *In vivo*, in experiments on transgenic

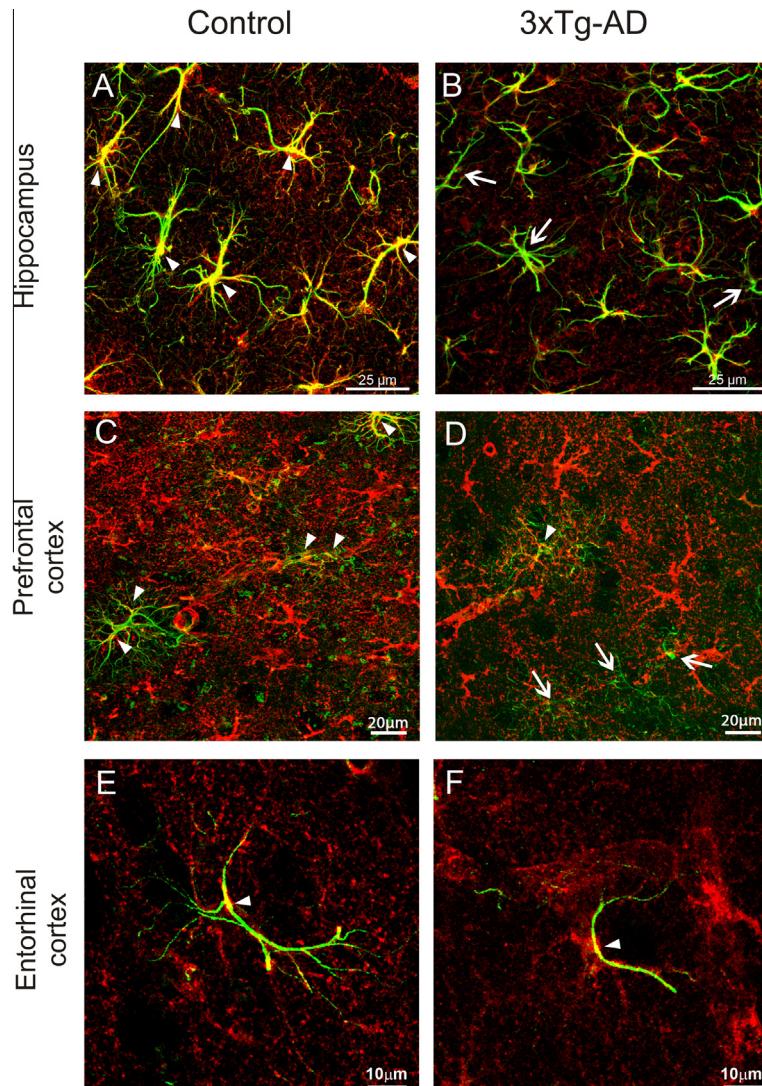


Fig. 5. Region-dependent changes in expression of glutamine synthetase in astrocytes from 3x-Tg-AD mice. Confocal images of astrocytes from hippocampus (A, B), prefrontal cortex (C, D) and entorhinal cortex (E, F) from wild-type control and 3xTg-AD mice; hippocampi were obtained from 18-month-old animals whereas PFC and EC preparations from 12-month-old animals. The cells were labeled for GFAP (green) and for GS (red). In the control wild-type mice GFAP and GS are co-expressed in almost all astrocytes (arrowheads), whereas GS expression is down regulated (arrows) in the GFAP-positive astrocytes from the hippocampus and prefrontal cortex but not from the entorhinal cortex of 3xTg-AD mice. Modified from (Olabarria et al., 2011; Kulijewicz-Nawrot et al., 2013; Yeh et al., 2013). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

AD animals (APP/PS1 mice carrying mutated genes for APP and PS1) revealed abnormally frequent $[Ca^{2+}]_i$ oscillations in reactive astrocytes associated with senile plaques: these abnormal Ca^{2+} transients gave rise to aberrant Ca^{2+} waves spreading through coupled astrocytes (Kuchibhotla et al., 2009). In another AD model, in mice expressing Swedish mutation of the APP gene, astrocytes showed higher frequency of spontaneous oscillations even before the appearance of amyloid plaques (Takano et al., 2007). Incidentally, intravenous administration of 0.4-mg/kg β -amyloid augmented astrocyte Ca^{2+} oscillations in healthy, wild-type control animals (Takano et al., 2007). Higher frequency of astroglial Ca^{2+} spiking was also observed in experiments *in situ* on slices acutely isolated from Tg2576 mice over-expressing a mutant form of APP, APPK670/671L (Riera et al., 2011).

