Astrocytic Face of Alzheimer's Disease

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Corresponding Author: Prof. Alexei Verkhratsky, MD, PhD

Corresponding Author's Institution: The University of Manchester

First Author: Robert Zorec

Order of Authors: Robert Zorec; Vladimir Parpura; Nina Vardjan; Alexei Verkhratsky, MD, PhD

Abstract: Ageing of the central nervous system (CNS) is the major risk factor for Alzheimer's disease (AD), a type of neurodegeneration that is associated with deficits in cognition and memory and clinically manifested as severe senile dementia. Numerous mental processes underlie cognition, including attention, producing and understanding language, learning, reasoning, problem solving, decision making and memory formation. In the past, neurones or their parts have been considered to be the exclusive cellular sites of memory and cognitive processes. However, it has become evident that astrocytes, the major homeostatic glial cell of the CNS, provide an essential contribution to memory formation, and astroglial failure may promote cognitive decline in AD. In response to the network reset mechanisms mediated by the noradrenergic projections of neurones located in the locus coeruleus, astrocytes get excited and participate in the morphological remodelling associated with synaptic plasticity, otherwise thought to represent a cellular mechanism of learning and memory. Astroglial morphological plasticity is an energy-demanding process requiring mobilisation of glycogen, which, in the CNS, is almost exclusively stored in astrocytes. Astroglia exhibit cytoplasmic excitability that engages ions (such as Ca^{2+} and Na^{+}) and second messengers (such as cAMP). These ions/molecules contribute to the reception of extracellular signals and coordinate the secretion of gliotransmitters, including peptides such as apolipoprotein E, which participates in lipid transport between glia and neurones. In this setting, astrocytes are positioned as spatio-temporal integrators of neural network coordination, which disintegrates during progression of AD.

Research highlights

Astrocytes are main homeostatic and defensive cells of the central nervous system

Astrocytes are endowed with morphological plasticity

Astroglial coverage defines, to a large extent, synaptic connectivity

Pathological remodelling of astroglia contributes to neurodegeneration.

Prof.Catherine Cook Kaczorowski
Managing Guest Editor
Behavioural Brain Research

RE: Ms. Ref. No.: BBR-D-16-00275;

Dear Catherine,

Further to your e-mail letter of 29 March 2016, regarding our manuscript entitled »Astrocytic Face of Alzheimer's Disease« by Robert Zorec; Vladimir Parpura; Nina Vardjan; Alexei Verkhatsky we have now addressed all the comments raised by the reviewers. Please find below the point-by-point response to the issues raised by both reviewers. Modifications in the manuscript are highlighted in grey. We are grateful to the reviewers for the criticisms which helped to improve the clarity of our review.

with best regards

Yours sincerely

Alexei

Point-by-point response to reviewer's comments.

Reviewer #1:

This review by Zorec et al., is very topical - the role of astrocyte physiology in AD is a growing area of interest and likely a critical contributor to the disease process. However, there are numerous issues that should be addressed.

Our reply: Thank you for this evaluation

There are many typos and grammatical errors throughout the manuscript that make it difficult to read. It would benefit greatly from editing and revision by a native English speaker.

Our reply: We performed professional proof reading

Although the discussion of the locus coeruleus adrenergic system is appropriate, given recent evidence that LC neuronal degeneration may be an early event in the pre-symptomatic phase of AD, there is little discussion of events in the hippocampus and entorhinal cortex, which are the most dramatically affected areas in AD, particularly with regard to memory loss.

There should be more discussion of the role of astrocytes in the hippocampus and entorhinal cortex in both physiological and pathophysiological conditions.

Our reply: We modified the text accordingly by adding suggested information

Similarly, there is little discussion of local metabolic, synaptic, and other factors involved in gliotransmission, which are likely to play a critically important role in neuroinflammation and neural metabolic support.

Our reply: In fact we were not focusing too much on gliotransmission but rather on astroglial plasticity that is relevant for memory, and hence we deliberately decided not to go into these (undeniable very interesting) topic

There is also an over reliance on review articles in the citations, more primary literature should be used. Also, there are several areas where there are insufficient citations.

Our reply: We tried our best to attend to this point

Reviewer #2: The manuscript from Zorec et al. is an excellent overview of an important topic in the field of Alzheimer's research. The authors bring together several lines of evidence in an informative review that I expect will be very well received by researchers working in this area. The manuscript is well written and includes some primary data that brings together some of their main points. I have only a few minor comments, most of which are typographical errors that should be corrected:

Our reply: Thank you

1. Abstract: I am not sure what is meant by the sentence "...interface the extracellular signal reception...." - perhaps this could be re-worded?

