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Astroglial asthenia and loss of function, rather than reactivity, contribute to the ageing of the brain

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Abstract

Astroglia represents a class of heterogeneous, in form and function, cells known as astrocytes, which provide for homoeostasis and defence of the central nervous system (CNS). Ageing is associated with morphological and functional remodelling of astrocytes with a prevalence of morphological atrophy and loss of function. In particular ageing is associated with (i) decrease in astroglial synaptic coverage; (ii) deficits in glutamate and potassium clearance; (iii) reduced astroglial synthesis of synaptogenic factors such as cholesterol; (iv) decrease in aquaporin 4 channels in astroglial endfeet with subsequent decline in the glymphatic clearance; (v) decrease in astroglial metabolic support through the lactate shuttle; (vi) decreased adult neurogenesis resulting from diminished proliferative capacity of radial stem astrocytes; (vii) decline in the astroglial-vascular coupling and deficient blood-brain barrier and (viii) decrease in astroglial ability to mount reactive astrogliosis. Decrease in reactive capabilities of astroglia is associated with increase in age-dependent neurodegenerative diseases. Astroglial morphology and function can be influenced and improved by lifestyle interventions such as intellectual engagement, social interactions physical exercise, caloric restriction, and healthy diet. These modifications of lifestyle are paramount for cognitive longevity.

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

The resilient ageing brain

The age-dependent decrease in brain function is often portrayed as the main outcome of ageing which stems from an intrinsic propensity of the nervous tissue to degenerate; the view on progressive loss of neurones with age is a commonplace while looming "pandemic" of dementia seems to erect impenetrable barrier for keeping the quality of life of the ageing humanity. Nonetheless, when compared with other organs and systems the brain ages most graciously; which has been documented already on one of the very first epidemiologic investigation of nearly 900 elderly (more than 80 years old), including 74 centenarians. This investigation, published in Cambridge in 1889, arrived to the following conclusion: "*Indeed, the brain in many held out as well or better than other organs - which may be regarded one of the bright rays, if not the brightest, in the centenarian landscape*."[1]

Looking into brain ageing from an unbiased perspective one need to consent with the obvious fact that many peripheral systems start functional decline much earlier than the cognitive abilities begun to suffer: intelligence at 40 is usually blossoming and continues to evolve, providing that an individual maintained healthy lifestyle, whereas at the same age man rarely successfully compete in physical abilities with youngsters. The long-lasting postnatal development of cognitive capacity is defined by the nature of the nervous tissue, and neuroplasticity which needs an environmental input - i.e. learning - to shape and remodel the cellular circuits and neuronal ensembles. Brain plasticity is operational throughout the lifespan; and many mechanisms supporting remodelling of brain connectivity last well into the adulthood. Excessive plasticity of the young brain creates an environment in which the brain is constantly changing and readjusting to the environmental conditions. This requires energy consumption and slows the decision-making. The plasticity of the brain decreases with age when brain wiring and synaptic strength is refined by experience. Hence, in the adult brain, plasticity is gradually replaced by network stability, which allows much faster performance in familiar settings at the expense of new skills acquisition. Thus, brain ageing can be viewed as a change of balance between plasticity and stability. Neuroglia play an important role in regulating both plasticity and stability of synapses and neurones, thus guiding and modulating the ageing of the nervous system.

Neuronal activity is highly energy demanding and hence throughout life the nervous tissue exists under continuous pressure of high production of reactive oxygen species. High protein synthesis requires effective system for clearance of by-products and proteinaceous waste, while ionic fluxes associated with neuronal excitability need a robust system regulating ion composition of the interstitial fluid. Finally, the brain is in need of defensive systems that may protect it against systemic influences or ongoing microtraumas. All in all, proper brain function presents a substantial logistical challenge that is met by specialised class of homoeostatic cells known as neuroglia.

