Astroglia in ageing

Alexei Verkhatsky

Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, M13 9PT, UK

Correspondence: Alexei.Verkhatsky@manchester.ac.uk

Received: 03.05.2021; Accepted: 05.05.2021; Published: 06.05.2021

Abstract. Astroglia are neural cells of ectodermal, neuroepithelial origin responsible for homeostasis and defence of the central nervous system (CNS). Ageing reduces the functional capacity of all organs, so does that of the nervous system, the latter is evident in the reduction of cognitive abilities, learning and memory. At the same time the progression of these deficits is very much individual and lifestyle dependent, indicating operation of mechanisms counterbalancing age-dependent decline. In physiological ageing astrocytes undergo morphological atrophy and functional asthenia; astrocytic paralysis facilitates progression of age-dependent neurodegenerative disorders. Astroglial status and homeostatic capabilities are influenced by lifestyle including intellectual engagement, social interactions, physical exercise, and healthy diet. Maintenance of healthy lifestyle is paramount for cognitive longevity.

Key words: Ageing; astrocyte; astroglial cradle; synaptic transmission; reactive astrogliosis; neuroplasticity

The resilient ageing brain

Ageing reduces the functionality of all organs and systems ultimately weakening the whole organism, reducing its capability to adaptation. Ageing of the nervous system impairs cognition, affects learning and weakens memory. Despite much popularised views on the inevitability and rapid progression of senile-related cognitive weakness, the human brain is nonetheless resilient to the ageing process: decline in cardiovascular or skeleto-muscular systems emerges at much younger ages. Plastic changes that remodel cellular circuitry of the brain occur throughout lifespan, while learning is capable to maintain brain functional capacity at high levels even at advanced ages. Mechanisms of brain ageing remain debatable; multiple changes occurring at all level of organisations of nervous tissues as well as systemic mechanisms contribute to brain senescence. Various theories of brain ageing highlight the importance of metabolic aberrations, neuroinflammation and cellular signalling [1-3]. Proper function of neurones, their axons and synaptic connection depend on maintenance of the homeostatic equilibrium in the nervous tissue; this homeostasis reflects functional activity of the specialised class of neural cells known as neuroglia.

Neuroglia: homeostatic and defensive arm of the nervous system

Neuroglia of the brain and the spinal cord are classified into (i) homeostatic astroglia; (ii) myelinating oligodendroglia and oligodendrocyte precursors also known as NG-2 glia and (iii) microglia [4-7]. Astrocytes are principal homeostatic cells of the central nervous system (CNS) [8], in addition astrocytes contribute to the defence of the nervous tissue through

evolutionary conserved programme or reactive astrogliosis [9-11]. Astrocytes are fundamental for maintaining synaptic connectivity: astrocytic processes enwrapping synaptic structures create the synaptic cradle which controls life of synapses from synaptogenesis to extinction and regulate homeostasis of ions and neurotransmitters [12-14]. Oligodendrocytes contribute to the brain connectome through myelination of axons in grey and white matter. Myelination in the human brain continues well into 4th or even 5th decade of life [15, 16]; probably activity-dependent myelination of the grey matter axons persists throughout life. Oligodendrocyte precursors, diffusely distributed through the CNS, maintain myelination in advanced ages [17, 18]. Finally, microglial cells (glia of myeloid origin) provide an innate immune defence for the CNS and contribute to neural plasticity [7, 19].

Figure 1. Astroglipathology: general principles (see text for details).

Pathophysiology of astroglia

Pathological changes in astroglia can generally be classified into (i) Reactive astrogliosis; (ii) Astroglial atrophy with loss of function; (iii) Pathological remodelling and (iv) Astroglial degeneration and death (Fig. 1, [20, 21]). Reactive astrogliosis is the evolutionary conserved response of astroglia to any pathological stimuli from acute trauma and infection to chronic neurodegeneration or epilepsy in which astrocytes launch molecular programs that result in biochemical, morphological and physiological remodelling of these cells with an ultimate goal of protection of the nervous tissue [10, 11, 22-27]. Fundamentally, reactive astrogliosis is always secondary to the lesion; initiation of reactive reprogramming by pathological insults results in emergence of multiple age-, context- and disease-specific reactive astrocytic phenotypes [11, 28-32]. Contrary to popular belief of detrimental outcome of astrocytic reactivity, astrogliosis is fundamentally neuroprotective, although reactive remodelling may be associated with loss of vital homeostatic functions [10]. To conclude: reactive astrogliosis is graded, context-dependent, reversible, and generally protective.

Astroglial atrophy is manifested by reduction of astrocytic complexity, decrease in astrocytic morphological profiles and loss of homeostatic functions. Astroglial atrophy is a fundamental part of pathophysiology of various diseases, including neuropsychiatric diseases, addictive disorders, epilepsy, and neurodegeneration including Alzheimer disease [24-27, 33, 34]. Some types of astrocytes are more prone to develop higher degree of atrophy: for example, Alzheimer's disease is associated with almost complete loss of interlaminar astrocytes [35]. Astroglial atrophy underlies results in decreased synaptic coverage and reduced synaptic support, which may contribute to the cognitive decline [24, 33].

Severe insults to the nervous tissue (such as trauma or ischemia) can trigger astrodegeneration and death. Morphologically astrodegeneration is manifested in the form of clasmotodendrosis (from Greek “κλάσμα”, fragment, “δένδρον”, tree, “ωσις”, process) characterised by fragmentation of astroglial processes, disappearance of distal fine processes, together with swelling and vacuolation of the cell body. Clasmotodendrotic astrocytes were identified in stroke, infectious encephalopathies, some psychiatric diseases and in ageing [36-38].

Morphology of aged astrocytes

Physiological ageing is not accompanied by changes in astrocytic numbers [39-41]. Morphological characterisation of aged astrocytes is mainly limited to analysis of cellular profiles stained with antibodies against glial fibrillary acidic protein, GFAP. The GFAP as a morphological marker is not ideal. First, in the healthy brain it labels only a sub-population of astrocytes; in many brain regions only 15 – 20% of astrocytes express GFAP at the level of detection [8]. Second, GFAP antibodies visualise only the cytoskeleton and do not label peripheral processes which account for the largest part of astrocyte surface [42]. Expression of GFAP generally increases with age; this inspired a widespread notion of the predominance of astroglial reactivity in old brains. Reactivity in turn has been linked to inflammation, hence increase in GFAP expression has been considered as an indication of age-dependent neuroinflammation. Data on morphology of GFAP astrocytic profiles, however, are controversial: both increase and decrease in size and complexity of astrocytes have been reported (see [40, 43] for comprehensive overview).

Figure 2. Reconstructions of hippocampal protoplasmic astrocytes from young, adult and old mice. Modified and reproduced from [49].

Labelling astrocytes with other techniques did not reveal age-dependent hypertrophy characteristic of astrogliosis. Golgi staining of old astrocytes did not show age-dependent changes [44]. Immunostaining of brain tissues from mice of different ages (3, 9, 18, 24 month old) with antibodies against GFAP, glutamate synthetase and protein S100B (all being canonical markers of astrocytes) revealed complex and region-dependent changes [45-48]. The GFAP-positive profiles were increased in hippocampus but substantially decreased in the entorhinal cortex (EC) of old animals. Astrocytes positive to GS were found to be smaller in old hippocampus but larger in old EC, and finally S100B-positive profiles from old animals demonstrated hypertrophy in EC, and almost no changes in hippocampus. In summary different sub-populations of astrocytes undergo distinct changes in ageing.