Our reply: Done

2. Page 4, first line of second paragraph. "Aggregates" could be used instead of "protein" since Abeta is a peptide and not a protein.

Our reply: Done

3. Page 5: Could a brief description of the neurodegenerative phenotype apparent in Ndufs4-/- mice be added to clarify the relevance of this model in the context of the discussion.

Our reply: Done

4. Typos Page 6 - line 5 "... function of this diffuse.. should be "...THESE diffuse...", line 9 "...presumably that indicated..." should be "...THE indicated...", line 11 add "is" after reference [44]. Page 10 line 15 "... glutamatergic vesicles motility is accelerated with an increase... should be "...VESICLE motility is accelerated BY..." Page 11 line 8 remove "THE" from "In the AD...".

Our reply: Done

5. Can the length of treatment be added to Fig. 1A legend?

Our reply: Done

6. I am not sure of the discussion on intellectual deficiency (starting at the bottom of page 11) for this review. Perhaps this could be removed?

Our reply: We politely disagree - we believe that these are common mechanisms on cognitive impairments, and commonality in astroglial responses and hence we feel it is indeed worth discussion.

Astrocytic Face of Alzheimer’s Disease

by Robert Zorec^{a,b}, Vladimir Parpura^c, Nina Vardjan^{a,b}, Alexej Verkhratsky^{a,b,d,e,f}

^aLaboratory of Neuroendocrinology and Molecular Cell Physiology, Institute of Pathophysiology, University of Ljubljana, Medical Faculty, Ljubljana, Slovenia

^bCelica, BIOMEDICAL, Ljubljana, Slovenia

^cDepartment 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 at Birmingham, Birmingham, AL 35294-0021, USA

^dFaculty of Life Sciences, University of Manchester, Manchester, UK

^eAchucarro Center for Neuroscience, IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

Send all correspondence to:
Professor Robert Zorec
email:
robert.zorec@mf.uni-lj.si (R. Zorec),
Or
Professpor Alexei Verkhratsky
alexej.verkhratsky@manchester.ac.uk (A. Verkhratsky).

Running title: Astroglia in cognitive decline

Abstract

Ageing of the central nervous system (CNS) is the major risk factor for Alzheimer's disease (AD), a type of neurodegeneration that is associated with deficits in cognition and memory and clinically manifested as severe senile dementia. Numerous mental processes underline cognition, including attention, producing and understanding language, learning, reasoning, problem solving, decision making and memory formation. In the past, neurones or their parts have been considered to be the exclusive cellular sites of memory and cognitive processes. However, it has become evident that astrocytes, the major homeostatic glial cell of the CNS, provide an essential contribution to memory formation, and astroglial failure may promote cognitive decline in AD. In response to the network reset mechanisms mediated by the noradrenergic projections of neurones located in the locus coeruleus, astrocytes get excited and participate in the morphological remodelling associated with synaptic plasticity, otherwise thought to represent a cellular mechanism of learning and memory. Astroglial morphological plasticity is an energy-demanding process requiring mobilisation of glycogen, which, in the CNS, is almost exclusively stored in astrocytes. Astroglia exhibit cytoplasmic excitability that engages ions (such as Ca^{2+} and Na^{+}) and second messengers (such as cAMP). These ions/molecules contribute to the reception of extracellular signals and coordinate the secretion of glio-signalling molecules, including peptides such as apolipoprotein E, which participates in lipid transport between glia and neurones. In this setting, astrocytes are positioned as spatio-temporal integrators of neural network coordination, which disintegrates during progression of AD.

Keywords: Astroglia; Memory; Morphology; Vesicle traffic; Metabolism; Alzheimer's disease

"All in all recaptured, we apparently appear to have in front of us a peculiar disease process. In recent years, such peculiar disease processes have been detected in growing numbers. This observation will have to suggest to us that we should not let ourselves satisfied with trying to include—with mobilisation of many an effort—any clinically unclear illness case into one of the diagnostic entities known to us. There are without any doubt many more psychic illnesses than listed in our textbooks. In some of these instances a later histological examination will subsequently reveal peculiarities of the specific case. Then, we will gradually arrive at a stage, when we will be able to separate out individual disease from the large illness categories of our textbooks; to delineate them clinically more accurately."

Alois Alzheimer (1907) Über eine eigenartige Erkrankung der Hirnrinde Vortrag in der Versammlung Südwestdeutscher Irrenärzte in Tübingen am 3. November 1906. Referiert (Eigenbericht) in: Allgemeine Zeitschrift für Psychiatrie und Psychisch-Gerichtliche Medizin 64. Bd. (1907) S. 148. Translation: Strassnig and Ganguli 2005.