The distinction between physiological brain ageing (with largely preserved cognitive capacity) and pathological brain ageing (which is associated with neurodegeneration and evolves towards dementia) is defined by the cognitive reserve. The cognitive

[1] George Murray Humphry, 1889, The Old Age. The results of information received respecting nearly nine hundred persons who had attained the age of eighty years, including seventy-four centenarians. MacMillan & Bowes, Cambridge, p. 48

reserve is an individual property of every organism; it reflects the lifelong interaction of genetic factors with environment and accumulated pathological damages. The cognitive reserve to a very large extent defines the cognitive outcome for different individuals and arguably defines the progression of neurodegenerative diseases [186; 226]. Conceptually, the cognitive reserve is a function of (i) neuronal reserve and (ii) neuronal compensation. The neuronal reserve reflects the functional structure of the brain as a result of life-long learning while neuronal compensation is determined by the capacity of the nervous tissue and the brain as an organ to defend itself, to limit the damage and to regenerate. Neuroglia largely defines neuronal compensation, and therefore ageing of neuroglia is fundamental for cognitive status of the aged brain. Furthermore, age-dependent decline in glial function may be the decisive factor in determining the resistance of the nervous tissue to neurodegeneration: failure of neuroglia facilitates the transition from physiological to pathological ageing [202].

Neuroglia defines cognitive reserve of the ageing brain

Neuroglia of the CNS are represented by three major cell classes (i) the homoeostatic astroglia; (ii) the myelinating oligodendroglia and their precursors also known as NG-2 glia and (iii) the microglia [87; 201]. Neuroglial cells contribute to cognition though multiple mechanisms. The oligodendrocytes are the central cellular element of the brain connectome though myelination of white matter tracts and grey matter axons. Myelination in the human brain spans through adulthood, probably into 4th or even 5th decade of life [9; 213]; the myelination of the grey matter axons can be operational even longer. The brain is populated by NG-2 glia, which arguably maintain myelination (through differentiation into oligodendrocytes) in advanced ages [26]. Microglial cells not only provide an innate immune defence for the CNS but also modulate synaptic transmission and eliminate redundant or non-functional synapses thus assisting in tailoring neuronal ensembles [86]. Finally, astrocytes are responsible for homoeostatic control of CNS on all levels of organisation [204] as well as for defence of nervous tissue through evolutionary conserved programme or reactive astrogliosis [143]. Astrocytes are indispensable elements of synaptic connectivity: astrocytic processes predominantly enwrap dendritic spines, in less degree presynaptic boutons and in the least degree dendritic shafts [55]. Astrocytic processes are classified into organelle-containing branches and branchlets, terminal organelle-free leaflets and blood-vessel-contacting endfeet [55]. Astroglial perisynaptic processes are predominantly leaflets [55] that cover ~60% of synapses in the brain and form astroglial synaptic cradle, which contributes to synaptogenesis, synaptic maturation, synaptic maintenance, and synaptic extinction [6; 203].

Ageing affects all types of glia. The white matter suffers the most even in the physiological ageing: reduction in white matter volume in old age reaches up to ~11%, while grey matter shrinks by only 3% [65]. This paralleled with decrease of numbers or atrophy of oligodendrocytes and loss of NG2 glia associated with the decline in its differentiating capacity [8; 50; 149; 161; 199]. Similarly, microglial cells undergo substantial changes in ageing which essentially decrease their protective potential. In humans, ageing is associated with dystrophy and degeneration of microglia; aged microglial cells have shorter and less branched processes, which often demonstrate fragmentation; these dystrophic microglia have reduced activation capacity [38; 187; 189; 190]. Arguably this deterioration of microglia lessens inflammatory capabilities of the old brain, reduces neuroprotection and facilitates neurodegeneration [188].

Astroglial function also diminishes with ageing as shall be detailed below. All in all, age-dependent decrease in neuroglial support impacts on cognitive reserve thus defining the speed of cognitive decline.