Morphometric analysis of astrocytes labelled with low molecular weight fluorescent probe Alexa Fluor 594 delivered through path-pipette into voltage-clamped cells in brain slices from 3, 9 and 24 months-old mice demonstrated an increase in the size and complexity of astrocytes in development from youth to adulthood. Ageing resulted in a significant decrease in size and complexity of astrocytes when compared to adult animals (Fig. 2). More detailed analysis of volume fraction of optically unresolved portions of the cell also revealed significant decrease in peripheral processes indicating reduced astroglial synaptic coverage in the old brain [49].

Figure 3. Ageing affects the density of plasmalemmal glutamate transporters and ionotropic receptor-mediated currents in acutely isolated single cortical astrocytes. A. Representative whole-cell currents elicited in the acutely isolated astrocytes by application of 100 μ M glutamate (left column), 10 μ M NMDA (middle column) and 10 μ M ATP/ α meATP (a potent and stable agonist at P2X₁, P2X₃, P2X_{2/3}, P2X_{1/5} and P2X_{4/6} receptors. It is also a weak partial agonist at human and mouse P2X₄ receptors, but an antagonist at the rat P2X₄ receptor; it has little or no effect at other P2X and P2Y receptors), at holding potential of -80 mV. Glutamate- and NMDA-evoked currents were inhibited by 10 μ M D-AP5, an NMDA antagonist, and 30 μ M CNQX, an AMPA receptor antagonist; ATP-evoked currents were inhibited by 10 μ M PPADS, a selective purinergic P2X antagonist. B. The density of currents mediated by P2X, NMDA and AMPA receptors and plasmalemmal glutamate transporters (GluT) in cortical astrocytes (mean $\pm$ SD for 9 - 12 cells for each age group); statistical significance of difference between average value for 1 month and corresponding values for 3 and 6 months $P < 0.02$ (ANOVA) for all types of currents. Reproduced with permission from [50]

Membrane physiology of aged astrocytes

Ageing is not associated with substantial changes in membrane physiology. Neither resting membrane potential nor membrane input resistance of cortical astrocytes change with ageing [49, 50]. Astrocytes from old humans and rodents express functional receptors to neurotransmitters and generate Ca²⁺ signals in response to appropriate stimulation [50-52]; parameters of these signals do not differ much from those observed in young or adult animals. The density of AMPA, NMDA and P2X receptors as well as the density of plasmalemmal glutamate transporter currents demonstrates bell-shaped age dependency (Fig. 3). There are some data hinting of age-dependent decrease in ATP-induced astroglial Ca²⁺ responses [53]. Old astrocytes seem to have less gap junctional coupling: substantial decrease in dye coupling between astrocytes was found in cortex and hippocampus of 20 -

27-months old mice [54]. Although the data are quite limited it seems that physiological ageing does not substantially affect fundamental physiological mechanisms in astrocytes.

Ageing is associated with loss of astrocyte homeostatic and defensive capabilities

Astrocytes control homeostasis of the CNS through numerous enzymatic cascades and membrane transporters [55]. Astrocytes are central for maintaining extracellular K^+ homeostasis thus preserving neuronal excitability; K^+ accumulation into astrocytes after periods of neuronal activity occurs mainly through Na^+/K^+ pump, whereas subsequent K^+ release is mediated by inwardly rectifying $K_{ir4.1}$ channels [56, 57]. Astrocytes are central for clearance and catabolism of major neurotransmitters. In particular, astrocytes provide for glutamate-(GABA)-glutamine shuttle, which maintains glutamatergic and GABAergic transmission in the CNS [58-60]. Astrocytes also remove and catabolise monoamines and adenosine, they secrete reactive oxygen species scavengers and regulate extracellular pH [8]. All these homeostatic functions are mediated through extended family of membrane transporters, which are mainly regulated by Na^+ signalling [61-64]. Ageing affects astrocytic homeostatic cascades thus reducing homeostatic support that may negatively impact on synaptic transmission and neural plasticity thus contributing to age-dependent cognitive deficits.

Neurotransmitter homeostasis

Glutamate, the main excitatory neurotransmitter in the CNS is synthesised in astrocytes; neurones do not express relevant enzymatic cascades being thus dependent on astrocytes for continuous supply of glutamate precursor glutamine [65, 66]. Glutamine, after entering neurones through glutamine transporters of SLC38A1-5 family, is converted into glutamate and subsequently into GABA to be used in neurotransmission. Glutamate released from neuronal terminals is removed to astrocytes by Na^+ -dependent excitatory amino acid transporters 1 and 2 (EAAT1/SLC1A6 and EAAT2/SLC1A2) [67]. In the cytosol of astrocytes glutamate is converted to glutamine by glutamine synthetase [60, 68] and glutamine is transported to neurones, where it is converted to glutamate, and by proxy of the latter to GABA. This glutamate (GABA)-glutamine shuttle is indispensable for both excitatory and inhibitory transmission in the brain.

Ageing is accompanied with an increase of the ratio of glutamate to glutamine in the brain indicating deficient glutamate (GABA)-glutamine shuttle [69, 70]. Expression of astrocytic glutamate transporters as well as the glutamate uptake is reduced in old rats [71]. In 18 - 21 months old mice the glutamate transporter current density is 7 – 10 times lower when compared to 6 months old animals [50]. The levels of glutamine synthetase are slightly decreased in the hippocampal astrocytes of 18 months old mice [72]. Decrease in astrocytic perisynaptic processes and synaptic coverage adds to the reduced glutamate uptake, and favours glutamate spillover; both these processes impair synaptic plasticity [49].

Astrocytes express most (in not all) of CNS monoamine oxidase-B (MAO-B), the principal enzyme of catecholamines catabolism [73, 74]. The level of MAO-B in aged brain is increased several folds [75]. Increase in MAO-B restricts bioavailability of noradrenaline, thus exacerbating deficient noradrenergic innervation associated with old age [76]. In aged astrocytes MAO-B also contributes to the putrescine-dependent synthesis of GABA, which is accompanied with the by-production of neurotoxic hydrogen peroxide [77-80].

Intracranial blood flow and blood-brain barrier

Astrocytes form the parenchymal portion of the blood brain barrier; astrocytic endfeet create *glia limitans vascularis*, which cover ~98% of parenchymal surface of brain blood vessels. Within the parenchyma protoplasmic astrocytes parcellate the nervous tissue into spatially segregated territorial domains [81] that integrate neurones, synapses, microglia and

neighbouring capillaries into the neurogliovascular (or neurovascular) unit [82]. Within the neurogliovascular unit astrocytes orchestrate multidirectional signalling between cellular components and contribute to the regulation of local blood flow through the release of vasoconstrictors and vasodilators that affect the tone of arterioles and capillaries [83-85]. In addition, astrocytes act as intracranial baroreceptors, being thus involved in regulation of arterial blood pressure [86]. Age-dependent atrophy of astrocytes may reduce their ability to monitor cerebral perfusion, and regulate circulation at the organ and local levels, which may contribute to age-related hypertonia and stroke.

The blood-brain barrier (BBB) which restricts molecular exchange between circulation and CNS parenchyma is composed by the continuous layer of brain capillary endothelial cells [87]. Ageing is associated with restructuring of brain blood vessels and decrease in blood supply, thus affecting metabolism [88, 89]. Physiological ageing is also associated with a decrease in BBB integrity, possibly associated with loss of astrocytic support [90-92].

Neurogenesis

Stem cells of adult neurogenic niches (located in dentate gyrus and subventricular zone of the hippocampus) are represented by radial astrocytes (also known as radial glia-like neural stem cells). These cells share properties of stem cells and homeostatic astrocytes: they are capable of symmetric division and production of committed neural precursors as well as of homeostatic support of neurones and synapses [93, 94]. Ageing leads to a decline in neurogenic capacity of radial astrocytes [95-97]. In pathological ageing and specifically Alzheimer's disease neurogenesis is even more severely suppressed [98-100].