Exposure of cultured astrocytes to β -amyloid was reported to trigger $[Ca^{2+}]_i$ transients and $[Ca^{2+}]_i$ oscillations (Abramov et al., 2003, 2004; Alberdi et al., 2013); although these findings were not universally confirmed and often β -amyloid did not trigger abnormal Ca^{2+} signaling in primary astroglia *in vitro* (e.g. (Toivari et al., 2011; Lim et al., 2013)). This discrepancy probably reflects differences in experimental conditions, for example differences in β -amyloid species and in β -amyloid concentrations used (Lim et al., *in press*), and, probably even more importantly, differences between astrocytes.

These differences can be quite substantial, as astrocytes from different regions of the brain show quite distinct physiology, and express different sets of neurotransmitter receptors. In the context of AD, profound differences were found in Ca^{2+} signaling toolkits between hippocampal astrocytes and astrocytes

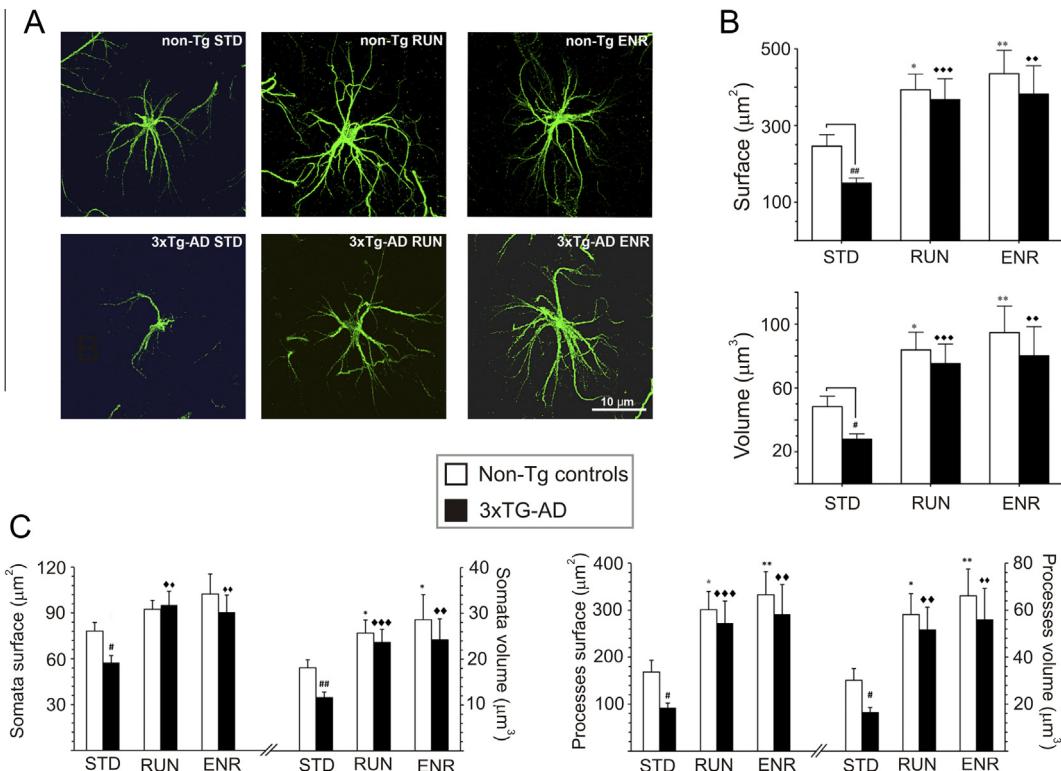


Fig. 6. Environmental stimulation restores astrogial morphology in AD animals. (A) Representative confocal images of GFAP-positive astroglial profiles in wild-type control (Non-Tg) and 3xTg-AD mice housed in standard conditions (STD) or subjected to voluntary running (RUN) or enriched environment (ENR). Note the morphological changes of the astrocytes induced by the different living conditions. The animals were 12 months old, being subjected for 9 months of physical or environmental stimulation. (B) Histograms showing difference of surface area and volume of GFAP-positive astrocytes in the dentate gyrus of wild-type control (Non-Tg) and 3xTg-AD mice housed under different housing conditions. (C) Histograms showing differences in surface area and volume of GFAP-positive astroglial somata and processes in non-Tg (wild-type controls) and 3xTg-AD mice housed under different housed conditions. Bars represent mean \pm SEM, # p < 0.05, ## p < 0.01 compared with non-Tg animals in same housing environment; * p < 0.05, ** p < 0.01 compared with non-Tg mice housed under STD; *** p < 0.01 and **** p < 0.001 compared with 3xTg-AD mice housed STD. Modified from (Rodríguez et al., 2013).