Transcriptome of ageing astrocytes

Recent decade has witnessed a surge in transcriptomic analysis of the brain; these started with microarray-based technologies, which were succeeded with RNA-sequencing and single-cell RNA sequencing approaches. Studies of astroglial transcriptomic profiles in both healthy and diseased brain tissues confirmed their remarkable regional heterogeneity and regional specialisation [74; 80; 138; 224]. Analysis of gene expression in ageing revealed that changes in glial transcriptome are much more substantial that in neurones, once more highlighting the fundamental role played by neuroglia in the process of brain ageing. Transcriptome profiling of post-mortem tissue obtained from 16 – 102 years old humans found prominent and complex changes in gene expression in oligodendrocytes and astrocytes; whereas neuronal patterns remained unchanged [184]. Aged mouse cortical astrocytes showed an increase in genes linked to an immune response with a decrease in expression of glial fibrillary acidic protein (GFAP) and genes related to neuroprotection and neuronal support [139]. Another study of the mouse brain (motor and visual cortex, hypothalamus and cerebellum) revealed age-dependent up-regulation of astroglial genes responsible for synapse elimination; these changes were the most prominent in hippocampus and cerebellum [16]. The singe-cell RNA sequencing of ~50000 single-cell transcriptomes from young (3-4 months) and old (21-23 months) mice demonstrated substantial regional heterogeneity of age-dependent changes indicating that ageing of neurones and glia may develop through distinct molecular pathways [223]. The transcriptomic data are now scrutinised by proteomic studies: the large-scale proteomic analysis of more than 2000 healthy and diseased human brain samples found that the group of proteins associated with neuroglia are the most affected in Alzheimer's disease (AD). Many of these proteins are associated with anti-inflammatory and neuroprotective cascades [81]. Our knowledge of genetic changes and variations with ageing is *in statu nascendi*; of course, linking transcriptomics and proteomics data to functional outcomes and physiological processes is a daunting task and yet information accumulated so far points towards substantial adaptive changes in astrocytic gene expression in the process of brain ageing.

Ageing astrocytes are not *bona fide* reactive

Pathophysiology of astrocytes: from degeneration and atrophy to reactive astrogliosis

Pathological changes in astroglia can generally be classified into several distinct groups (Fig. 1). These groups are: (i) Reactive astrogliosis; (ii) Astroglial atrophy with loss of function; (iii) Pathological remodelling and (iv) Astroglial degeneration and death.

Reactive astrogliosis is one of the most researched and universally accepted classes of astroglial pathology. The concept of astrogliosis as one of the most frequent response of astrocyte to brain lesions has been developed in 1920s [40; 146]. Reactive astrogliosis is the evolutionary conserved response of astroglia to pathological stimuli (of whatever nature, from acute trauma and infection to chronic neurodegeneration or

epilepsy) in which astrocytes undergo activation of molecular programs that instigate biochemical, morphological and physiological remodelling of these cells with an ultimate goal of protection of the nervous tissue [143; 181; 182; 210]. Reactive astrogliosis is, by definition, secondary to the lesion; initiation of reactive reprogramming by pathological insults results in emergence of multiple disease-specific reactive phenotypes. Recently proposed ideas of binary division of reactive astrocytes into "neuroprotective" or "neurotoxic" (so called A1/A2 dichotomy), originated from over-interpretation of limited data sets, have been misleading and detrimental to the field; numerous investigations had demonstrated remarkable diversity of reactive astrocytic phenotypes [2; 61; 69; 218]. Contrary to common neurological believes of deleterious reactive astrocytes, astrogliosis is fundamentally neuroprotective, and direct astroglial toxicity (i.e. secretion of neurotoxic agents) has not been unequivocally demonstrated hitherto. The detrimental effects of chronic astrogliosis [143; 144] are most likely associated with loss of astroglial homoeostatic and protective function. In summary, the reactive astrogliosis is graded, context-dependent and reversible.

Astroglial atrophy as general pathological entity begun to receive attention in recent decade; morphological atrophy of astrocytes often associated with loss of homoeostatic functions contribute to numerous diseases, including neuropsychiatric diseases, addictive disorders, epilepsy, acute neurodegeneration such as Wernicke-Corsakoff syndrome and neurodegenerative diseases, including Alzheimer disease [51; 180; 206]. Astroglial atrophy may be primary, when it drives pathology, and secondary, when atrophic changes arise from injury or astrodegeneration; sometimes shrinkage of astroglial peripheral processes may even accompany astrogliosis [151]. Some astroglial sub-populations show higher degree of atrophy: for example, severe neurodegeneration in human brain is associated with almost complete loss of processes of interlaminar astrocytes [32]. Astroglial morphological atrophy underlies reduced synaptic coverage and diminished synaptic support, which in turn may contribute to the cognitive decline [205]. Since astrocytic Ca^{2+} activity strongly depends on astrocyte morphology, Ca^{2+} signalling is reduced in these cells [151; 221]. Because astrocytic Ca^{2+} activity is linked to number of physiological properties of this cells including synaptic coverage [196], such reduction in Ca^{2+} signalling may further exacerbate astrocytic atrophy.