1. Neuroglia facets of Alzheimer's disease

The most common cause of dementia in the elderly is Alzheimer's disease (AD), a progressive, multistage, ultimately fatal neurodegenerative disease with preclinical phase of a decade or longer [1]. Many features appear at different stages of AD, but the most familiar one, presenting at a late stage, is linked to the histopathological presence of extracellular deposits of fibrillar β -amyloid peptide ($A\beta$), and intraneuronal accumulation of aggregates of hyper-phosphorylated Tau protein [2]. Degeneration and loss of synapses preceding neuronal death also occur [3,4]. Neurodegeneration occurs gradually and dementia may reflect the end stage of an accumulation of pathological changes that may start to develop decade(s) before the onset of the clinical symptoms [5,6].

The nature of the preclinical stages of AD remains obscure. Although the current view is that neurodegeneration in AD reflects neurone-specific deficits, it is possible that preceding or concomitant changes in neuroglia may also contribute to this process [1,6–9]. Neuroglia sustain brain homeostasis at the organ, cellular, micro-architectural, vascular, metabolic, neurotransmitter and ion levels [10–15] and, by definition, homeostatic failure results in disease [7]. The role of neuroglia in dementia was recognised by Alois Alzheimer, who found glial cells in close contact with damaged neurones: "The glia have produced abundant fibres; concurrently many glia show large fatty sacks" [16–18]. In post-mortem tissue from patients with AD, astroglial hypertrophy (reflecting reactive astrogliosis accompanied by increased levels of glial fibrillary acidic protein (GFAP) and S100, a calcium binding protein) is often observed, particularly in astrocytes associated with senile plaques [19–23]. Consistent with a recent study in patients with AD [6], distinct time- and brain-specific morphological alterations were reported in an animal mouse model of familial AD, i.e. astroglial atrophy in addition to hypertrophy was detected in certain brain areas [24–27]. Astroglial asthenia preceded the appearance of senile plaques and appeared first in the entorhinal cortex, the region earliest affected by AD pathology [26]. Although the pathological developments

associated with AD are of particular interest, studies in humans are difficult. Some facets of this pathology can be, to some extent, reproduced in animal models, where the evolution time of AD-like peculiarities is shorter and hence more amenable for experimentation. Thus, the aforementioned mouse models of AD offer certain opportunities for studying AD-related neuropathology. Besides mice, there are many other animal models of AD including nematodes, *Drosophila* flies, rabbits, canines, and non-human primates, and each model recapitulates different aspects of AD to some extent (reviewed in [28]).

In addition to the accumulation of peptides, such as A β , and morphological alterations of neuroglia, AD progression is also associated with brain tissue remodelling, which is often defined as neuroinflammation, because the accumulation of misfolded proteins provokes an innate immune response in the central nervous system (CNS) [1]. This response is in part associated with the A β deposits, because hydrophobic proteins may bind apolipoprotein E (ApoE), a major cholesterol carrier in the CNS that is synthesized and released mainly by astrocytes [29–31]. Polymorphic alleles of ApoE are the main genetic determinants of sporadic AD; individuals carrying the ϵ 4 allele are at increased risk of AD compared with those carrying the more common ϵ 3 allele, whereas the ϵ 2 allele decreases the risk of developing the disease [32]. The mechanism(s) by which the particular alleles are associated with AD are unknown, but they may be related to the capacity of the respective ApoE isoforms to prevent neuroinflammation. It has been claimed that the ApoE lipoprotein isoform function associated with neuroinflammation arises from differential binding to hydrophobic proteins, such as A β , regulating their aggregation and clearance in the brain [33].

ApoE-mediated effects may also be related to a general deregulation of cell metabolism and fundamental failure in neuronal–glial interaction [34]. The key mechanism of neuronal damage by reactive oxygen species and mitochondrial dysfunction in *Drosophila* was associated with impaired lipid metabolism in glia before or at the onset of neurodegeneration. This likely indicates impairment in lipid traffic, carried by ApoE or similar carriers, between glia and neurones, a primary defect that leads to neurodegeneration. This was further supported by experiments in which human amyloid precursor protein (APP) was expressed in neuronal cells of *Drosophila*, which promoted neurodegeneration; the latter could be attenuated with mimetic ApoE peptides [35]. Before neurodegeneration, lipid droplet accumulation was shown to be present in the astrocytes of an animal model of neurometabolic disorder (Leigh’s disease) that affects the CNS. *Ndufs*^{−/−} mutant mice exhibit mitochondrial dysfunction, which further implies impairment of brain lipid metabolism in the early stages of neurodegeneration [34]; the *Ndufs4s* gene otherwise encodes a nucleus-encoded accessory subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (complex I, or NADH:ubiquinone oxidoreductase). The ApoE-mimetic peptides derived from the receptor-binding region of ApoE were selected based on their ability to mimic the functional anti-inflammatory and neuroprotective effects of the intact ApoE protein seen in different animal models of neurological diseases [36].