from the entorhinal cortex. In astrocytes isolated from the hippocampus of 3xTg-AD transgenic animals increase in metabotropic Ca^{2+} signaling and in store-operated Ca^{2+} entry was associated with an increased expression of metabotropic glutamate receptors mGluR5, inositol 1,4,5-trisphosphate (InsP_3)-receptors type 1 and store-operated channels of transient receptor potential canonical (channel) 1 (TRPC1) and Orai types (Grolla et al., 2013; Ronco et al., 2014). Similar modification of Ca^{2+} signaling toolkit and Ca^{2+} dynamics could be induced in cultured hippocampal astrocytes from healthy controls that were exposed to β -amyloid (100 nM for 72 h). An up-regulation of mGluR5 in plaque-associated astrocytes has been shown in AD model mice expressing mutant PS1 (Shrivastava et al., 2013), as well as in post-mortem AD human brains (Lim et al., 2013). In contrast, astrocytes isolated from the EC of 3xTg-AD mice did not display similar differences with control cells: entorhinal astrocytes exposed to β -amyloid neither demonstrated modified Ca^{2+} signaling toolkit nor modified Ca^{2+} dynamics (Grolla et al., 2013; Ronco et al., 2014). Incidentally, in astrocytes isolated from the neocortex of mice with genetic deletion of APP, a decrease in store-operated Ca^{2+} entry was observed (Linde et al., 2011).

Absence of remodeling of metabotropic Ca^{2+} signaling toolkit in entorhinal astrocytes could be linked to the absence of astrogliosis in the EC of AD animal models in response to β -amyloid accumulation (Yeh et al., 2011). There are indications that the InsP_3 -dependent Ca^{2+} signaling cascade is critical for initiation of astrogliosis (Kanemaru et al., 2013) and absence of β -amyloid effects on the InsP_3 -dependent toolkit in astroglial cells from the EC may be associated with their astrogliotic deficiency.

NEUROGLIA AS A POTENTIAL THERAPEUTIC TARGET

Pathological remodeling of astrocytes that affects their homeostatic and neuroprotective functions is an important component of pathogenesis of neurodegenerative diseases including AD. This pathological potential of astrocytes makes them legitimate and auspicious therapeutic targets. The development of glia-aiming drugs remains in a nascent state, and indeed identifying glia-specific and therapeutically relevant molecules representing the largest challenge. Nonetheless astrocytes can be affected by a variety of

treatments, the simplest being an environmental stimulation or dietary modifications.

Chronic (for 9 months) exposure of 3xTg mice to either physical activity or enriched environment, resulted in a reversal of morphological atrophy of astrocytes in the hippocampus, which happened in parallel with the reduction of β -amyloid burden and cognitive improvement (Fig. 6 and (Beauquis et al., 2013; Rodriguez et al., 2013)). Incidentally, this treatment restored neurogenesis that is generally suppressed in the AD progression (Rodriguez et al., 2008, 2009)).

Specific targeting of astroglial homeostatic cascades represents another therapeutic strategy. In particular manipulations with astroglial glutamate uptake has been considered as an approach to reduce excitotoxicity and normalize neurotransmission. Expression of astroglial glutamate transporters was found to be augmented by a neuroprotective drug Riluzole (Frizzo et al., 2004) or following treatment with β -lactam antibiotics (Ji et al., 2005). Another possible strategy may be aimed at controlling GFAP expression (Biran et al., 2009), although the therapeutic outcomes remain controversial. An alternative therapeutic avenue is associated with direct interference with astroglial genes. In experimental settings, for instance, viral transfection of astrocytes from APP/PS1 AD mice model with a peptide that interferes with the immune/inflammatory calcineurin/nuclear factor of activated T-cells (NFAT) signaling cascades ameliorated cognitive deficits and lowered β -amyloid deposits (Furman et al., 2012). Another possible therapeutic strategy may be aimed at manipulations with FGAP expression and hence with astrogliotic response.

Acknowledgments—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 and J3 4146 from the Slovenian Research Agency (ARRS) and the EduGlia ITN EU grant to R.Z. and A.V., and 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.); and the Government of the Basque Country grants AE-2010-1-28, AEGV10/16 and GV-2011111020 to J.J.R.

