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Abstract: Ageing of the brain is the major risk factor for neurodegenerative disorders that result in cognitive decline and senile dementia. Ageing astrocytes undergo complex and region specific remodelling which can reflect life-long adaptive plasticity. In neurodegeneration astroglial cells similarly are subject for morpho-functional changes hampering the homoeostasis, defence and regeneration of the central nervous system. Region-specific astroglial atrophy with the loss of function and astroglial reactivity have been reported in virtually all forms of neurodegenerative pathologies. Modulating these astroglia changes may represent a fertile ground for novel therapeutic intervention strategies to prevent, delay progression and/or ameliorate pathology. While at present this bodacious goal represents a wishful thinking, further understanding of astroglial role in ageing and neurodegeneration could bring us closer to laying the foundations for such cell-specific therapeutic approaches.

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05 August 2015

Professor Diana Amantea,
Section Editor
Current Opinion in Pharmacology

Dear Prof
Amantea

Here we are submitting commissioned paper entitled " Astroglia dynamics in aging and Alzheimer's disease" by Alexei Verkhratsky, Robert Zorec, J.J. Rodríguez & Vladimir Parpura. The body of the paper is ~2000 words as instructed and it is illustrated with 1 figure.

We confirm that this manuscript has not been submitted to any other journal..

We are very much looking forward to hearing from you soon.

On behalf of the authors,
With best regards, I shall remain,

Yours sincerely

A handwritten signature in blue ink, appearing to read 'A. Verkhratsky', with a stylized flourish at the end.

Conflict of interest

Authors declare no conflict of interest

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Highlights

Astrocytes are the housekeeping cells of the CNS controlling homeostasis and defence

In ageing astrocytes undergo complex region-specific remodelling

In AD generalised astrodegeneration may contribute to synaptic deficits

Astroglia dynamics in aging and Alzheimer's disease

by Alexei Verkhratsky^{1,2,3}, Robert Zorec^{4,5}, J.J. Rodríguez² & Vladimir Parpura⁶

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Running title: Astrocytes in aging and AD

Abstract

Ageing of the brain is the major risk factor for neurodegenerative disorders that result in cognitive decline and senile dementia. Ageing astrocytes undergo complex and region specific remodelling which can reflect life-long adaptive plasticity. In neurodegeneration astroglial cells similarly are subject for morpho-functional changes hampering the homoeostasis, defence and regeneration of the central nervous system. Region-specific astroglial atrophy with the loss of function and astroglial reactivity have been reported in virtually all forms of neurodegenerative pathologies. Modulating these astroglia changes may represent a fertile ground for novel therapeutic intervention strategies to prevent, delay progression and/or ameliorate pathology. While at present this bodacious goal represents a wishful thinking, further understanding of astroglial role in ageing and neurodegeneration could bring us closer to laying the foundations for such cell-specific therapeutic approaches.

Key words: Neuroglia; astrocytes; astrogliopathology; ageing; neurodegeneration; Alzheimer's disease; Huntington disease

Highlights

Astrocytes are the housekeeping cellular population of the CNS responsible for control of homeostasis and defence

In ageing astrocytes undergo complex region specific remodelling that may define the cognitive survival of the senescent brain

In Alzheimer's disease reactive astrocytes surround senile plaques, whereas generalised astrodegeneration may contribute to synaptic deficits

Introduction: The resilient ageing brain

Contrary to the general belief, the senescence-associated cognitive decline is far from being the universal signature of human ageing. The mental capabilities of aged people vary tremendously, and while many develop senile dementia, a considerable number remains perfectly cognitively competent. Surprisingly, the prevalence of age-associated cognitive impairment seems to become significant only in comparatively recent times; the health survey of elderly conducted by British Medical Council in the late 19th century, found memory impairments only in 22% of people older than 80 years [1]; in modern world about 40 - 65% of individuals older than 75 are cognitively impaired with ~35% of 85 year-old suffering from dementia [2]. Whatever the reason for this evolution, the human brain withstands the effects of ageing much better than, for example, cardio-vascular and skeletal systems; most of men attaining their intellectual summit in relatively old age.

So, how does the human/mammalian brain resist ageing? Considering exceptionally high energy metabolism of the brain (which consumes ~20% of all energy needed to sustain a human organism accounting for mere 2% of the total body mass [3]) the resulting oxidative stress pressure, should, at least in theory, induce substantial cellular damage within this organ. In addition, neural activity requires release of high quantities of glutamate, this being a highly toxic endogenous substance. Thus, for the brain to function and withstand the ageing, a dedicated protective system must be in existence. This system is represented by neuroglia, which act as the homeostatic/defensive system of the CNS, with a particular contribution of astrocytes that are responsible for neural homeostasis as well as neuroprotection.