Age-dependent decline of the glymphatic system

Astrocytes are fundamental for operations of the brain-specific glymphatic clearance system responsible for life-long waste collection [101, 102]. At the level of arterioles, two basement membranes, the parenchymal (facing astrocytic endfeet) and vascular (attached to endothelial cells) create the perivascular space which exchanges fluids with the interstitium. Water transport between the perivascular space and the brain parenchyma is mediated by aquaporin 4 water channels that concentrate in astrocytic endfeet. Ageing, as well as many neuropathologies, is associated with loss of aquaporin 4 polarisation to endfeet, which impacts upon normal operation of the glymphatic clearance [103]. Of note, even more severe disruptions in AQP4 polarisation and by proxy in glymphatic operation occurs in neurodegenerative disorders and Alzheimer's disease in particular [104].

Ageing impairs astroglial metabolic support

Astrocytes supply neurones with lactate as an energy substrate; lactate production is regulated by neuronal activity and is important for neural plasticity, learning and memory [105]. Ageing increases astroglial oxidative metabolism, thus limiting lactate production [106, 107].

Ageing astrocytes and mitochondria

Mitochondrial function declines in ageing [108, 109]. Mitochondrial senescence is linked to an increase in mutations of mitochondrial RNA and decrease in ATP production. Astrocytes contribute to neuroprotection through transmitophagy: astrocytes collect damaged mitochondria from neurones and supply the latter with functional organelles [110, 111]. Age-dependent astroglial dystrophy and decline in astrocytic support affects this pathway thus compromising neuroprotection.

Cholesterol synthesis

Cholesterol, ultimately required for synaptogenesis and morphological plasticity, does not pass the blood-brain barrier and the brain therefore depends on cholesterol synthesis that

occur almost entirely in astrocytes [112, 113]. In ageing the main cholesterol synthesising enzyme HMG-CoA reductase or Hmgcr, is down-regulated; in parallel the level of the receptors for cholesterol transport in astrocytes increases with age [114]. These findings indicate disrupted cholesterol metabolism that may contribute to the deficient synaptic plasticity in the old nervous tissue [115].

Ageing affects astrocytic antioxidant defence

Astrocytes are principal elements of the brain antioxidant system, which relies on glutathione and ascorbic acid [116]. Obligatory precursors of glutathione synthesis in neurones are cysteine or glutamylcysteine, which both are supplied by astrocytes. Neurones do not have membrane transport system for accumulating cystine, whereas astrocytes specifically express the Sxc⁻ glutamate/cystine exchanger [117]. This exchanger is used to accumulate cystine, which is reduced to cysteine or converted into glutamylcysteine; both can be utilised for glutathione synthesis. Astrocytes secrete cysteine and glutathione which is, when outside of the cell, converted into glutamylcysteine. Cysteine and glutamylcysteine are taken up by neurones by EAAT2/3 transporters [118]. Neuronal glutathione production is severely affected in the absence of astrocytes because of the lack of substrate. Depletion of a neuronal-astroglial co-cultures of astrocytes significantly reduces neuronal glutathione production, thus promoting neuronal oxidative damage. In mixed co-cultures an individual astrocyte provides antioxidative protection to 20 neurones [119]. Ageing is associated with decrease in the brain levels of glutathione because of decreased production in astrocytes [120, 121]. In a toxic model of ageing and neurodegeneration that used chronic injection of D-galactose resulting in impaired memory and learning, astrocytes demonstrated signs of substantial damage, associated with decreased level of reduced glutathione [122].

Age-dependent decline of reactive astrogliosis

Reactive astrogliosis is a fundamental mechanism of brain defence; reactive astrocytes change their gene expression, biochemistry, morphology, secretome and physiology in order to protect the nervous tissue; the ultimate result of such protection is glial scarring, which fences the damaged area thus saving the healthy nervous tissue; inhibition of astrogliosis often exacerbates neuropathology and neurological outcome [10, 123]. Glial reactivity (both astro- and microgliosis) is declining with age, and this glial paralysis creates the permissive landscape for progression of neurodegenerative diseases and Alzheimer's disease in particular [33, 46]. Early stages of Alzheimer's disease, associated with mild cognitive impairment, are characterised with prominent astrogliosis [124, 125]. The switch from mild cognitive impairment to dementia is associated with declining astroglial reactivity: demented brains have less reactive astrocytes [125, 126]. In depth analysis of the brain tissues of patients with Alzheimer's disease demonstrated that patients with preserved cognitive abilities possess a population of reactive astrocytes with thick and long processes and an increased level of expression of glutamate transporter EAAT2; demented patients did not have such astrocytes [127]. In conclusion, ageing is associated with decline of defensive and neuroprotective capabilities of astrocytes thus increasing brain vulnerability neuropathologies including neurodegeneration.

Mens sana in corpore sana: Lifestyle changes promote astrocytes complexity and extend cognitive ageing

How to extend cognitive longevity? This question is directly linked to the concept of cognitive reserve, the individual property of an individual organism determined by a lifelong interactions between genetic background and environment and reflecting the balance between acquired plastic changes (memory) and accumulated pathological damage [76, 128]. Mechanistically, cognitive reserve is a function of neuronal reserve and neuronal

compensation. The neuronal reserve is accumulated knowledge and associated rewiring of neuronal ensembles, whereas neuronal compensation reflects the capacity of brain defensive systems, and is mainly determined by the status of neuroglia. Conceptually, in the context of ageing, neuroglial failure determines the switch from physiological to pathological ageing [33].

The only plausible way to modify cognitive reserve involves modification of the lifestyle. It is a truth universally acknowledged, that physical exercise, combined with intellectual and social engagement and constant challenge of the brain with learning and new visual and sensory stimuli, is indeed the most powerful non-invasive and non-pharmacological approach to delay cognitive decline associated with ageing. At the cellular level, constant physical, mental and social challenges, which generally can be defined as exposure to enriched environment, instigates numerous molecular, cellular, and structural changes that increase cognitive reserve thus extending cognitive longevity [129-132]. Holistic therapies involving intellectual stimulation and physical activity restore cognitive abilities in patients with Alzheimer's disease [133, 134].

Exposure of experimental animals to various forms of enriched environments (which to a certain degree imitate polytherapies applicable to human patients) affect neural cells, in particular increasing size, complexity, and functional performance of astrocytes, while, at the same time, improving cognitive processes in animals indicating that astrocytic plasticity is a target and the mechanism of positive effects of environmental stimulation [135, 136]. Astrocytic changes translate to increased synaptic support and hence in the increased number of synaptic contacts [135, 137]. In old triple-transgenic Alzheimer's disease mice, exposure to running and enriched environment resulted in a significant increase of astrocytic complexity and reverse of morphological atrophy [138]. Likewise, enriched environment increased astroglial complexity and improved cognitive performance in healthy animals when compared to those housed in standard conditions [136]. Finally, enriched environment and physical exercise improves neurogenesis in both normal ageing and in AD-type pathology [139].

Food intake and dieting represent another life style factor that may influence cognitive longevity. Caloric restriction in particular was demonstrated to increase life span of many species from worms to primates and improve health in ageing [140]. Astrocytes are affected by caloric restriction: exposure of mice to calorie restricted diet increases astrocytic complexity and volume of peripheral processes thus increasing synaptic coverage. This in turn increases the efficacy of K^+ and glutamate clearance thus stimulating synaptic plasticity [141].

Conclusions

Astrocytes define brain homeostasis and contribute to brain defence. Paralysis of astrocytes represents a leading factor determining pathological brain ageing. Astrocytic plasticity is highly sensitive to environmental factors: modifications of life style (intellectual engagement, physical activity and dieting) affect structure and function of astrocytes thus boosting homeostatic support and improving synaptic plasticity. These astrocytic changes translate into increased brain reserve and hence into an increased cognitive longevity.