ApoE as a cholesterol-carrying protein likely contributes to cholesterol brain homeostasis. The brain is the most cholesterol-rich organ in the body [37]. Due to the blood–brain barrier, there is little uptake of cholesterol by circulating lipoproteins. However, cholesterol synthesis (which occurs mainly in glia [38]), takes place in the brain. In addition to de novo synthesis, cholesterol homeostasis in the brain also includes its enzymatic conversion to 24(S)-hydroxycholesterol [37], which readily crosses the blood–brain barrier; this represents the major route for elimination of cholesterol from the brain. It is appealing to speculate that

neurodegeneration may be initiated by defects in components of cell metabolism at a very early stage of AD.

2. Failure of the locus coeruleus network reset system in Alzheimer's disease

Neurones in the locus coeruleus (LC) degenerate at the early stages of AD [39]. These neurones are the primary source of noradrenaline (NA), which is known to be a generic inhibitor of neuroinflammation [40]. Thus, deficient noradrenergic innervation in the CNS may affect the progression of AD through reduced inhibition of neuroinflammation, engaging a rather diffuse innervation of the brain provided by the LC as elaborated below.

The LC localized in the brainstem contains noradrenergic neurones, which project to most, if not to all, areas in the CNS [41]; cortical areas receive more abundant innervation [42]. The function of these diffuse projections lies in the synchronous activation of target neural ensembles, acting thus as a functional reset [43,44]. Activation of LC projections is associated with a variety of processes, such as arousal, attention and memory, the sleep–wake cycle, behavioural flexibility, behavioural inhibition and stress, cognitive control, emotions, neuroplasticity, posture and balance [41]; presumably these processes would be affected via activation of specific subgroups of LC projections. Simultaneous activation of all the LC projections [44] is likely reflected by gamma waves on an electroencephalogram [43].

Noradrenaline released by the LC neurones acts through α - and β -adrenergic receptors (α/β -ARs), which are expressed in neurones, microglia and astrocytes. Stimulation of α -ARs initiates Ca^{2+} signalling in astroglial cells [45–47]. Astroglial Ca^{2+} signals and Ca^{2+} waves propagating through astroglial syncytia were observed in vivo after stimulation of the LC in anesthetized animals [48]. Similarly, in awake animals, stimulation of LC neurones triggered (by activation of α_1 -ARs) widespread astroglial Ca^{2+} signals, which were generated in almost all astrocytes in the field of study [49]. This response may represent the means by which neural networks are activated coherently in space and time. In AD, however, astrocytic Ca^{2+} homeostasis is altered [50,51], which may affect APP processing and A β clearance [52]. Impaired Ca^{2+} signalling with subsequent glutamate toxicity may also be implicated in AD [52,53].

The pool of α -AR is activated in parallel with the β -AR [47]. These latter receptors are coupled to the cAMP-dependent pathway, which regulates degradation of glycogen, which otherwise acts as the energy reserve in the brain [54,55]. Furthermore, β -AR/cAMP signalling controls astroglial morphological plasticity [56]. Morphological changes in astrocytes have important implications in memory formation [57] and are central for reduction of astrocytic cell oedema mediated by adrenergic receptor [58].

3. Astrocytes and memory formation

Memory is defined as the process of retention and reconstruction of learned (acquired) knowledge. As recently discussed [57], both declarative (explicit) memory for facts and events, for people, places, and objects (knowing that) and non-declarative (implicit) memory for perceptual and motor skills (knowing how) [59], rely on similar mechanisms associated with reinforcement of synaptic transmission, which involve morphological changes at the synapses that outlast memory stabilisation [60]. While this morphology-based mechanism was suggested more than a century ago [61], contemporary views assume that memory formation

occurs in the form of molecular events at the level of an individual synaptic connection, which is termed synaptic plasticity. Synaptic changes then integrate through multiple synaptic connections involving neuronal networks, and are finally expressed at the behavioural level [62]. Memory formation is not exclusively related to neurones and their parts but also involves astrocytes [57], which actively participate in information processing through cytosolic Ca^{2+} [63,64] and cAMP signals, as well as by elaborate secretory pathways [65,66] although in a different time domain from neuronal communications [47,67]. It is easy to consider interactions between astrocytes and neurones, since a single astrocyte is intimately associated with many neurones and with their synaptic contacts [68,69]. Human hippocampal protoplasmic astrocytes are substantially larger than their rat namesakes [70]; they are associated with ~2 million synapses [71], one order of magnitude higher than in rats [70]. Abundant morphological interactions of astrocytic processes with neurones are not restricted to the hippocampus; they are a widespread property of CNS tissue.