Astroglia: the homeostatic and protective cell of the CNS

Astrocytes are highly heterogeneous population of neural cells that share their developmental origin with neurones being scions of neuroepithelial cells [4,5]. The density, morphology and physiological properties of astrocytes vary significantly between brain regions [6-9], reflecting developmental specialisation most likely controlled by the immediate neurochemical environment [10]. Astrocytes define the cyto-architecture of the grey matter by parcelling it into relatively independent neuro-glio-vascular units [11], enwrapping synapses by perisynaptic processes [12] and performing almost every conceivable homeostatic task needed for maintaining the CNS operational [4,5,13]. Astrocytes also act as "endocranial" secretory cells releasing, through multiple mechanisms, neurotransmitters, neuromodulators, hormones, trophic and immune factors [14,15] Furthermore, astroglial endfeet plaster blood vessels and create a perivascular "glymphatic" system that promotes a removal of metabolites from CNS parenchyma [16]. Astrocytes also appear as main neuroprotective cells which, either through the homeostatic response, or by mounting a complex and multifaceted reactive astrogliosis, contain environmental insults and promote post-lesion regeneration of neural networks [17-19].

Being a primary homeostatic system of the CNS astroglia inevitably contributes to pathophysiology of all neurological diseases, being either a primary target (for example, in Alexander disease or in many forms of toxic encephalopathies, including Wernicke-Korsakoff syndrome or hepatoencephalopathy) or mounting a reactive response to a pathological lesion [20]. Failure of the astroglial defence, arguably,

exacerbates the damage and facilitates pathological progression [18,21]. In chronic neuropathologies, astrocytes may also undergo atrophic changes, reducing their morphological profiles and/or their homeostatic functions. Astroglial atrophy accompanies major psychiatric disorders [22,23] and is observed in neurodegeneration [24], whereas compromised astroglial glutamate uptake represents the fundamental element of excitotoxicity in amyotrophic lateral sclerosis [25,26]. Arguably, the astroglial performance defines the defensive landscape of the brain, either preventing or facilitating pathological progression.

Ageing astrocytes

Although many cellular mechanisms of brain ageing remains enigmatic, the two diverging traits in human senescence are clearly distinguishable: the brain may undergo physiological ageing in which adaptive plasticity compensates for age-dependent detrition, or ageing can take a pathological route manifested by neurodegeneration and ultimately dementia [27,28]. Age-dependent astroglial remodelling, either protective or maladaptive, may represent an important component for determining this switch between physiology and pathology.

Little is known about ageing of astroglia, and the scarce data available are often controversial. In human post-mortem analysis, the changes in astroglial cell counts were insignificant in old vs. young-adult brains [29,30]. In old rodents, an increase [31], a decrease [32] and no change [33-35] in the number of glial fibrillary acidic protein (GFAP)-positive astroglial cells in the hippocampus were reported. Age-dependent changes in astroglial morphology were also identified. Hence, in the hippocampus of 21-month-old rats, the volume of astrocytes labelled with antibodies against glutamine synthetase (GS) was ~ 2 times larger than in 5-month-old animals [35]. Morphological remodelling of astroglia in the aged brain was also found to be region-specific; the GFAP-positive astroglial profiles appear to be significantly increased in the CA1 region and dentate gyrus (DG) of the hippocampus, whereas they were significantly smaller in the entorhinal cortex (EC) (Fig. 1A). At the same time morphological profiles of astrocytes labelled with anti-S100 β antibodies were found to be increased in the aged DG and EC but not in the CA1 hippocampal region, whereas GS-positive profiles were smaller in the old CA1 and DG, while no changes in the EC were identified [34]. The age-dependent increase in GFAP expression may also reflect astroglial adaptive plasticity. It has been widely reported that environmental stimulation (i.e. physical activity or housing animals in the enriched environment) increased expression of GFAP overall astroglial profiles and complexity of astroglial processes in the hippocampus [36,37]; importantly, morpho-functional cellular changes coincided with cognitive improvement [36]. These astroglial remodelling in response to environmental stimulation may contribute to experience-dependent neuroplasticity [38].

Age-associated changes in astroglial physiology include a decreased expression of ionotropic glutamate and purine receptors, which is paralleled with a decrease in Ca²⁺ signals in astroglial cells studied in acute slices isolated from old (18-21 months) animals [39]. Expression of aquaporin 4, forming water channels, was similarly decreased in old astrocytes, which may contribute to age-dependent deficits in the glymphatic system responsible for the protein waste clearance [40]. Finally, there is emerging evidence for astrocytes acquiring senescence-associated secretory

phenotype characterised by an increased expression of cytokines and accumulation of proteotoxic aggregates [41]. Age-related changes in membrane receptors and channels as well as in the secretory phenotype of astrocytes may, at least in part, reflect altered vesicle traffic dynamics, which depends on cytoskeleton including GFAP [42-44]. Moreover, age-related changes in vesicle dynamics may contribute to the morphological alterations of astrocytes as well [45] and changes in metabolic activity of astrocytes, key glycogen storage cells in the CNS.