Information about Author:

Alexei Verkhratsky - MD, PhD, DSc, MAE, ML, MPAN, MSASA, Prof., Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, M13 9PT, U K, AchucarroCenter for Neuroscience IKERBASQUE, 48011 Bilbao, Spain; <https://orcid.org/0000-0003-2592-9898>

References

1. V. Pizza, A. Agresta, C.W. D'Acunto, M. Festa, A. Capasso, Neuroinflammation and ageing: current theories and an overview of the data, *Rev Recent Clin Trials* **2011**, *6*, 189-203.
2. E.C. Toescu, A. Verkhratsky, The importance of being subtle: small changes in calcium homeostasis control cognitive decline in normal aging, *Aging Cell* **2007**, *6*, 267-273.
3. A. Verkhratsky, M.P. Mattson, E.C. Toescu, Aging in the mind, *Trends Neurosci* **2004**, *27*, 577-578.
4. A. Verkhratsky, A.M. Butt, Glial Physiology and Pathophysiology, Wiley-Blackwell, Chichester, 2013.
5. H. Kettenmann, B. Ransom, Neuroglia, Oxford University Press, Oxford, **2013**, pp. 864.
6. A. Verkhratsky, M.S. Ho, R. Zorec, V. Parpura, The Concept of Neuroglia, *Adv Exp Med Biol* **2019**, *1175*, 1-13.
7. H. Kettenmann, U.K. Hanisch, M. Noda, A. Verkhratsky, Physiology of microglia, *Physiol Rev* **2011**, *91*, 461-553.
8. A. Verkhratsky, M. Nedergaard, Physiology of Astroglia, *Physiol Rev* **2018**, *98*, 239-389.
9. V. Parpura, M.T. Heneka, V. Montana, S.H. Olie, A. Schousboe, P.G. Haydon, R.F. Stout, Jr., D.C. Spray, A. Reichenbach, T. Pannicke, M. Pekny, M. Pekna, R. Zorec, A. Verkhratsky, Glial cells in (patho)physiology, *J Neurochem* **2012**, *121*, 4-27.
10. M. Pekny, M. Pekna, A. Messing, C. Steinhäuser, J.M. Lee, V. Parpura, E.M. Hol, M.V. Sofroniew, A. Verkhratsky, Astrocytes: a central element in neurological diseases, *Acta Neuropathol* **2016**, *131*, 323-345.
11. C. Escartin, E. Galea, A. Lakatos, J.P. O'Callaghan, G.C. Petzold, A. Serrano-Pozo, C. Steinhäuser, A. Volterra, G. Carmignoto, A. Agarwal, N.J. Allen, A. Araque, L. Barbeito, A. Barzilai, D.E. Bergles, G. Bonvento, A.M. Butt, W.T. Chen, M. Cohen-Salmon, C. Cunningham, B. Deneen, B. De Strooper, B. Diaz-Castro, C. Farina, M. Freeman, V. Gallo, J.E. Goldman, S.A. Goldman, M. Gotz, A. Gutierrez, P.G. Haydon, D.H. Heiland, E.M. Hol, M.G. Holt, M. Iino, K.V. Kastanenka, H. Kettenmann, B.S. Khakh, S. Koizumi, C.J. Lee, S.A. Liddelow, B.A. MacVicar, P. Magistretti, A. Messing, A. Mishra, A.V. Molofsky, K.K. Murai, C.M. Norris, S. Okada, S.H.R. Olie, J.F. Oliveira, A. Panatier, V. Parpura, M. Pekna, M. Pekny, L. Pellerin, G. Perea, B.G. Perez-Nievas, F.W. Pfrieger, K.E. Poskanzer, F.J. Quintana, R.M. Ransohoff, M. Riquelme-Perez, S. Robel, C.R. Rose, J.D. Rothstein, N. Rouach, D.H. Rowitch, A. Semyanov, S. Sirko, H. Sontheimer, R.A. Swanson, J. Vitorica, I.B. Wanner, L.B. Wood, J. Wu, B. Zheng, E.R. Zimmer, R. Zorec, M.V. Sofroniew, A. Verkhratsky, Reactive astrocyte nomenclature, definitions, and future directions, *Nat Neurosci* **2021**, *24*, 312-325.
12. M. Augusto-Oliveira, G.P. Arrifano, P.Y. Takeda, A. Lopes-Araujo, L. Santos-Sacramento, D.C. Anthony, A. Verkhratsky, M.E. Crespo-Lopez, Astroglia-specific contributions to the regulation of synapses, cognition and behaviour, *Neurosci Biobehav Rev* **2020**, *118*, 331-357.
13. A. Verkhratsky, M. Nedergaard, Astroglial cradle in the life of the synapse, *Philos Trans R Soc Lond B Biol Sci* **2014**, *369*, 20130595.
14. M. Nedergaard, A. Verkhratsky, Artifact versus reality - how astrocytes contribute to synaptic events, *Glia* **2012**, *60*, 1013-1023.
15. G. Bartzokis, M. Beckson, P.H. Lu, K.H. Nuechterlein, N. Edwards, J. Mintz, Age-related changes in frontal and temporal lobe volumes in men: a magnetic resonance imaging study, *Arch Gen Psychiatry* **2001**, *58*, 461-465.
16. K.B. Walhovd, H. Johansen-Berg, R.T. Karadottir, Unraveling the secrets of white matter - Bridging the gap between cellular, animal and human imaging studies, *Neuroscience* **2014**, *276C*, 2-13.
17. A.M. Butt, M. Papanikolaou, A. Rivera, Physiology of Oligodendroglia, *Adv Exp Med Biol* **2019**, *1175*, 117-128.
18. I. Vanzulli, M. Papanikolaou, I.C. De-La-Rocha, F. Pieropan, A.D. Rivera, D. Gomez-Nicola, A. Verkhratsky, J.J. Rodriguez, A.M. Butt, Disruption of oligodendrocyte progenitor cells is an early sign of pathology in the triple transgenic mouse model of Alzheimer's disease, *Neurobiol Aging* **2020**, *94*, 130-139.
19. H. Kettenmann, F. Kirchhoff, A. Verkhratsky, Microglia: new roles for the synaptic stripper, *Neuron* **2013**, *77*, 10-18.