Close morphological apposition allows astrocytes to receive signals from the synaptic cleft and feed back by releasing their own signalling molecules. Release of many of these molecules occurs through a secretory pathway that uses cytoplasmic vesicles, which store chemical messengers. On stimulation, the vesicle membrane fuses with the plasmalemma, a process termed regulated exocytosis. The secretory role of neuroglia was proposed by Hans Held [72] and Jean Nageotte [73] who observed secretory granules in glial preparations. This hypothesis has been confirmed experimentally in the last two decades, and it is generally recognized that vesicle-based mechanisms participate in the heterocellular signalling that occurs at a morpho-functional unit known as the tri/multipartite synapse [74–76]. Moreover, astroglia-derived signalling molecules [65] are secreted into the extracellular space and may be transported throughout the tissue parenchyma to distant places in the CNS, likely taking advantage of the glymphatic convective system [77]. Conceivably, alterations in the shape of astrocytes may determine the long-range convective communication by glio-signalling molecules.

During implicit memory consolidation of Pavlovian threat conditioning, astrocytic processes retract from synapses in the lateral amygdala, allowing these synapses to enlarge, suggesting that contact with astroglial processes opposes synapse growth during memory consolidation [69]. In other words, if astrocytic processes enwrap synapses and the latter need to expand (or otherwise change structurally) during memory formation, astrocytes may hinder this metamorphosis, demonstrating how astrocytic structural plasticity enables morphological remodelling of synapses associated with memory formation. Under physiological conditions, including reproduction, sensory stimulation and learning, astrocytes display remarkable structural plasticity. Distal astrocytic processes can undergo morphological changes in a matter of minutes, thus modifying the geometry and diffusion properties of the extracellular space and relationships with adjacent neuronal elements, especially with synapses. This type of astroglial plasticity has important functional consequences because it modifies extracellular ionic homeostasis and neurotransmission, thus ultimately modulating neuronal function at the cellular and system levels [78–80].

Recently it has been reported that humans with AD have increased levels of the Gs-coupled adenosine receptor $\text{A}_{2\text{A}}$ in astrocytes. Conditional genetic removal of these receptors enhanced long-term memory in young and ageing mice and increased the levels of Arc (activity-regulated cytoskeleton-associated protein; also known as Arg3.1), a member of an immediate-early gene family, that is required for long-term memory (and is also expressed in astrocytes [81]). Chemogenetic activation of astrocytic Gs-coupled signalling reduced long-

term memory in mice without affecting learning. Similarly to humans with AD, ageing mice expressing human mutant APP (hAPP) showed increased levels of astrocytic A_{2A} receptors. Conditional genetic removal of these receptors enhanced memory in ageing hAPP mice [82].

4. Dynamics of astrocyte morphology and metabolism in Alzheimer's disease

The mechanisms responsible for morphological changes in astrocytes are not known, but these likely involve LC-originated adrenergic signals and generation of cAMP [56], which (at least in vitro) drives astrocytes into a stellate morphology with numerous processes (Fig. 1) [56,83–88]. This remodelling occurs within the time frame of memory consolidation (minutes to hours) and involves cytoskeletal reorganisation, including the restructuring of actin filaments, microtubules, and intermediate filaments [89,90]. Similar morphological plasticity may take place in vivo in long-term memory formation because NA operates as a neuromodulator in Hebbian learning [91]. Under similar training conditions, changes in astrocytic shape have indeed been observed in situ [69]. Hence, if during the early stage of AD, the availability of NA becomes reduced, this may affect astroglial morphological dynamics and consequently memory formation [8].