Astroglial atrophy and reactivity in AD: Regional differences

Pathological potential of neuroglia in the context of neurodegenerative diseases at large and Alzheimer's disease (AD) in particular has received specific attention in the recent decade, with an accumulation of experimental data highlighting the role of glia in neuroinflammation and neuroprotection associated with pathological progression of AD [46]. Astrocytes undergo complex and region-specific changes in the AD-type pathology. The total number of astrocytes (labelled with antibodies against GFAP, S100 β or GS) remained stable in AD model animals of increasing age of up to 24 month [33,47]. In both post-mortem human tissues and in experimental AD animal models, the senile plaques are surrounded by reactive astrocytes [33,48]. These reactive astrocytes are characterised by an up-regulation of GFAP and morphological hypertrophy, although without the loss of domain organisation, i.e., the astroglial response remains relatively mild (being an example of isomorphic astrogliosis) with no signs of the glial scar formation [48]. The reactive astrocytes are likely to play a protective role, and the inhibition of reactivity (by, for example, a genetic knockout of astroglial intermediate filaments GFAP and vimentin) exacerbates β -amyloid pathology [21]. Astroglial reactivity may appear relatively early, even at pre-plaque stages [49-51], and it has even been hypothesised that an accumulation of reactive astrocytes in the AD brain lays down the localisation of emerging senile plaques [51]. Incidentally, reactive astrocytes surrounding β -amyloid depositions demonstrate an increased expression of $\alpha 7$ nicotinic cholinergic receptors, which may be involved in pathological development as these receptors can bind β -amyloid with high affinity [52]. Pathologically modified astrocytes are likely to contribute to both amyloidogenesis and neuroinflammation [46,51,53], while healthy astrocytes can be β -amyloid scavengers [54].

In parallel with astrogliosis, the AD progression is also associated with astrodegeneration. Morphological atrophy of astrocytes (Fig. 1B) have been observed at pre-plaque stages in the hippocampus, prefrontal and entorhinal cortices of mouse AD models including the 3xTG-AD animals (expressing mutant genes for the APPSwe, the PS1M146V and the tauP301L) and in PDAPP-J20 mice (expressing the mutant APPSwe); this atrophy was documented as a decrease in cell and somata volume and surface, along with a decrease in the number of primary processes [55-58]. In the 3xTg-AD animals the astroglial atrophy developed first in the entorhinal cortex (at 1 month of age); subsequently, it was detected in the prefrontal cortex (at 3 months of age) and hippocampus (9 - 12 months of age) [33,56,57] (Fig 1D). This morphological astrodegeneration in 3xTG-AD transgenic mice was associated by functional deficits, seen as compromised astroglial response. Astroglial reactivity is manifest in cells accumulating around β -amyloid deposits in the hippocampus, but not in the entorhinal and prefrontal cortices [33,56,57]. This region-differential reactivity may determine the specific vulnerability of different parts of the brain to AD type

pathology [59]. The morphological atrophy of astrocytes in transgenic AD animals can be restored by a long-lasting exposure to environmental stimulation, which arguably may coincide with certain cognitive improvement [37,55].

Pathological remodelling of astroglia in the context of AD affects various signalling cascades, the most notably astroglial Ca^{2+} signalling. There are compelling data demonstrating that exposure of astrocytes to β -amyloid *in vitro* or in organotypic slices triggers aberrant Ca^{2+} transients or oscillations (see [60] for detailed overview). Similarly, *in vitro* aberrant Ca^{2+} waves have been found to originate in astrocytes surrounding senile plaques [61]. Evolution of aberrant Ca^{2+} signalling results from modifications of astroglial Ca^{2+} signalling toolkit, which however differs between astroglial cells in different regions of the brain [62].

Concluding remarks

Ageing of neuroglia can be a fundamental factor in defining the senescent brain [63,64], as well as in determining the susceptibility to neurodegeneration and cognitive decline. Our knowledge about astroglia ageing is still rudimentary and obscured by regional differences and complexity of brain structures. In neurodegeneration (that could be defined as a pathological brain ageing) astroglia undergoes multifaceted changes which are generally manifested by reactive remodelling of cells surrounding β -amyloid depositions and chronic atrophy of astrocytes in β -amyloid free parenchyma. Astrodegeneration can reflect overall exhaustion of astroglial homeostatic and protective capabilities as well as reduction in synaptic coverage by astroglial perisynaptic processes, which in turn may contribute to synaptic weakening and synaptic loss, the earliest histopathological changes observed in AD [65-67]. Reactive astrocytes in turn are associated with senile plaques where they seemingly contain the spread of β -amyloid pathology; deficits in this astroglial response, which may occur in certain regions of the CNS, can arguably define distinct vulnerability of brain regions to AD pathology.

In conclusion, astrocytes are important components of physiological and pathological ageing and further understanding of their pathological potential may lay foundations for emerging cell-specific therapies of AD and other neurodegenerative disorders.

Conflict of interest statement

Nothing declared.

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Figure legend**Figure 1.** Astroglial morphological dynamics in ageing and in AD.

A: Representative confocal images showing glial fibrillary acidic protein–immunoreactive astrocytes in the dentate gyrus (DG) and in the enthorhinal cortex (EC) of mice at 3 months and 24 months of age; note hypertrophy of GFAP-positive profiles in the DG and atrophy of GFAP-positive profiles in the EC.

B: Confocal images illustrating decreased GFAP-positive astroglial profiles in the hippocampal DG, in the EC and in the prefrontal cortex (PFC) in 3xTG-AD mice compared with control animals.

Figure 1
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