20. A. Verkhratsky, R. Zorec, V. Parpura, Stratification of astrocytes in healthy and diseased brain, *Brain Pathol* **2017**, 27, 629-644.
21. A. Verkhratsky, M.S. Ho, N. Vardjan, R. Zorec, V. Parpura, General Pathophysiology of Astroglia, *Adv Exp Med Biol* **2019**, 1175, 149-179.
22. M.V. Sofroniew, Molecular dissection of reactive astrogliosis and glial scar formation, *Trends Neurosci* **2009**, 32, 638-647.
23. M.V. Sofroniew, Astrogliosis, *Cold Spring Harb Perspect Biol* **2014**, 7, a020420.
24. A. Verkhratsky, J.J. Rodrigues, A. Pivoriunas, R. Zorec, A. Semyanov, Astroglial atrophy in Alzheimer's disease, *Pflugers Arch* **2019**, 471, 1247-1261.
25. I. Ferrer, Diversity of astroglial responses across human neurodegenerative disorders and brain aging, *Brain Pathol* **2017**, 27, 645-674.
26. B.M. Siemsen, C.M. Reichel, K.C. Leong, C. Garcia-Keller, C.D. Gipson, S. Spencer, J.A. McFaddin, K.N. Hooker, P.W. Kalivas, M.D. Scofield, Effects of Methamphetamine Self-Administration and Extinction on Astrocyte Structure and Function in the Nucleus Accumbens Core, *Neuroscience* **2019**, 406, 528-541.
27. A. Verkhratsky, M. Olabarria, H.N. Noristani, C.Y. Yeh, J.J. Rodriguez, Astrocytes in Alzheimer's disease, *Neurotherapeutics* **2010**, 7, 399-412.
28. M.A. Wheeler, I.C. Clark, E.C. Tjon, Z. Li, S.E.J. Zandee, C.P. Couturier, B.R. Watson, G. Scalisi, S. Alkawai, V. Rothhammer, A. Rotem, J.A. Heyman, S. Thaploo, L.M. Sanmarco, J. Ragoussis, D.A. Weitz, K. Petrecca, J.R. Moffitt, B. Becher, J.P. Antel, A. Prat, F.J. Quintana, MAFG-driven astrocytes promote CNS inflammation, *Nature* **2020**, 578, 593-599.
29. O. Al-Dalahmah, A.A. Sosunov, A. Shaik, K. Ofori, Y. Liu, J.P. Vonsattel, I. Adorjan, V. Menon, J.E. Goldman, Single-nucleus RNA-seq identifies Huntington disease astrocyte states, *Acta Neuropathol Commun* **2020**, 8, 19.
30. A. Grubman, G. Chew, J.F. Ouyang, G. Sun, X.Y. Choo, C. McLean, R.K. Simmons, S. Buckberry, D.B. Vargas-Landin, D. Poppe, J. Pflueger, R. Lister, O.J.L. Rackham, E. Petretto, J.M. Polo, A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation, *Nat Neurosci* **2019**, 22, 2087-2097.
31. D. Henrik Heiland, V.M. Ravi, S.P. Behringer, J.H. Frenking, J. Wurm, K. Joseph, N.W.C. Garrelfs, J. Strahle, S. Heynckes, J. Grauvogel, P. Franco, I. Mader, M. Schneider, A.L. Potthoff, D. Delev, U.G. Hofmann, C. Fung, J. Beck, R. Sankowski, M. Prinz, O. Schnell, Tumor-associated reactive astrocytes aid the evolution of immunosuppressive environment in glioblastoma, *Nat Commun* **2019**, 10, 2541.
32. M. Pekny, U. Wilhelmsson, M. Pekna, The dual role of astrocyte activation and reactive gliosis, *Neurosci Lett* **2014**, 565, 30-38.
33. A. Verkhratsky, A. Marutle, J.J. Rodriguez-Arellano, A. Nordberg, Glial Asthenia and Functional Paralysis: A New Perspective on Neurodegeneration and Alzheimer's Disease, *Neuroscientist* **2015**, 21, 552-568.
34. A. Verkhratsky, J.J. Rodriguez, L. Steardo, Astrogliopathology: a central element of neuropsychiatric diseases?, *Neuroscientist* **2014**, 20, 576-588.
35. J.A. Colombo, B. Quinn, V. Puissant, Disruption of astroglial interlaminar processes in Alzheimer's disease, *Brain Res Bull* **2002**, 58, 235-242.
36. D.J. Sahlas, J.M. Bilbao, R.H. Swartz, S.E. Black, Clasmatodendrosis correlating with periventricular hyperintensity in mixed dementia, *Ann Neurol* **2002**, 52, 378-381.
37. M. Tachibana, I. Mohri, I. Hirata, A. Kuwada, S. Kimura-Ohba, K. Kagitani-Shimono, H. Fushimi, T. Inoue, M. Shiomi, Y. Kakuta, M. Takeuchi, S. Murayama, M. Nakayama, K. Ozono, M. Taniike, Clasmatodendrosis is associated with dendritic spines and does not represent autophagic astrocyte death in influenza-associated encephalopathy, *Brain Dev* **2019**, 41, 85-95.
38. R.E. Hulse, J. Winterfield, P.E. Kunkler, R.P. Kraig, Astrocytic clasmatodendrosis in hippocampal organ culture, *Glia* **2001**, 33, 169-179.
39. D.P. Pelvig, H. Pakkenberg, A.K. Stark, B. Pakkenberg, Neocortical glial cell numbers in human brains, *Neurobiol Aging* **2008**, 29, 1754-1762.

40. A. Verkhratsky, M. Augusto-Oliveira, A. Pivoriunas, A. Popov, A. Brazhe, A. Semyanov, Astroglial asthenia and loss of function, rather than reactivity, contribute to the ageing of the brain, *Pflugers Arch* **2021**, 473, 753-774.
41. K. Fabricius, J.S. Jacobsen, B. Pakkenberg, Effect of age on neocortical brain cells in 90+ year old human females--a cell counting study, *Neurobiol Aging* **2013**, 34, 91-99.
42. A. Reichenbach, A. Derouiche, F. Kirchhoff, Morphology and dynamics of perisynaptic glia, *Brain Res Rev* **2010**, 63, 11-25.
43. J.J. Rodriguez-Arellano, V. Parpura, R. Zorec, A. Verkhratsky, Astrocytes in physiological aging and Alzheimer's disease, *Neuroscience* **2016**, 323, 170-182.
44. A.J. Castiglioni Jr., M.E. Legare, B. D.L., E. Tiffany-Castiglioni, Morphological changes in astrocytes of aging mice fed normal or caloric restricted diets, *Age* **1991**, 14, 102-106.
45. J.J. Rodriguez, C.Y. Yeh, S. Terzieva, M. Olabarria, M. Kulijewicz-Nawrot, A. Verkhratsky, Complex and region-specific changes in astroglial markers in the aging brain, *Neurobiol Aging* **2014**, 35, 15-23.
46. A. Verkhratsky, R. Zorec, J.J. Rodriguez-Arellano, V. Parpura, Neuroglia in Ageing, *Adv Exp Med Biol* **2019**, 1175, 181-197.
47. J.J. Rodriguez, A.M. Butt, E. Gardenal, V. Parpura, A. Verkhratsky, Complex and differential glial responses in Alzheimer's disease and ageing, *Curr Alzheimer Res* **2016**, 13, 343-358.
48. A. Verkhratsky, R. Zorec, J.J. Rodriguez, V. Parpura, Astroglia dynamics in ageing and Alzheimer's disease, *Curr Opin Pharmacol* **2016**, 26, 74-79.
49. A. Popov, A. Brazhe, P. Denisov, O. Sutyagina, L. Li, N. Lazareva, A. Verkhratsky, A. Semyanov, Astrocyte dystrophy in ageing brain parallels impaired synaptic plasticity, *Aging Cell* **2021**, 20, e13334.
50. U. Lalo, O. Palygin, R.A. North, A. Verkhratsky, Y. Pankratov, Age-dependent remodelling of ionotropic signalling in cortical astroglia, *Aging Cell* **2011**, 10, 392-402.
51. M. Gomez-Gonzalo, M. Martin-Fernandez, R. Martinez-Murillo, S. Mederos, A. Hernandez-Vivanco, S. Jamison, A.P. Fernandez, J. Serrano, P. Calero, H.S. Futch, R. Corpas, C. Sanfeliu, G. Perea, A. Araque, Neuron-astrocyte signaling is preserved in the aging brain, *Glia* **2017**, 65, 569-580.
52. M. Navarrete, G. Perea, L. Maglio, J. Pastor, R. Garcia de Sola, A. Araque, Astrocyte calcium signal and gliotransmission in human brain tissue, *Cereb Cortex* **2013**, 23, 1240-1246.
53. U. Lalo, A. Bogdanov, Y. Pankratov, Age- and Experience-Related Plasticity of ATP-Mediated Signaling in the Neocortex, *Front Cell Neurosci* **2019**, 13, 242.
54. O. Peters, C.G. Schipke, A. Philipps, B. Haas, U. Pannasch, L.P. Wang, B. Benedetti, A.E. Kingston, H. Kettenmann, Astrocyte function is modified by Alzheimer's disease-like pathology in aged mice, *J Alzheimers Dis* **2009**, 18, 177-189.
55. A. Verkhratsky, M. Nedergaard, L. Hertz, Why are astrocytes important?, *Neurochem Res* **2015**, 40, 389-401.
56. B.R. Larsen, M. Assentoft, M.L. Cotrina, S.Z. Hua, M. Nedergaard, K. Kaila, J. Voipio, N. MacAulay, Contributions of the Na⁺/K⁺-ATPase, NKCC1, and Kir4.1 to hippocampal K⁺ clearance and volume responses, *Glia* **2014**, 62, 608-622.
57. K. Breslin, J.J. Wade, K. Wong-Lin, J. Harkin, B. Flanagan, H. Van Zalinge, S. Hall, M. Walker, A. Verkhratsky, L. McDaid, Potassium and sodium microdomains in thin astroglial processes: A computational model study, *PLoS Comput Biol* **2018**, 14, e1006151.
58. L. Hertz, D.L. Rothman, Glutamine-Glutamate Cycle Flux Is Similar in Cultured Astrocytes and Brain and Both Glutamate Production and Oxidation Are Mainly Catalyzed by Aspartate Aminotransferase, *Biology (Basel)* **2017**, 6.
59. L. Hertz, T.B. Rodrigues, Astrocytic-Neuronal-Astrocytic Pathway Selection for Formation and Degradation of Glutamate/GABA, *Front Endocrinol (Lausanne)* **2014**, 5, 42.
60. C.F. Rose, A. Verkhratsky, V. Parpura, Astrocyte glutamine synthetase: pivotal in health and disease, *Biochem Soc Trans* **2013**, 41, 1518-1524.
61. C.R. Rose, D. Ziemens, A. Verkhratsky, On the special role of NCX in astrocytes: Translating Na⁺-transients into intracellular Ca²⁺ signals, *Cell Calcium* **2020**, 86, 102154.
62. A. Verkhratsky, C.R. Rose, Na⁺-dependent transporters: The backbone of astroglial homeostatic function, *Cell Calcium* **2020**, 85, 102136.