REFERENCES

- 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.
- 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.
- 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.
- Allaman I, Gavillet M, Belanger M, Laroche T, Vieret D, Lashuel HA, Magistretti PJ (2010) Amyloid- β aggregates cause alterations of astrocytic metabolic phenotype: impact on neuronal viability. *J Neurosci* 30:3326–3338.
- Alzheimer A (1910) Beiträge zur Kenntnis der pathologischen Neuroglia und ihrer Beziehungen zu den Abbauvorgängen im Nervengewebe. In: Histologische und histopathologische Arbeiten über die Grosshirnrinde mit besonderer Berücksichtigung der pathologischen Anatomie der Geisteskrankheiten, vol. 1–3 (Nissl F, Alzheimer A, eds.), pp 401–562 Jena: Gustav Fischer.
- Apelt J, Ach K, Schliebs R (2003) Aging-related down-regulation of neprilisin, a putative β -amyloid-degrading enzyme, in transgenic Tg2576 Alzheimer-like mouse brain is accompanied by an astroglial upregulation in the vicinity of β -amyloid plaques. *Neurosci Lett* 339:183–186.
- Beach TG, McGeer EG (1988) Lamina-specific arrangement of astrocytic gliosis and senile plaques in Alzheimer's disease visual cortex. *Brain Res* 463:357–361.
- Beauquis J, Pavia P, Pomilio C, Vinuela 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.
- Bell RD, Zlokovic BV (2009) Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. *Acta Neuropathol* 118:103–113.
- Bezprozvanny I, Mattson MP (2008) Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. *Trends Neurosci* 31:454–463.
- Biran Y, Masters CL, Barnham KJ, Bush AI, Adlard PA (2009) Pharmacotherapeutic targets in Alzheimer's disease. *J Cell Mol Med* 13:61–86.
- Braak H, Braak E, Bohl J, Bratzke H (1998a) Evolution of Alzheimer's disease related cortical lesions. *J Neural Trans Supplement* 54:97–106.
- Braak H, de Vos RA, Jansen EN, Bratzke H, Braak E (1998b) Neuropathological hallmarks of Alzheimer's and Parkinson's diseases. *Prog Brain Res* 117:267–285.
- Burda JE, Sofroniew MV (2014) Reactive gliosis and the multicellular response to CNS damage and disease. *Neuron* 81:229–248.
- Butterworth RF (2010) Altered glial-neuronal crosstalk: cornerstone in the pathogenesis of hepatic encephalopathy. *Neurochem Int* 57:383–388.
- Calhoun ME, Kurth D, Phinney AL, Long JM, Hengemihle J, Mouton PR, Ingram DK, Jucker M (1998) Hippocampal neuron and synaptophysin-positive bouton number in aging C57BL/6 mice. *Neurobiol Aging* 19:599–606.
- Cerbai F, Lana D, Nosi D, Petkova-Kirova P, Zecchi S, Brothers HM, Wenk GL, Giovannini MG (2012) The neuron-astrocyte-microglia triad in normal brain ageing and in a model of neuroinflammation in the rat hippocampus. *PLoS One* 7:e45250.
- Chalermpalanupap T, Kinhead B, Hu WT, Kummer MP, Hammerschmidt T, Heneka MT, Weinshenker D, Levey AI (2013) Targeting norepinephrine in mild cognitive impairment and Alzheimer's disease. *Alzheimers Res Ther* 5:21.
- Coleman P, Federoff H, Kurlan R (2004) A focus on the synapse for neuroprotection in Alzheimer disease and other dementias. *Neurology* 63:1155–1162.
- Crunelli V, Carmignoto G, Steinhauser C (2014) Novel astrocyte targets: New avenues for the Therapeutic Treatment of Epilepsy. *Neuroscientist* 21:62–83.
- Cruz NF, Ball KK, Dienel GA (2010) Astrocytic gap junctional communication is reduced in amyloid-beta-treated cultured astrocytes, but not in Alzheimer's disease transgenic mice. *ASN Neuro* 2:e00041.
- DeKosky ST, Scheff SW (1990) Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Ann Neurol* 27:457–464.
- Dickstein D, Kabaso D, Rocher A, Luebke J, Wearne S, Hof P (2006) Changes in the structural complexity of the aged brain. *Aging Cell* 6:275–284.
- Ding F, O'Donnell J, Thrane AS, Zeppenfeld D, Kang H, Xie L, Wang F, Nedergaard M (2013) α_1 -Adrenergic receptors mediate coordinated Ca^{2+} signaling of cortical astrocytes in awake, behaving mice. *Cell Calcium* 54:387–394.