Astrodegeneration is the most extreme form of astrogliopatholgy; it may appear in a form of clasmatodendrosis or astroglial death, apoptotic or necrotic. Clasmatodendrosis (from Greek (from Greek "κλάσμα", fragment, "δένδρον", tree, "ωσις", process) represents a specific form of astrodegeneration. Initially characterised by Alzheimer and Cajal (see [146]) it was for a long time neglected, although several recent studies described clasmatodendrosis in ischaemia, infectious encephalopathies, stroke, psychiatric diseases and ageing [71; 172; 195]. Clasmatodendrosis is characterised by fragmentation of astroglial processes, disappearance of distal fine processes, together with swelling and vacuolation of the cell body. Finally lesions to the brain can cause astroglial death by necrosis or apoptosis.

Morphology of astrocytes in the old brain

Studies of GFAP expression in the old brain almost universally revealed an increase in its global expression at both mRNA and protein levels (Table 1), while other archetypal astroglial markers such as protein S100B or glutamine synthetase (GS) remain unchanged. It is generally agreed that physiological ageing is not associated with any substantial decrease in astroglial cell numbers across CNS regions, although the data are not uniform (Table 2). Similarly, the data on the morphological appearance of astrocytes in the old brains are contradictory: both increase and decrease in size and complexity of astrocytes have been reported (Table 2). It has to be specifically noted that absolute majority of morphological studies have been performed on astrocytes immunostained for GFAP. The GFAP as a morphological marker is not ideal; it is labelling the cytoskeleton and completely misses peripheral processes that account for the largest part of astrocyte surface [157]. Moreover, an increase in GFAP-positive profile does not necessarily reports the true astrocyte hypertrophy; the territorial domains of reactive astrocytes remain largely unchanged [219]. Furthermore, an increase in GFAP expression and a cytoskeletal hypertrophy, although being widely recognised as a marker of astrogliosis, is not always linked to astroglial reactivity. For instance, expression of GFAP undergoes circadian fluctuations in the supraoptical nerve [11; 75]. Moreover, GFAP and astroglial morphology respond to environmental stress: both physical exercise and exposure to enriched environment is known to substantially increase GFAP-positive astroglial profiles in various regions of the brain [43; 164; 175]. Another important parameter that has to be carefully considered is the choice of age groups. Maximal increase in GFAP expression, size and complexity of astrocytes occurs in postnatal development, during the first year of life of rodents ([14; 90], see also Fig. 2). A substantial increase in GFAP expression as well as in size and complexity of astroglial profiles has been observed during first year of life of African giant rats [136]; similar remarkable increase in astrocytic size and complexity characterised the transition from juvenile to adolescent monkeys [158]. In consequence, comparing old and young animals may reveal a dramatic increase in size of old astrocytes; whereas comparing the same old astrocytes with cells from adult animals may not reveal a major difference.

There are scarcely a handful of studies analysing astrocytes labelled with markers other than GFAP. The Golgi staining of old astrocytes (which in skilful hands produce detailed morphological images) did not reveal any age-dependent changes [28]. Probing of astrocytes from mice of different ages (3, 9, 18, 24 months old) with antibodies against GFAP, glutamate synthetase and protein S100B showed rather complex and region-dependent changes (Fig. 3 and [166]). The GFAP-positive profiles were increased in CA1 region and in dentate gyrus of old hippocampus but substantially decreased in the entorhinal cortex (EC); GS-positive astrocytes were smaller in old hippocampus but larger in old EC, and finally S100B-positive profiles from old animals demonstrated an increase in EC, much smaller increase in DG and no changes in CA1. In summary, it seems that different astroglial sub-population may undergo distinct changes in ageing.

Finally, the morphology of astrocytes in brain slices from mice of different ages (3, 9 and 24 months) was visualised using intercellular perfusion with low molecular weight fluorescent probe Alexa Fluor 594 [152]. This fluorescent probe diffuses through the cytosol and penetrates in the most distant parts of the cell labelling even tiny perisynaptic processes. Two-photon imaging with subsequent 3D reconstruction of Alexa Fluor 594 labelled astrocytes revealed substantial increase in the size and