63. C.R. Rose, A. Verkhratsky, Principles of sodium homeostasis and sodium signalling in astroglia, *Glia* **2016**, *64*, 1611-1627.
64. S. Kirischuk, V. Parpura, A. Verkhratsky, Sodium dynamics: another key to astroglial excitability?, *Trends Neurosci* **2012**, *35*, 497-506.
65. L. Hertz, R. Dringen, A. Schousboe, S.R. Robinson, Astrocytes: glutamate producers for neurons, *J Neurosci Res* **1999**, *57*, 417-428.
66. A. Schousboe, S. Scafidi, L.K. Bak, H.S. Waagepetersen, M.C. McKenna, Glutamate metabolism in the brain focusing on astrocytes, *Adv Neurobiol* **2014**, *11*, 13-30.
67. N.C. Danbolt, Glutamate uptake, *Prog Neurobiol* **2001**, *65*, 1-105.
68. M.D. Norenberg, A. Martinez-Hernandez, Fine structural localization of glutamine synthetase in astrocytes of rat brain, *Brain Res* **1979**, *161*, 303-310.
69. J.M. Duarte, K.Q. Do, R. Gruetter, Longitudinal neurochemical modifications in the aging mouse brain measured in vivo by 1H magnetic resonance spectroscopy, *Neurobiol Aging* **2014**, *35*, 1660-1668.
70. J.L. Harris, H.W. Yeh, R.H. Swerdlow, I.Y. Choi, P. Lee, W.M. Brooks, High-field proton magnetic resonance spectroscopy reveals metabolic effects of normal brain aging, *Neurobiol Aging* **2014**, *35*, 1686-1694.
71. B. Potier, J.M. Billard, S. Riviere, P.M. Sinet, I. Denis, G. Champeil-Potokar, B. Grintal, A. Jouvenceau, M. Kollen, P. Dutar, Reduction in glutamate uptake is associated with extrasynaptic NMDA and metabotropic glutamate receptor activation at the hippocampal CA1 synapse of aged rats, *Aging Cell* **2010**, *9*, 722-735.
72. M. Olabarria, H.N. Noristani, A. Verkhratsky, J.J. Rodriguez, Age-dependent decrease in glutamine synthetase expression in the hippocampal astroglia of the triple transgenic Alzheimer's disease mouse model: mechanism for deficient glutamatergic transmission?, *Mol Neurodegener* **2011**, *6*, 55.
73. P. Levitt, J.E. Pintar, X.O. Breakefield, Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons, *Proc Natl Acad Sci U S A* **1982**, *79*, 6385-6389.
74. K.N. Westlund, R.M. Denney, R.M. Rose, C.W. Abell, Localization of distinct monoamine oxidase A and monoamine oxidase B cell populations in human brainstem, *Neuroscience* **1988**, *25*, 439-456.
75. M.J. Kumar, J.K. Andersen, Perspectives on MAO-B in aging and neurological disease: where do we go from here?, *Mol Neurobiol* **2004**, *30*, 77-89.
76. R. Zorec, V. Parpura, A. Verkhratsky, Preventing neurodegeneration by adrenergic astroglial excitation, *FEBS J* **2018**, *285*, 3645-3656.
77. M. Strolin Benedetti, P. Dostert, Monoamine oxidase, brain ageing and degenerative diseases, *Biochem Pharmacol* **1989**, *38*, 555-561.
78. H. Chun, H. Im, Y.J. Kang, Y. Kim, J.H. Shin, W. Won, J. Lim, Y. Ju, Y.M. Park, S. Kim, S.E. Lee, J. Lee, J. Woo, Y. Hwang, H. Cho, S. Jo, J.H. Park, D. Kim, D.Y. Kim, J.S. Seo, B.J. Gwag, Y.S. Kim, K.D. Park, B.K. Kaang, H. Cho, H. Ryu, C.J. Lee, Severe reactive astrocytes precipitate pathological hallmarks of Alzheimer's disease via H₂O₂ production, *Nat Neurosci* **2020**, *23*, 1555-1566.
79. M. Lee, E.G. McGeer, P.L. McGeer, Mechanisms of GABA release from human astrocytes, *Glia* **2011**, *59*, 1600-1611.
80. O. Garaschuk, A. Verkhratsky, GABAergic astrocytes in Alzheimer's disease, *Aging (Albany NY)* **2019**, *11*, 1602-1604.
81. E.A. Bushong, M.E. Martone, Y.Z. Jones, M.H. Ellisman, Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains, *J Neurosci* **2002**, *22*, 183-192.
82. C. Iadecola, The Neurovascular Unit Coming of Age: A Journey through Neurovascular Coupling in Health and Disease, *Neuron* **2017**, *96*, 17-42.
83. M. Zonta, M.C. Angulo, S. Gobbo, B. Rosengarten, K.A. Hossmann, T. Pozzan, G. Carmignoto, Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation, *Nat Neurosci* **2003**, *6*, 43-50.
84. S.J. Mulligan, B.A. MacVicar, Calcium transients in astrocyte endfeet cause cerebrovascular constrictions, *Nature* **2004**, *431*, 195-199.
85. J.A. Filosa, A.D. Bonev, S.V. Straub, A.L. Meredith, M.K. Wilkerson, R.W. Aldrich, M.T. Nelson, Local potassium signaling couples neuronal activity to vasodilation in the brain, *Nat Neurosci* **2006**, *9*, 1397-1403.