- Diniz DG, Foro CA, Rego CM, Gloria DA, de Oliveira FR, Paes JM, de Sousa AA, Tokuhashi TP, Trindade LS, Turiel MC, Vasconcelos EG, Torres JB, Cunningham C, Perry VH, Vasconcelos PF, Diniz CW (2010) Environmental impoverishment and aging alter object recognition, spatial learning, and dentate gyrus astrocytes. *Eur J Neurosci* 32:509–519.
- Erickson CA, Barnes CA (2003) The neurobiology of memory changes in normal aging. *Exp Gerontol* 38:61–69.
- Fabricius K, Jacobsen JS, Pakkenberg B (2013) Effect of age on neocortical brain cells in 90+ year old human females - a cell counting study. *Neurobiol Aging* 34:91–99.
- Franceschi C (2007) Inflammaging as a major characteristic of old people: can it be prevented or cured? *Nutr Rev* 65:S173–176.
- Frizzo ME, Dall'Osso LP, Dalcin KB, Souza DO (2004) Riluzole enhances glutamate uptake in rat astrocyte cultures. *Cell Mol Neurobiol* 24:123–128.
- Frohlich N, Nagy B, Hovhannisan A, Kukley M (2011) Fate of neuron-glia synapses during proliferation and differentiation of NG2 cells. *J Anat* 219:18–32.
- Fuller S, Munch G, Steele M (2009) Activated astrocytes: a therapeutic target in Alzheimer's disease? *Expert Rev Neurother* 9:1585–1594.
- Furman JL, Sama DM, Gant JC, Beckett TL, Murphy MP, Bachstetter AD, Van Eldik LJ, Norris CM (2012) Targeting astrocytes ameliorates neurologic changes in a mouse model of Alzheimer's disease. *J Neurosci* 32:16129–16140.
- Genisman Y, Ganeshina O, Yoshida R, Berry RW, Disterhoft JF, Gallagher M (2004) Aging, spatial learning, and total synapse number in the rat CA1 stratum radiatum. *Neurobiol Aging* 25:407–416.
- Gourine AV, Kasparov S (2011) Astrocytes as brain interoceptors. *Exp Physiol* 96:411–416.
- Griffin WS, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ, White 3rd CL, Araoz C (1989) Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc Natl Acad Sci USA* 86:7611–7615.
- Grolla AA, Sim JA, Lim D, Rodriguez JJ, Genazzani AA, Verkhratsky A (2013) 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.
- Grosche A, Grosche J, Tackenberg M, Scheller D, Gerstner G, Gumprecht A, Pannicke T, Hirrlinger PG, Wilhelmsson U, Huttmann K, Hartig W, Steinhäuser C, Pekny M, Reichenbach A (2013) Versatile and simple approach to determine astrocyte territories in mouse neocortex and hippocampus. *PLoS One* 8:e69143.
- Haug H, Eggers R (1991) Morphometry of the human cortex cerebri and corpus striatum during aging. *Neurobiol Aging* 12:336–338.
- Hazell AS (2009) Astrocytes are a major target in thiamine deficiency and Wernicke's encephalopathy. *Neurochem Int* 55:129–135.
- Hedden T, Gabrieli JD (2004) Insights into the ageing mind: a view from cognitive neuroscience. *Nat Rev Neurosci* 5:87–96.
- 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 Neuroinflamm* 2:22.
- Heneka MT, O'Banion MK, Terwel D, Kummer MP (2010a) Neuroinflammatory processes in Alzheimer's disease. *Journal of neural transmission* 117:919–947.
- Heneka MT, Rodriguez JJ, Verkhratsky A (2010b) Neuroglia in neurodegeneration. *Brain Res Rev* 63:189–211.
- Hertz L, Chen Y, Gibbs ME, Zang P, Peng L (2004) Astrocytic adrenoceptors: a major drug target in neurological and psychiatric disorders? *Curr Drug Targets CNS Neurol Disord* 3:239–267.
- Iliff JJ, Nedergaard M (2013) Is there a cerebral lymphatic system? *Stroke* 44:S93–95.
- Ji HF, Shen L, Zhang HY (2005) Beta-lactam antibiotics are multipotent agents to combat neurological diseases. *Biochem Biophys Res Commun* 333:661–663.