complexity of astrocytes in development from youth to adulthood; and significant decrease in size and complexity of astrocytes in old animals (Fig. 2). Furthermore, using Alexa Fluor 594 fluorescence in combination with two-photon microscopy, we were able to characterise the status of peripheral and perisynaptic astroglial processes. To this end we measure the volume fraction of peripheral processes as the fluorescence ratio of unresolved processes area to the astrocyte soma [151; 153]. This approach presumes that fluorescence measured from the soma reflects 100% of astrocyte space occupancy, whereas the fluorescence of unresolved area is proportional to the volume fraction of optically irresolvable astrocyte processes in any given area [114]. It turned out that the volume fraction occupied by peripheral processes diminishes with age, thus indicating decreased astroglial synaptic coverage in the old brain [152]. Astroglial peripheral processes are one of the main contributors to the neuropil [194], and their shrinkage is associated with an increase in diffusion channels and hence to an increase in mean diffusivity of the grey matter (Fig. 4), which is observed in elderly humans with diffusion tensor imaging [173].

To summarise, ageing is accompanied with changes in gene expression and structure of astrocytes; these changes are region-specific and multifaceted. Increase in expression of GFAP and increase in astroglial cytoskeleton appears in some parts of the brain, notably in hippocampus. These changes, however, do not signal increase in astrocytic reactivity but may reflect long-term adaptive changes. At the same time, the complexity and full expend of astroglial arborisation seem to decrease in old brains, which also reduces the glial contribution to neuropil underlying an increase in the diffusivity of the grey matter. These structural changes coincide with functional decline of astroglia discussed in the ensuing chapters.

Ageing astrocytes: Physiology

Astroglial physiology is defined by highly hyperpolarized (-80 to -85 mV) resting membrane potential, by inability to generate action potentials, by high activity of plasmalemmal transporters underlying astroglial homoeostatic responses and by intracellular ionic excitability [204]. Astroglial membrane potential lies close to the equilibrium potential for K^+ ions; astroglial membrane behaves as an almost ideal K^+ electrode because of high density of several types of K^+ channels (inward and delayed rectifying, voltage-dependent and voltage independent [135; 178; 208]), which ensure the stability of membrane potential even upon substantial depolarising stimuli. Gap junction connectivity of astrocytes also contributes to the isopotentiality of astroglial syncytia [105] and astrocyte input resistance [1; 153]. Steep transmembrane voltage and ionic gradients provide an electro-chemical driving force for homoeostatic transporters localised in astroglial membrane; most of these transporters are controlled by transmembrane gradient for Na^+ [88; 207] and can be affected by K^+ mediated membrane depolarisation [99]. Astroglial Na^+/K^+ pump is of fundamental importance for maintaining both transmembrane ionic gradients and hyperpolarised membrane potential. Stimulation of astrocytes with mechanical or neurochemical means trigger complex changes in the intracellular ion concentrations; these ionic signals represent the substrate for glial excitability [204]. Astroglial Ca^{2+} and Na^+ signalling is well characterised [168; 179] with astroglial Na^+/Ca^{2+} exchangers linking both signalling systems together [169]. Signalling potential for other ions, including K^+ and Cl^-, in astrocytes are now being considered [21; 209].

The data on age-dependent changes in physiological properties of astrocytes are quite limited (Table 3). Neither resting membrane potential nor membrane input resistance of astrocytes in cortical astrocytes was affected by ageing (mice aged between 1 and 21 months) but input resistance somewhat increased in aged hippocampal astroglial cells (from 9-12 and 20 - 24 month) [97; 152]. This increase in input resistance may reflect both age-dependent astrocyte shrinkage and uncoupling through the gap-junctions. Astrocytes in aged brains of humans and rodents express functional receptors to neurotransmitters and generate Ca^{2+} signals in response to appropriate stimulation [59; 97; 127]. The density of AMPA, NMDA and P2X receptors as well as the density of plasmalemmal glutamate transporter currents demonstrates bell-shaped age dependency (Fig. 5). There are some indications about aberrant Ca^{2+} signalling in aged astrocytes. In 20 months old mice, the spontaneous Ca^{2+} oscillations have been found to occur about 20 times more frequent than in young 2.5 months old controls [110]. There are also some indications of age-dependent decrease in ATP-induced astroglial Ca^{2+} responses [96]. Old astrocytes seem to weaken their syncytial organisation: substantial decrease in astroglial coupling was reported for both cortex and hippocampus of 20 – 27-months old mice [150]. All in all, and even despite the scarcity of experimental data, we may conclude that physiological ageing does not substantially affect fundamental physiological mechanisms in astrocytes.

Astroglial senescence: does it exist in the ageing brain?