86. N. Marina, I.N. Christie, A. Korsak, M. Doronin, A. Brazhe, P.S. Hosford, J.A. Wells, S. Sheikhabaiei, I. Humoud, J.F.R. Paton, M.F. Lythgoe, A. Semyanov, S. Kasparov, A.V. Gourine, Astrocytes monitor cerebral perfusion and control systemic circulation to maintain brain blood flow, *Nat Commun* **2020**, *11*, 131.
87. M.D. Sweeney, Z. Zhao, A. Montagne, A.R. Nelson, B.V. Zlokovic, Blood-Brain Barrier: From Physiology to Disease and Back, *Physiol Rev* **2019**, *99*, 21-78.
88. S. Amin-Hanjani, X. Du, D.K. Pandey, K.R. Thulborn, F.T. Charbel, Effect of age and vascular anatomy on blood flow in major cerebral vessels, *J Cereb Blood Flow Metab* **2015**, *35*, 312-318.
89. S.B. Jessen, C. Mathiesen, B.L. Lind, M. Lauritzen, Interneuron Deficit Associates Attenuated Network Synchronization to Mismatch of Energy Supply and Demand in Aging Mouse Brains, *Cereb Cortex* **2017**, *27*, 646-659.
90. A. Montagne, S.R. Barnes, M.D. Sweeney, M.R. Halliday, A.P. Sagare, Z. Zhao, A.W. Toga, R.E. Jacobs, C.Y. Liu, L. Amezcua, M.G. Harrington, H.C. Chui, M. Law, B.V. Zlokovic, Blood-brain barrier breakdown in the aging human hippocampus, *Neuron* **2015**, *85*, 296-302.
91. J. Duncombe, R.J. Lennen, M.A. Jansen, I. Marshall, J.M. Wardlaw, K. Horsburgh, Ageing causes prominent neurovascular dysfunction associated with loss of astrocytic contacts and gliosis, *Neuropathol Appl Neurobiol* **2017**, *43*, 477-491.
92. D.A. Nation, M.D. Sweeney, A. Montagne, A.P. Sagare, L.M. D'Orazio, M. Pachicano, F. Sepehrband, A.R. Nelson, D.P. Buennagel, M.G. Harrington, T.L.S. Benzinger, A.M. Fagan, J.M. Ringman, L.S. Schneider, J.C. Morris, H.C. Chui, M. Law, A.W. Toga, B.V. Zlokovic, Blood-brain barrier breakdown is an early biomarker of human cognitive dysfunction, *Nat Med* **2019**, *25*, 270-276.
93. Z. Mirzadeh, F.T. Merkle, M. Soriano-Navarro, J.M. Garcia-Verdugo, A. Alvarez-Buylla, Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain, *Cell Stem Cell* **2008**, *3*, 265-278.
94. J. Moss, E. Gebara, E.A. Bushong, I. Sanchez-Pascual, R. O'Laoi, I. El M'Ghari, J. Kocher-Braissant, M.H. Ellisman, N. Toni, Fine processes of Nestin-GFP-positive radial glia-like stem cells in the adult dentate gyrus ensheath the local synapses and vasculature, *Proc Natl Acad Sci U S A* **2016**, *113*, E2536-2545.
95. N.M. Ben Abdallah, L. Slomianka, A.L. Vyssotski, H.P. Lipp, Early age-related changes in adult hippocampal neurogenesis in C57 mice, *Neurobiol Aging* **2010**, *31*, 151-161.
96. M. Bouab, G.N. Paliouras, A. Aumont, K. Forest-Berard, K.J. Fernandes, Aging of the subventricular zone neural stem cell niche: evidence for quiescence-associated changes between early and mid-adulthood, *Neuroscience* **2011**, *173*, 135-149.
97. A.M. Nicaise, C.M. Willis, S.J. Crocker, S. Pluchino, Stem Cells of the Aging Brain, *Front Aging Neurosci.* **2020**, *12*, 247.
98. J.J. Rodriguez, V.C. Jones, M. Tabuchi, S.M. Allan, E.M. Knight, F.M. LaFerla, S. Oddo, A. Verkhratsky, Impaired adult neurogenesis in the dentate gyrus of a triple transgenic mouse model of Alzheimer's disease, *PLoS One* **2008**, *3*, e2935.
99. J.J. Rodriguez, A. Verkhratsky, Neurogenesis in Alzheimer's disease, *J Anat* **2011**, *219*, 78-89.
100. J.J. Rodriguez, V.C. Jones, A. Verkhratsky, Impaired cell proliferation in the subventricular zone in an Alzheimer's disease model, *Neuroreport* **2009**, *20*, 907-912.
101. J.J. Iliff, M. Wang, Y. Liao, B.A. Plogg, W. Peng, G.A. Gundersen, H. Benveniste, G.E. Vates, R. Deane, S.A. Goldman, E.A. Nagelhus, M. Nedergaard, A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta, *Sci Transl Med* **2012**, *4*, 147ra111.
102. M. Nedergaard, Neuroscience. Garbage truck of the brain, *Science* **2013**, *340*, 1529-1530.
103. B.T. Kress, J.J. Iliff, M. Xia, M. Wang, H.S. Wei, D. Zeppenfeld, L. Xie, H. Kang, Q. Xu, J.A. Liew, B.A. Plog, F. Ding, R. Deane, M. Nedergaard, Impairment of paravascular clearance pathways in the aging brain, *Ann Neurol* **2014**, *76*, 845-861.
104. W. Peng, T.M. Achariyar, B. Li, Y. Liao, H. Mestre, E. Hitomi, S. Regan, T. Kasper, S. Peng, F. Ding, H. Benveniste, M. Nedergaard, R. Deane, Suppression of glymphatic fluid transport in a mouse model of Alzheimer's disease, *Neurobiol Dis* **2016**, *93*, 215-225.
105. L. Pellerin, P.J. Magistretti, Sweet sixteen for ANLS, *J Cereb Blood Flow Metab* **2012**, *32*, 1152-1166.