- Jiang T, Cadenas E (2014) Astrocytic metabolic and inflammatory changes as a function of age. *Aging Cell* 13:1059–1067.
- 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 USA* 110:11612–11617.
- Kettenmann H, Kirchhoff F, Verkhratsky A (2013) Microglia: new roles for the synaptic stripper. *Neuron* 77:10–18.
- Kettenmann H, Ransom BR, editors. *Neuroglia*. Oxford: Oxford University Press.
- Kraft AW, Hu X, Yoon H, Yan P, Xiao Q, Wang Y, Gil SC, Brown J, Wilhelmsson U, Restivo JL, Cirrito JR, Holtzman DM, Kim J, Pekny M, Lee JM (2013) Attenuating astrocyte activation accelerates plaque pathogenesis in APP/PS1 mice. *FASEB J* 27:187–198.
- Kress BT, Iliff JJ, Xia M, Wang M, Wei H, Zeppenfeld D, Xie L, Kang H, Xu Q, Liew J, Plog BA, Ding F, Deane R, Nedergaard M (2014) Impairment of paravascular clearance pathways in the aging brain. *Ann Neurol* 76:845–861.
- Kuchibhotla KV, Lattarulo CR, Hyman BT, Bacskai BJ (2009) Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice. *Science* 323:1211–1215.
- Kulijewicz-Nawrot M, Sykova E, Chvatal A, Verkhratsky A, Rodriguez JJ (2013) Astrocytes and glutamate homeostasis in Alzheimer's disease: a decrease in glutamine synthetase, but not in glutamate transporter-1, in the prefrontal cortex. *ASN Neuro* 5:273–282.
- 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.
- Lalo U, Palygin O, North RA, Verkhratsky A, Pankratov Y (2011) Age-dependent remodelling of ionotropic signalling in cortical astroglia. *Aging Cell* 10:392–402.
- Lalo U, Rasooli-Nejad S, Pankratov Y (2014) Exocytosis of gliotransmitters from cortical astrocytes: implications for synaptic plasticity and aging. *Biochem Soc Trans* 42:1275–1281.
- Lim D, Iyer A, Ronco V, Grolla AA, Canonico PL, Aronica E, Genazzani AA (2013) Amyloid beta deregulates astroglial mGluR5-mediated calcium signaling via calcineurin and Nf- κ B. *Glia* 61:1134–1145.
- Lim D, Ronco V, Grolla AA, Verkhratsky A, Genazzani AA (2014) Glial calcium signalling in Alzheimer's disease. *Rev Physiol Biochem Pharmacol* (in press).
- Linde CI, Baryshnikov SG, Mazzocco-Spezia A, Golovina VA (2011) Dysregulation of Ca^{2+} signaling in astrocytes from mice lacking amyloid precursor protein. *Am J Physiol* 300:C1502–1512.
- Logan JM, Sanders AL, Snyder AZ, Morris JC, Buckner RL (2002) Under-recruitment and nonselective recruitment: dissociable neural mechanisms associated with aging. *Neuron* 33:827–840.
- Lynch AM, Murphy KJ, Deighan BF, O'Reilly JA, Gun'ko YK, Cowley TR, Gonzalez-Reyes RE, Lynch MA (2010) The impact of glial activation in the aging brain. *Aging Dis* 1:262–278.
- Malarkey EB, Parpura V (2008) Mechanisms of glutamate release from astrocytes. *Neurochem Int* 52:142–154.
- Martineau M, Parpura V, Mothet JP (2014) Cell-type specific mechanisms of D-serine uptake and release in the brain. *Front Synaptic Neurosci* 6:12.
- Meda L, Baron P, Scarlato G (2001) Glial activation in Alzheimer's disease: the role of Abeta and its associated proteins. *Neurobiol Aging* 22:885–893.
- Medvedev ZA (1990) An attempt at a rational classification of theories of ageing. *Biol Rev Camb Philos Soc* 65:375–398.
- Messing A, Brenner M, Feany MB, Nedergaard M, Goldman JE (2012) Alexander disease. *J Neurosci* 32:5017–5023.
- Mosconi L, Pupi A, De Leon MJ (2008) Brain glucose hypometabolism and oxidative stress in preclinical Alzheimer's disease. *Ann N Y Acad Sci* 1147:180–195.
- Nagele RG, D'Andrea MR, Lee H, Venkataraman V, Wang HY (2003) Astrocytes accumulate A β 42 and give rise to astrocytic amyloid plaques in Alzheimer disease brains. *Brain Res* 971:197–209.