Alterations of astroglial morphology may have a number of consequences. Tight association between the synaptic membranes and astrocytes is considered essential for homeostatic control of the synaptic cleft, including rapid removal of glutamate [92,93] and potassium from the extracellular space [15,94]. Thus, retraction of astrocytic membrane from the synapse during memory formation [69] may facilitate the spillover of neurotransmitter and thus affect synaptic plasticity [95]. When these events are altered, as is likely the case during disease, homeostatic control by astrocytes is impaired. In the context of AD, astroglial cells undergo complex morphological alterations that differ between brain regions and occur at distinct stages of the disease. In the post-mortem brain, both reactive astrocytes (associated mainly with senile plaques) and degenerated, atrophic astrocytes coexist [23]. In transgenic models of AD, in PDAPP-J20 mice (carrying the Swedish and Indiana APP human mutations [96]) and in 3xTG-AD mice (carrying mutated PS1, APP and tau genes), atrophic, degenerative forms of astroglial cells have been identified [25]. These astroglial atrophies were manifested by a decrease in the surface area and volume of astroglial GFAP-positive profiles and a reduced number of primary and secondary processes [8,23,26]. Astroglial atrophy had a peculiar temporal progression. It started in the entorhinal cortex (at 1 month of age), appeared somewhat later in the prefrontal cortex (between 3 and 6 months of age) and became evident in the hippocampus between 9 and 12 months of age [24,26,27]. In the later stages of AD, emerging extracellular depositions of A β trigger an astrogliotic response; although reactive hypertrophic astrocytes are mainly associated with senile plaques, astrocytes distant to the plaques remain atrophic [26]. This astroglial reactivity, however, was observed only in the hippocampus; and was absent in entorhinal and prefrontal cortices [24,26]. Incidentally, both entorhinal and prefrontal cortices are highly vulnerable to AD pathology; this vulnerability may reflect the absence of defensive astroglial reactivity [51]. Astroglial reactivity, in turn, may be associated with differential modification of Ca²⁺ signalling toolkits. A β induces a reactive response through release of intracellular Ca²⁺ [97] and the latter is suppressed in entorhinal astrocytes when compared with hippocampal astrocytes [98]. Morphological remodelling of astrocytes may contribute to memory impairment through reduced synaptic coverage [57] and homeostatic support, which together may lead to synaptic weakness and synaptic demise, thus affecting the connectivity of neuronal networking and associated information processing. Indeed, neuronal circuits become dysfunctional in response to high A β levels. Recent experimental evidence obtained in mouse model studies suggests that

1 disruption in the balance of excitation and inhibition underlies both the functional impairment
2 of local neuronal circuits and that of large-scale networks in the amyloid-depositing brain
3 [99].

4
5 At the same time, astroglial deficits lead to impairment of memory formation, which is
6 associated with morphological growth of synaptic elements together with enhanced protein
7 synthesis and rearrangement of receptor proteins, all of which increase the energy
8 consumption [100]. Energy substrates required for morphological synaptic plasticity are likely
9 provided by astrocytes. With extensive end feet plastering of blood vessels, astrocytes are
10 well positioned to take up glucose from the blood and distribute glucose or pyruvate or lactate
11 derived from glucose to astrocytic processes surrounding synapses, possibly even through
12 astroglial syncytia by diffusion through gap junctions [101]. Experimentally quantified
13 diffusion of glucose within astrocytes is relatively rapid [102] and may well support glucose
14 delivery via interconnected astrocytes in situ. Although synapses are the main energy
15 consumers in the brain, glycogen, an important CNS energy storage system, is present mainly,
16 if not exclusively, in astroglia. Memory consolidation in young chickens requires
17 glycogenolysis [103,104]. The successful consolidation of memory from short-term to long-
18 term memory also requires neuronal NA release [105]. Therefore, it appears that NA, released
19 from neurones, initiates astrocytic morphological changes, which involve vesicles (discussed
20 in the next section) and activates astroglial energy metabolism. Astrocytes are also the main
21 source of cholesterol in the CNS, which is transported to neurones by ApoE carriers in the
22 extracellular space. While it is still unclear how NA affects lipid metabolism in astrocytes, it
23 is safe to consider that NA signalling integrates glycolysis, morphology and function of
24 astrocytes. Moreover, it was recently shown that NA may also prevent cell swelling in a
25 pathological scenario [58].
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31 **5. Impaired astrocytic vesicle dynamics in Alzheimer's disease**