The cellular senescence which is defined as irreversible growth arrest accompanied with some specific changes in gene expression, in cellular phenotypes and in molecular markers. The senescence of various cell types has been demonstrated to increase in ageing, while manipulation with cellular senescence can accelerate or retard (at least some of) age-dependent processes [7; 77; 125]. Historically the concept of cellular senescence goes all the way back to 1881 when August Weismann suggested that "*death takes place because a worn-out tissue cannot forever renew itself, and because a capacity for increase by means of cell division is not ever-lasting but finite*" [216]. The restricted cell division capacity, which ceases, in culture, after 40 – 60 divisions was experimentally proven by Leonard Heyflick [68] and "Hayflick limit" become a thoroughly popular term (this term was coined by Macfarlane Burnett [24]). At a functional level, the cellular senescence is linked to the "senescence-associated secretory phenotype" reflecting increased release of various proinflammatory factors, including growth factors, chemokines, cytokines and proteases [33; 132]. This phenomenon, in turn, complements the concept of "inflammageing" that regards ageing as a chronic immunopathology [53].

Most (if not all) of the evidence favouring astroglial senescence (recently overviewed by [31]) has been obtained *in vitro*, in malignant glial cells, in cell lines and in primary astrocytes. Primary cultured astrocytes *in vitro* demonstrated replicative senescence and expressed classical markers of cell senescence including β- galactosidase activity expression of p21 expression, and p53-dependent growth arrest [49]. Cultured astrocytes from post-mortem sample of AD patients showed an increase in senescence markers p16 and p21 as well as replication arrest [15]; similarly, senescent astroglial phenotype was described in cells subjected to oxidative stress [13; 185]. Co-culturing senescent astrocytes with neurones revealed reduced astroglial homoeostatic support and neuroprotection [84; 148]. Can this *in vitro* evidence be translated to the *in vivo* brain and physiological ageing? This remains an

unanswered question. Astrocytes are profoundly postmitotic cells, with very low rates of proliferation: astroglial proliferation has been documented for many brain regions albeit the percentage of proliferating astrocytes varies between 0.090% - 0.30% in the cerebral cortex and 0.445% in the corpus callosum [34; 56; 57]. To summarise: the role of astroglial senescence in the ageing brain and its contribution to cognitive decline remains to be studied and contemplated before the final verdict can be made.

Ageing astrocytes: Loss of functions

Although basic physiology of astrocytes in the old brain does not seem to be substantially affected, there is a wealth of evidence for decline of certain vital astroglial functions with ageing (Fig. 6). This decrease in astroglial support may impact on neuronal performance, on synaptic transmission and neuroplasticity, on neuroprotection and supply of neurones with energy substrates. Thus, age-dependent asthenia of astrocytes contributes to cognitive decline of the senescent brain.

Neurotransmitter homeostasis

(i) Glutamate. Astrocytes are central elements for glutamate turnover in the brain. Neurones, which (in contrast to astrocytes) are unable to synthesise glutamate *de novo* from glucose, rely on astrocytes for continuous supply of glutamine [70; 176], which, after being transported to neurones through the complementary system of export and import glutamine transporters of SLC38A1-5 family, is converted into glutamate and subsequently into GABA to be used in neurotransmission. Astrocytes are also central for glutamate removal from the synaptic cleft through Na^+-dependent excitatory amino acid transporters 1 and 2 (EAAT1/SLC1A6 and EAAT2/SLC1A2) which clear ~ 80% of all glutamate released in the course of neurotransmission [35]. After being accumulated into astrocytes glutamate is converted to glutamine by glutamine synthetase [131; 167] and glutamine is again transported to neurones. Thus astrocytes accomplish glutamate turnover through the operation of the glutamate (GABA)-glutamine shuttle.

Ageing is generally accompanied with an increase of the ratio of glutamate to glutamine in the brain indicating possible abnormalities in the operation of the glutamate (GABA)-glutamine shuttle [45; 64]. Expression of astrocytic glutamate transporters as well as the efficacy of glutamate uptake is reduced in old (24 – 27 months old) rats when compared to young adults (3 - 5 months old) [154]. In 18 - 21 months old mice the glutamate transporter current density was only ~10-15% of that in 6 months old animals; although there was no difference between old and very young (1 month old) mice (Fig. 5, [97]). The levels of glutamate synthetase was somewhat (not significantly) lower in the hippocampal astrocytes of 18 months old mice [134]. As alluded to before, aged astrocytes have diminished peripheral and perisynaptic processes; this decreased synaptic coverage may also contribute to the reduced efficacy of astroglial glutamate uptake, increased spillover with subsequent impairment of synaptic plasticity [152].