106. T. Jiang, E. Cadenas, Astrocytic metabolic and inflammatory changes as a function of age, *Aging Cell* **2014**, *13*, 1059–1067.
107. J.L. Harris, I.Y. Choi, W.M. Brooks, Probing astrocyte metabolism in vivo: proton magnetic resonance spectroscopy in the injured and aging brain, *Front Aging Neurosci* **2015**, *7*, 202.
108. M. Rockstein, K.F. Brandt, Enzyme changes in flight muscle correlated with aging and flight ability in the male housefly, *Science* **1963**, *139*, 1049-1051.
109. B.A. Payne, P.F. Chinnery, Mitochondrial dysfunction in aging: Much progress but many unresolved questions, *Biochim Biophys Acta* **2015**, *1847*, 1347-1353.
110. C.H. Davis, K.Y. Kim, E.A. Bushong, E.A. Mills, D. Boassa, T. Shih, M. Kinebuchi, S. Phan, Y. Zhou, N.A. Bihlmeyer, J.V. Nguyen, Y. Jin, M.H. Ellisman, N. Marsh-Armstrong, Transcellular degradation of axonal mitochondria, *Proc Natl Acad Sci U S A* **2014**, *111*, 9633-9638.
111. K. Hayakawa, E. Esposito, X. Wang, Y. Terasaki, Y. Liu, C. Xing, X. Ji, E.H. Lo, Transfer of mitochondria from astrocytes to neurons after stroke, *Nature* **2016**, *535*, 551-555.
112. D.H. Mauch, K. Nagler, S. Schumacher, C. Goritz, E.C. Muller, A. Otto, F.W. Pfrieger, CNS synaptogenesis promoted by glia-derived cholesterol, *Science* **2001**, *294*, 1354-1357.
113. J.M. Dietschy, S.D. Turley, Cholesterol metabolism in the brain, *Curr Opin Lipidol* **2001**, *12*, 105-112.
114. M.M. Boisvert, G.A. Erikson, M.N. Shokhirev, N.J. Allen, The Aging Astrocyte Transcriptome from Multiple Regions of the Mouse Brain, *Cell Rep* **2018**, *22*, 269-285.
115. A.L. Palmer, S.S. Ousman, Astrocytes and Aging, *Front Aging Neurosci* **2018**, *10*, 337.
116. T.K. Makar, M. Nedergaard, A. Preuss, A.S. Gelbard, A.S. Perumal, A.J. Cooper, Vitamin E, ascorbate, glutathione, glutathione disulfide, and enzymes of glutathione metabolism in cultures of chick astrocytes and neurons: evidence that astrocytes play an important role in antioxidative processes in the brain, *J Neurochem* **1994**, *62*, 45-53.
117. R.J. Bridges, N.R. Natale, S.A. Patel, System xc⁻ cystine/glutamate antiporter: an update on molecular pharmacology and roles within the CNS, *Br J Pharmacol* **2012**, *165*, 20-34.
118. Y. Chen, R.A. Swanson, The glutamate transporters EAAT2 and EAAT3 mediate cysteine uptake in cortical neuron cultures, *J Neurochem* **2003**, *84*, 1332-1339.
119. S. Desagher, J. Glowinski, J. Premont, Astrocytes protect neurons from hydrogen peroxide toxicity, *J Neurosci* **1996**, *16*, 2553-2562.
120. U.E. Emir, S. Raatz, S. McPherson, J.S. Hodges, C. Torkelson, P. Tawfik, T. White, M. Terpstra, Noninvasive quantification of ascorbate and glutathione concentration in the elderly human brain, *NMR Biomed* **2011**, *24*, 888-894.
121. P. Maher, The effects of stress and aging on glutathione metabolism, *Ageing Res Rev* **2005**, *4*, 288-314.
122. M. Lei, X. Hua, M. Xiao, J. Ding, Q. Han, G. Hu, Impairments of astrocytes are involved in the d-galactose-induced brain aging, *Biochem Biophys Res Commun* **2008**, *369*, 1082-1087.
123. M.V. Sofroniew, Astrocyte Reactivity: Subtypes, States, and Functions in CNS Innate Immunity, *Trends Immunol* **2020**, *41*, 758-770.
124. S.F. Carter, M. Scholl, O. Almkvist, A. Wall, H. Engler, B. Langstrom, A. Nordberg, Evidence for astrocytosis in prodromal Alzheimer disease provided by ¹¹C-deuterium-L-deprenyl: a multitracers PET paradigm combining ¹¹C-Pittsburgh compound B and ¹⁸F-FDG, *J Nucl Med* **2012**, *53*, 37-46.
125. E. Rodriguez-Vieitez, L. Saint-Aubert, S.F. Carter, O. Almkvist, K. Farid, M. Scholl, K. Chiotis, S. Thordardottir, C. Graff, A. Wall, B. Langstrom, A. Nordberg, Diverging longitudinal changes in astrocytosis and amyloid PET in autosomal dominant Alzheimer's disease, *Brain* **2016**, *139*, 922-936.
126. R. Mathur, P.G. Ince, T. Minett, C.J. Garwood, P.J. Shaw, F.E. Matthews, C. Brayne, J.E. Simpson, S.B. Wharton, M.R.C.C. Function, G. Ageing Neuropathology Study, A reduced astrocyte response to beta-amyloid plaques in the ageing brain associates with cognitive impairment, *PLoS One* **2015**, *10*, e0118463.
127. E. Kobayashi, M. Nakano, K. Kubota, N. Himuro, S. Mizoguchi, T. Chikenji, M. Otani, Y. Mizue, K. Nagaishi, M. Fujimiya, Activated forms of astrocytes with higher GLT-1 expression are associated with cognitive normal subjects with Alzheimer pathology in human brain, *Sci Rep* **2018**, *8*, 1712.
128. Y. Stern, Cognitive reserve, *Neuropsychologia* **2009**, *47*, 2015-2028.

129. M. Leon, C. Woo, Environmental Enrichment and Successful Aging, *Front Behav Neurosci* **2018**, *12*, 155.
130. F. Mora, Successful brain aging: plasticity, environmental enrichment, and lifestyle, *Dialogues Clin Neurosci* **2013**, *15*, 45-52.
131. T.J. Dause, E.D. Kirby, Aging gracefully: social engagement joins exercise and enrichment as a key lifestyle factor in resistance to age-related cognitive decline, *Neural Regen Res* **2019**, *14*, 39-42.
132. F. Mora, G. Segovia, A. del Arco, Aging, plasticity and environmental enrichment: structural changes and neurotransmitter dynamics in several areas of the brain, *Brain Res Rev* **2007**, *55*, 78-88.
133. D.E. Bredesen, Reversal of cognitive decline: a novel therapeutic program, *Aging (Albany NY)* **2014**, *6*, 707-717.
134. Q. Meng, M.S. Lin, I.S. Tzeng, Relationship Between Exercise and Alzheimer's Disease: A Narrative Literature Review, *Front Neurosci* **2020**, *14*, 131.
135. D.G. Diniz, M.A. de Oliveira, C.M. de Lima, C.A. Foro, M.C. Sosthenes, J. Bento-Torres, P.F. da Costa Vasconcelos, D.C. Anthony, C.W. Diniz, Age, environment, object recognition and morphological diversity of GFAP-immunolabeled astrocytes, *Behav Brain Funct* **2016**, *12*, 28.
136. P. Sampedro-Piquero, P. De Bartolo, L. Petrosini, C. Zancada-Menendez, J.L. Arias, A. Begega, Astrocytic plasticity as a possible mediator of the cognitive improvements after environmental enrichment in aged rats, *Neurobiology of learning and memory* **2014**, *114*, 16-25.
137. G.G. Viola, L. Rodrigues, J.C. Americo, G. Hansel, R.S. Vargas, R. Biasibetti, A. Swarowsky, C.A. Goncalves, L.L. Xavier, M. Achaval, D.O. Souza, O.B. Amaral, Morphological changes in hippocampal astrocytes induced by environmental enrichment in mice, *Brain Res* **2009**, *1274*, 47-54.
138. J.J. Rodriguez, S. Terzieva, M. Olabarria, R.G. Lanza, A. Verkhratsky, Enriched environment and physical activity reverse astroglial degeneration in the hippocampus of AD transgenic mice, *Cell Death Dis* **2013**, *4*, e678.
139. J.J. Rodriguez, H.N. Noristani, M. Olabarria, J. Fletcher, T.D. Somerville, C.Y. Yeh, A. Verkhratsky, Voluntary running and environmental enrichment restores impaired hippocampal neurogenesis in a triple transgenic mouse model of Alzheimer's disease, *Curr Alzheimer Res* **2011**, *8*, 707-717.
140. J.A. Mattison, R.J. Colman, T.M. Beasley, D.B. Allison, J.W. Kemnitz, G.S. Roth, D.K. Ingram, R. Weindruch, R. de Cabo, R.M. Anderson, Caloric restriction improves health and survival of rhesus monkeys, *Nat Commun* **2017**, *8*, 14063.
141. A. Popov, P. Denisov, M. Bychkov, A. Brazhe, E. Lyukmanova, Z. Shenkarev, N. Lazareva, A. Verkhratsky, A. Semyanov, Caloric restriction triggers morphofunctional remodeling of astrocytes and enhances synaptic plasticity in the mouse hippocampus, *Cell Death Dis* **2020**, *11*, 208.