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34 Many gliotransmitter molecules that are stored in membrane-bound vesicles are secreted by
35 astrocytes on the merger of the vesicle and plasma membrane [65]. In addition to molecules
36 stored in the vesicle lumen, ion channels, membrane receptors and transporters, such as major
37 histocompatibility complex II (MHC-II [106]) and excitatory amino acid transporter 2 (EAAT2
38 [107]) are delivered to the plasma membrane by vesicle traffic [108], which brings vesicles
39 from the site of synthesis, deep in the cytoplasm, to the cell surface. This traffic is maintained
40 by an elaborate system regulated by increases in $[Ca^{2+}]_i$ [108,109]. The complexity of vesicle
41 traffic regulation in astrocytes is characterized by two typical, yet opposing, properties of
42 vesicles that contain peptides, such as atrial natriuretic peptide, and those that carry amino
43 acids and are labelled by the vesicular glutamate transporter VGLUT1 [109,110]. Namely,
44 glutamatergic vesicle motility is accelerated by an increase in $[Ca^{2+}]_i$ [111], whereas the same
45 increase in $[Ca^{2+}]_i$ slows down peptidergic vesicles and endolysosomes [112]. Such regulation
46 also applies to recycling peptidergic vesicles that have merged with the plasma membrane and
47 subsequently re-entered the cytoplasm. The mobility of recycling peptidergic vesicles was
48 studied in cultured astrocytes [113] and those residing in intact brain slices [114]. At rest,
49 peptidergic vesicles moved faster and more directionally, linked to cytoskeletal elements, than
50 after the exposure of astrocytes to ionomycin to increase $[Ca^{2+}]_i$ [113]. The effect of increased
51 $[Ca^{2+}]_i$ was remarkable. The movement of vesicles was almost halted, with only a jitter
52 remaining (that was associated with random diffusional movement). At least some of the
53 peptidergic vesicles carry ATP and a similar attenuation was observed in their mobility when
54 astrocytes were stimulated [115].
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1 Astrocytes from a mouse model of AD (3xTg-AD) isolated in the pre-symptomatic phase of
2 the disease exhibit alterations in vesicle traffic (Fig. 2). Spontaneous mobility of peptidergic
3 and endolysosomal vesicles as well as ATP-evoked, Ca^{2+} -dependent vesicle mobility were
4 diminished in diseased astrocytes. Similar impairment of peptidergic vesicle trafficking was
5 observed in healthy rat astrocytes transfected with familial AD-associated mutated presenilin
6 1 (PS1_{M146V}). The stimulation-dependent peptide discharge from single vesicles was less
7 efficient in 3xTg-AD and PS1_{M146V}-expressing astrocytes than in respective controls. The
8 impaired vesicle dynamics and reduced evoked secretion of the signalling peptides may both
9 contribute to the development of AD [50]. Although atrial natriuretic peptide-containing
10 vesicles were examined in that study, it is likely that all peptidergic vesicles exhibit similar
11 changes. Hence, vesicular traffic of ApoE, important for cholesterol transport between
12 astrocytes and neurones, is also likely to be affected and may in the long term contribute to
13 the presumed anti-inflammatory and clearing functions of ApoE in the brain. This remains to
14 be investigated.
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18 In AD, astrocytes associated with plaques and A β deposits in the hippocampus appear
19 reactive with morphological hypertrophy and upregulated expression of intermediate
20 filaments [116]. These reactive astrocytes may contribute to the neuroinflammatory response.
21 After exposure to the pro-inflammatory cytokine interferon- γ (IFN- γ), previously
22 immunologically silent astrocytes start to express MHC-II molecules and antigens on their
23 surface and act as nonprofessional antigen-presenting cells (APCs). Intracellular traffic of
24 astrocytic vesicles, including endolysosomes, serves to deliver MHC-II proteins to the plasma
25 membrane [106,117]. The delivery of MHC-II molecules from MHC-II compartments to the
26 cell surface of APCs is mediated via a cytoskeletal network and is most likely accomplished
27 by the fusion of MHC-II-carrying late endolysosomes with the plasma membrane. Actin
28 microfilaments [118], microtubules [119,120], and their motor proteins [119,121] have all
29 been shown to mediate trafficking of MHC-II. Recently, the role of intermediate filaments in
30 MHC-II trafficking was investigated in IFN- γ -activated reactive (i.e. overexpressing
31 intermediate filament GFAP) astrocytes [106]. Upregulation of intermediate filaments allows
32 faster and therefore more efficient delivery of MHC-II molecules to the cell surface. Reduced
33 mobility of late endolysosomes at high $[\text{Ca}^{2+}]_i$ may increase their probability of docking to
34 and fusion with the plasma membrane [112]. In astrocytes acting as APCs, this process may
35 serve as an additional regulatory mechanism that controls the onset of late endolysosomal
36 fusion and final delivery of MHC-II molecules to the cell surface [106]. Besides IFN- γ ,
37 endogenous suppressors, including NA, have been shown to regulate the expression of MHC-
38 II molecules in astrocytes [40,122]. Thus, when LC neurones degenerate and the amount of
39 NA in the CNS is consequently reduced, this facilitates inflammatory processes.
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46 Impaired astrocytic vesicle traffic is not only associated with neurodegenerative diseases,
47 such as AD, but it is similarly affected in neurodevelopmental disorders, such as intellectual
48 deficiency (ID) [123]. Symptoms of ID appear early in life and the disability affects about 2%
49 of the population. Family studies have demonstrated a relatively large number of X
50 chromosome-linked forms of ID (XLID) with an incidence of about 0.9 to 1.4 in 1000 males
51 [124]. One of the first genes found to be mutated in patients with XLID is *GDII* [125], which
52 encodes for guanine nucleotide dissociation inhibitor (α GDI), a protein physiologically
53 involved in retrieving inactive GDP-bound RAB proteins from the membrane. The
54 identification of *GDII* association with ID suggested that vesicular traffic in neural cells is an
55 important pathway for the development of cognitive functions [126,127]. Although the
56 importance of α GDI in neuronal function has been demonstrated, it is unclear whether its role
57 in glia vesicle trafficking contributes to the disease. The α GDI protein regulates the function
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of RAB GTPases, such as RAB 4 and RAB 5, which have been shown to regulate vesicle dynamics in astrocytes [128], and it is likely that impaired vesicle traffic in astrocytes contributes to ID, which is linked to impaired cognitive processes involving memory formation.