(ii) Noradrenaline. Astroglial cells are central for catabolism of noradrenaline and other catecholamines because the principal enzyme monoaminoxidase-B (MAO-B) is mainly present in protoplasmic and radial astrocytes [103; 217]. The level of MAO-B in aged brain is increased two to three fold [95], which may have several damaging

consequences. First, increase in MAO-B decreases the levels of noradrenaline; the noradrenergic excitation is provided by neurones from locus coeruleus, which is particularly vulnerable to age-dependent neurodegeneration. Increase in MAO-B therefore may exacerbate potential harmful effects of reduced noradrenergic innervation and hence potentially aggravate neurodegeneration [226]. Second, the major by-product of MAO-B is hydrogen peroxide, which gives rise to highly toxic hydroxyl radicals thus exacerbating the oxidative stress and damaging neurones [191].

(iii) γ-aminobutyric acid (GABA). Another consequence of elevated MAO-B content in aged astrocytes is linked to synthesis of main inhibitory neurotransmitter GABA. Healthy astrocytes do not have GABA synthesising enzymes and even when GABA is accumulated through dedicated transporters it is rapidly consumed by Krebs cycle [177]. Astrocytic GABA is increased in aged astrocytes and especially in astrocytes from AD patients and AD animal models [19; 100; 222], Thus may be linked to increase in MAO-B catalysed GABA production from putrescine. Astroglial GABA can be released through reversed GABA transporters or by diffusion through Bestrophine-1 Cl^- channels thus contributing to neurotransmitter imbalance and negatively impacting on cognition [54; 79], although the latter mechanism is debatable and is frequently questioned [137].

Neurogliovascular unit and the blood-brain barrier

Protoplasmic astrocytes parcellate the nervous tissue into spatially segregated territorial domains [25]. Within these domains astrocytes integrate neurones, synapses, microglial cells and neighbouring capillaries into the neurogliovascular (or neurovascular) unit [72]. Astroglial endfeet form perivascular glia limitans that provide for parenchymal coverage of blood vessels in the brain. At the level of intra-parenchymal arterioles and capillaries astrocytes contribute to the regulation of local blood flow through the release of vasoconstrictors and vasodilatators [52; 124; 225]. In particular, vasoactive agents are related to the activity of Ca^{2+}-sensitive phospholipase A2 that produces arachidonic acid; the latter is converted to prostaglandins and to epoxyeicosatrienoic acids. The exact mechanisms translating astrocytic Ca^{2+} signals into release of eicosanoids and regulation of functional hyperaemia remain debatable; in particular the role of fast local Ca^{2+} signals not associated with ER Ca^{2+} release has been proposed [140]. Astrocytes function as intracranial baroreceptors and control of arterial blood pressure, hence, brain blood flow [109]. Ageing reduces brain stiffness in old human in sex and region-specific manner [5] and affects viscoelastic properties of the human brain [171] The astrocytes atrophy may be also considered in this context as changes in brain viscoelastisity may affect astrocyte ability to monitor cerebral perfusion and control systemic circulation. This phenomenon can be linked to age-related hypertonia and consequently to heart diseases and stroke.

The blood-brain barrier (BBB) separates the circulation from the brain microenvironment and is formed by the continuous layer of specialized brain capillary endothelial cells. These cells are functionally coupled with other vascular (pericytes, vascular smooth muscle cells), and neural (neurones, astrocytes, microglia) cells constituting the neurogliovascular unit [193]. At the capillary level, endothelial cells, pericytes and astroglial endfeet share a common basement membrane. At the level of arterioles, two basement membranes, the parenchymal (in contact with astroglial