- Nedergaard M, Ransom B, Goldman SA (2003) New roles for astrocytes: redefining the functional architecture of the brain. *Trends Neurosci* 26:523–530.
- Nedergaard M, Rodriguez JJ, Verkhratsky A (2010) Glial calcium and diseases of the nervous system. *Cell Calcium* 47:140–149.
- Nicholson DA, Yoshida R, Berry RW, Gallagher M, Geinisman Y (2004) Reduction in size of perforated postsynaptic densities in hippocampal axospinous synapses and age-related spatial learning impairments. *J Neurosci* 24:7648–7653.
- Oddy S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM (2003a) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron* 39:409–421.
- Oddy S, Caccamo A, Kitazawa M, Tseng BP, LaFerla FM (2003b) Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. *Neurobiol Aging* 24:1063–1070.
- Olabarria M, Noristani HN, Verkhratsky A, Rodriguez JJ (2010) Concomitant astrogliat atrophy and astrogliosis in a triple transgenic animal model of Alzheimer's disease. *Glia* 58:831–838.
- 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 Neurodegen* 6:55.
- Orre M, Kamphuis W, Osborn LM, Jansen AH, Kooijman L, Bossers K, Hol EM (2014) Isolation of glia from Alzheimer's mice reveals inflammation and dysfunction. *Neurobiol Aging* 35:2746–2760.
- Palygin O, Lalo U, Verkhratsky A, Pankratov Y (2010) Ionotropic NMDA and P2X_{1/5} receptors mediate synaptically induced Ca²⁺ signalling in cortical astrocytes. *Cell Calcium* 48:225–231.
- Parpura V, Grubisic V, Verkhratsky A (2011) Ca²⁺ sources for the exocytotic release of glutamate from astrocytes. *Biochim Biophys Acta* 1813:984–991.
- Parpura V, Heneka MT, Montana V, Oliet SH, Schousboe A, Haydon PG, Stout Jr RF, 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.
- Parpura V, Verkhratsky A (2012) Homeostatic function of astrocytes: Ca²⁺ and Na⁺ signalling. *Transl Neurosci* 3:334–344.
- Pascual O, Ben Achour S, Rostaing P, Triller A, Bessis A (2012) Microglia activation triggers astrocyte-mediated modulation of excitatory neurotransmission. *Proc Natl Acad Sci USA* 109:E197–205.
- Pekny M, Wilhelmsson U, Pekna M (2014) The dual role of astrocyte activation and reactive gliosis. *Neurosci Lett* 565:30–38.
- Pelvig DP, Pakkenberg H, Stark AK, Pakkenberg B (2008) Neocortical glial cell numbers in human brains. *Neurobiol Aging* 29:1754–1762.
- Peters A, Sethares C (2004) Oligodendrocytes, their progenitors and other neuroglial cells in the aging primate cerebral cortex. *Cereb Cortex* 14:995–1007.
- Peters O, Schipke CG, Philippus A, Haas B, Pannasch U, Wang LP, Benedetti B, Kingston AE, Kettenmann H (2009) Astrocyte function is modified by Alzheimer's disease-like pathology in aged mice. *J Alzheimer's Dis* 18:177–189.
- Potokar M, Stenovec M, Jorgačevski J, Holen T, Kreft M, Ottersen OP, Zorec R (2013a) Regulation of AQP4 surface expression via vesicle mobility in astrocytes. *Glia* 61:917–928.
- Potokar M, Vardjan N, Stenovec M, Gabrijel M, Trkov S, Jorgačevski J, Kreft M, Zorec R (2013b) Astrocytic vesicle mobility in health and disease. *Int J Mol Sci* 14:11238–11258.
- Rajkowska G, Stockmeier CA (2013) Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. *Curr Drug Targets* 14:1225–1236.
- Reichenbach A, Bringmann A (2010) Müller cells in the healthy and diseased retina. Heidelberg: Springer.
- Riera J, Hatanaka R, Uchida T, Ozaki T, Kawashima R (2011) Quantifying the uncertainty of spontaneous Ca²⁺ oscillations in astrocytes: particulars of Alzheimer's disease. *Biophys J* 101:554–564.
- Rodriguez JJ, Jones VC, Tabuchi M, Allan SM, Knight EM, LaFerla FM, Oddo S, Verkhratsky A (2008) Impaired adult neurogenesis in the dentate gyrus of a triple transgenic mouse model of Alzheimer's disease. *PLoS One* 3:e2935.
- Rodriguez JJ, Jones VC, Verkhratsky A (2009) Impaired cell proliferation in the subventricular zone in an Alzheimer's disease model. *NeuroReport* 20:907–912.
- 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.
- Rodriguez JJ, Verkhratsky A (2011) Neuroglial roots of neurodegenerative diseases? *Mol Neurobiol* 43:87–96.
- Rodriguez JJ, Yeh CY, Terzieva S, Olabarria M, Kulijewicz-Nawrot M, Verkhratsky A (2014) Complex and region-specific changes in astrogliat markers in the aging brain. *Neurobiol Aging* 35:15–23.
- 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.
- Rose CF, Verkhratsky A, Parpura V (2013) Astrocyte glutamine synthetase: pivotal in health and disease. *Biochem Soc Trans* 41:1518–1524.
- 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.
- Sampedro-Piquero P, De Bartolo P, Petrosini L, Zancada-Menendez C, Arias JL, Begega A (2014) Astrocytic plasticity as a possible mediator of the cognitive improvements after environmental enrichment in aged rats. *Neurobiol Learn Mem* 114:16–25.
- Samuel W, Masliah E, Hill LR, Butters N, Terry R (1994) Hippocampal connectivity and Alzheimer's dementia: effects of synapse loss and tangle frequency in a two-component model. *Neurology* 44:2081–2088.
- 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.
- Simpson JE, Ince PG, Lace G, Forster G, Shaw PJ, Matthews F, Savva G, Brayne C, Wharton SB (2010) Astrocyte phenotype in relation to Alzheimer-type pathology in the ageing brain. *Neurobiol Aging* 31:578–590.
- Smith TD, Adams MM, Gallagher M, Morrison JH, Rapp PR (2000) Circuit-specific alterations in hippocampal synaptophysin immunoreactivity predict spatial learning impairment in aged rats. *J Neurosci* 20:6587–6593.
- Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. *Trends Neurosci* 32:638–647.
- 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.
- Streit WJ, Xue QS (2013) Microglial senescence. *CNS Neurol Disord Drug Targets* 12:763–767.
- Stutzmann GE (2007) The pathogenesis of Alzheimer's disease is it a lifelong "calciumopathy"? *Neuroscientist* 13:546–559.
- Takano T, Han X, Deane R, Zlokovic B, Nedergaard M (2007) Two-photon imaging of astrocytic Ca²⁺ signaling and the microvasculature in experimental mice models of Alzheimer's disease. *Ann N Y Acad Sci* 1097:40–50.
- Terry RD (2000) Cell death or synaptic loss in Alzheimer disease. *J Neuropathol Exp Neurol* 59:1118–1119.
- Terwel D, Steffensen KR, Vergheze PB, Kummer MP, Gustafsson JA, Holtzman DM, Heneka MT (2011) Critical role of astroglial apolipoprotein E and liver X receptor-alpha expression for microglial Abeta phagocytosis. *J Neurosci* 31:7049–7059.
- Thrane AS, Rangroo Thrane V, Nedergaard M (2014) Drowning stars: reassessing the role of astrocytes in brain edema. *Trends Neurosci* 37:620–628.