6. Conclusions

Astrocytes provide homeostatic regulation in the CNS to support many processes, including memory formation via changes in shape, metabolic support to neurones and cell-to-cell signalling by vesicle-based mechanisms. Even very small defects in any of the processes may, over a long period of time, contribute significantly to the development of diseased states. In this article, we have highlighted multiple facets by which astrocytes contribute to AD, which also include dynamic alterations in astrocytic vesicles, which change the signalling landscape of astrocytes related to neuroinflammation, energy support and cell-to-cell communication.

Conflict of interest statement

None declared.

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Figure legends

Figure 1. (A) Remodelling of astrocyte morphology (stellation) by the β -adrenergic receptor (β -AR) agonist isoprenaline (Iso), which increases cAMP. Green fluorescing astrocytes transfected with the cAMP nanosensor Epac1-camps (top) and their corresponding differential interference contrast images (bottom) before (left) and 30 min after 1 μ M β -AR agonist isoprenaline (Iso). Note the thinning and elongation of processes indicating astrocyte stellation. Scale bar, 20 μ m. Astrocytes were cultured from rat cortex. Modified from Vardjan et al. [56] with permission. (B) Time course of cytosolic levels of cAMP. Norepinephrine (NA) persistently increases intracellular cAMP levels in astrocytes. Representative time courses of the Epac1-camps (i.e. a Förster resonance energy transfer (FRET)-based cAMP sensor) from three cells after the addition of 1 μ M NA. Changes in FRET are expressed as percentages relative to the initial values. (C) Time course of cytosolic levels of Ca^{2+} . The application of fingolimod (FTY720) evokes prolonged transient increases (oscillations). Superimposed time-resolved fluorescence intensity obtained in five cells treated with FTY720 (white bar). The thin dotted line indicates the zero fluorescence level (F_0). Modified with permission from [56,67].

Figure 2. Attenuated 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 ANP.emd, stored in individual vesicles, observed as bright fluorescent puncta; scale bars, 10 μ m. (C) Vesicle tracks ($N=50$) obtained in a 15-s epoch of imaging representative of control (wt) and (D) 3xTg-AD astrocytes expressing ANP.emd. 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 $f=a \times \exp(-0.5 \times (x/x_0)/b)^2/x$, where $a=17.88 \pm 0.00$, $b=0.07 \pm 0.00 \mu\text{m}^{-0.5}$, $x_0=0.07 \pm 0.00 \mu\text{m}$ (black curve) and $a=6.53 \pm 0.13$, $b=0.19 \pm 0.01 \mu\text{m}^{-0.5}$, $x_0=0.31 \pm 0.01 \mu\text{m}$ (grey curve) in wt astrocytes, and with the function $f=a \times \exp(-0.5 \times (\ln x/x_0)/b)^2/x$, where $a=1.96 \pm 0.04$, $b=0.92 \pm 0.02 \mu\text{m}^{-0.5}$, $x_0=0.10 \pm 0.00 \mu\text{m}$ (black curve) in 3xTg-AD astrocytes. The vertical dashed line indicates the step length of 0.2 μ m obtained close to the intersection of distributions (black and grey curve) in wt astrocytes to discriminate small ($<0.2 \mu\text{m}$) from large ($\geq 0.2 \mu\text{m}$) steps. Note the higher proportion (%) of smaller step lengths in the 3xTg-AD astrocytes 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 bars (mean $\pm$ SEM) indicate the number of vesicles analysed; the numbers at the bottom of the bars indicate the number of cells analysed. Modified with permission from [50].

Figure 1
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Figure 2
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