- 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.
- Tremblay ME, Zettel ML, Ison JR, Allen PD, Majewska AK (2012) Effects of aging and sensory loss on glial cells in mouse visual and auditory cortices. *Glia* 60:541–558.
- Unger JW (1998) Glial reaction in aging and Alzheimer's disease. *Microsc Res Tech* 43:24–28.
- 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.
- Vardjan N, Kreft M, Zorec R (2014) Dynamics of β -adrenergic/cAMP signaling and morphological changes in cultured astrocytes. *Glia* 62:566–579.
- Verkhratsky A, Butt AM (2013) Glial physiology and pathophysiology. Chichester: Wiley-Blackwell.
- Verkhratsky A, Nedergaard M (2014) Astroglial cradle in the life of the synapse. *Phil Trans R Soc Ser B* 369:20130595.
- Verkhratsky A, Olabarria M, Noristani HN, Yeh CY, Rodriguez JJ (2010) Astrocytes in Alzheimer's disease. *Neurotherapeutics* 7:399–412.
- Verkhratsky A, Marutle A, Rodriguez-Arellano JJ, Nordberg A (2014a) Glial asthenia and functional paralysis: a new perspective on neurodegeneration and Alzheimer's disease. *Neuroscientist* (in press). <http://dx.doi.org/10.1177/1073858414547132>.
- Verkhratsky A, Parpura V, Pekna M, Pekny M, Sofroniew M (2014) Glia in the pathogenesis of neurodegenerative diseases. *Biochem Soc Trans* 42:1291–1301.
- Verkhratsky A, Rodriguez JJ, Parpura V (2013a) Astroglia in neurological diseases. *Future Neurol* 8:149–158.
- Verkhratsky A, Rodriguez JJ, Steardo L (2013b) Astroglial pathology: a central element of neuropsychiatric diseases? *Neuroscientist* (in press).
- 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.
- Walhovd KB, Johansen-Berg H, Karadottir RT (2014) Unraveling the secrets of white matter – bridging the gap between cellular, animal and human imaging studies. *Neuroscience* 276C:2–13.
- West MJ (1993) Regionally specific loss of neurons in the aging human hippocampus. *Neurobiol Aging* 14:287–293.
- Wharton SB, O'Callaghan JP, Savva GM, Nicoll JA, Matthews F, Simpson JE, Forster G, Shaw PJ, Brayne C, Ince PG (2009) Population variation in glial fibrillary acidic protein levels in brain ageing: relationship to Alzheimer-type pathology and dementia. *Dement Geriatr Cogn Disord* 27:465–473.
- Wyss-Coray T, Loike JD, Brionne TC, Lu E, Anankov R, Yan F, Silverstein SC, Husemann J (2003) Adult mouse astrocytes degrade amyloid- β *in vitro* and *in situ*. *Nat Med* 9:453–457.
- Wyss-Coray T, Rogers J (2012) Inflammation in Alzheimer disease—a brief review of the basic science and clinical literature. *Cold Spring Harb Perspect Med* 2:a006346.
- Xavier AL, Menezes JR, Goldman SA, Nedergaard M (press) Fine-tuning the central nervous system: microglial modelling of cells and synapses. *Phil Trans R Soc Ser B* 369:20130593.
- Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiagarajan M, O'Donnell J, Christensen DJ, Nicholson C, Iliff JJ, Takano T, Deane R, Nedergaard M (2013) Sleep drives metabolite clearance from the adult brain. *Science* 342:373–377.
- 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.
- Yeh CY, Verkhratsky A, Terzieva S, Rodriguez JJ (2013) Glutamine synthetase in astrocytes from entorhinal cortex of the triple transgenic animal model of Alzheimer's disease is not affected by pathological progression. *Biogerontology* 14:777–787.
- Zlokovic BV (2008) The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron* 57:178–201.

(Accepted 6 January 2015)
 (Available online xxxx)