endfeet) and vascular (in contact with endothelial cells) create the perivascular space used by glymphatic clearance pathway to eliminate waste products from brain parenchyma [214]. Astrocytes act in concert with other cells to support formation and integrity of the BBB and to ensure proper functioning of neurovascular and neurometabolic coupling mechanisms [193]. Energy requirements are not uniform throughout the brain and depend on the local neuronal activity. Neurovascular coupling ensure a rapid and localised on-demand increase of cerebral blood flow sufficient delivery of oxygen and glucose and simultaneous removal of metabolites such as lactic acid and carbon dioxide; the phenomenon generally known as functional hyperaemia [89; 122; 170]. At the level of arteries and arteriolae, local blood flow is mainly regulated by the nitric oxide produced by neurones, whereas at the level of capillaries vasoconstriction and vasodilatation are regulated by both neuronal and astroglial mechanisms. Neurovascular coupling is tightly coordinated with dynamic metabolic changes regulating energy consumption and utilisation of metabolites, a mechanism known as neurometabolic coupling [215]. It is becoming increasingly clear that mechanisms of neurovascular and neurometabolic coupling are tightly coordinated with mechanisms regulating BBB permeability to nutrients and toxins.

Brain ageing is associated with restructuring of blood vessels and decrease in blood supply [4]. This translates in declining oxygen supply and causes imbalance in neural cells metabolism [76]. There are some indications that ageing affects glial secretion of arachidonic acid and eicosanoids, which may affect local neurovascular coupling [85; 197]. Another age-dependent mechanism may be associated with deficits in circulating levels of Insulin-like growth factor 1 (IGF-1), which promotes multiple cerebromicrovascular alterations [183]. Decrease in IGF-1 levels affect astrocytic eicosanoid production and hence may impair upon astroglial control of local functional hyperaemia [198].

Physiological brain ageing is accompanied with decay in BBB and disruption of neurovascular and neurometabolic coupling [46; 118; 126], into which astroglial dysfunction most likely plays a major role. Ultrastructural studies of the BBB in aged rats showed increased thickness of capillary walls and basement membranes; furthermore, in the old animal the area of the astrocyte endfeet surrounding the capillaries was significantly higher than in the young animal [17]. There are also some indications that astrocytes loos their ability to regulate tight junctions expression in the context of neurodegeneration [94]; whether a similar loss of function occurs in physiological ageing remains to be investigated. In conclusion, brain ageing is accompanied by progressive decline in the BBB deterioration and accumulating evidence indicate that astroglial dysfunction plays complex and multifaceted role in this process. Future studies specifically focusing on the perivascular astroglial subpopulations may significantly improve our understanding about the role of astroglia during ageing-related BBB pathologies.

Neurogenesis

Neural stem cells which dwell and operate in the adult neurogenic niches of the subventricular zone and hippocampus are, in essence, radial astrocytes (also known as radial glia-like neural stem cells). These cells combine features of stem cells and homoeostatic astrocytes: they, for example, send processes to form perivascular

endfeet and at the same time their peripheral processes provide for perisynaptic coverage [117; 121]. Ageing is associated with a significant decline in neurogenic capacity in all neurogenic zones due to a cessation of asymmetric division of radial stem astrocytes [12; 18; 129]. This neurogenic silencing may be associated with accumulation of damaged proteins [211]. Of note, pathological ageing and specifically Alzheimer disease, is associated with severe suppression of radial stem cell functions and in early decrease in neurogenesis [162; 165].

Control of proliferation of stem radial astrocytes also decreases with ageing and astrocytes contribute to this process. Astrocytes regulate, at least in part, asymmetric division of stem cells through Wnt-mediated survivin signalling and this regulation is compromised by ageing, leading to a decreased proliferative capacity [116]. Furthermore astroglial secretion of Wnt3 is reduced with ageing, disrupting the regulation of genes such as NeuroD1 and doublecortin [133]. These findings suggest that preservation of astrocytic functions play essential roles to protect hippocampal function during ageing process.

Age-dependent decline of the glymphatic system

Astrocytes are central elements of the brain specific glymphatic system responsible for life-long waste collection [73; 128]. Astroglial endfeet form the perivascular space and aquaporin 4 (AQP4) water channels localised in these endfeet mediate water transport into the brain parenchyma. Polarised expression of astroglial AQP4 which are concentrated in the endfeet is a key for normal operation of the glymphatic flow. In ageing, this polarisation is impaired and AQP4 channels migrate away from the endfeet which is associated with almost 40% decline in operational capacity of the glymphatic system [93]. Of note, even more severe decline is accompanying neurodegeneration and Alzheimer's disease in particular [147].

Ageing impairs astroglial metabolic support

Ageing increases astroglial in oxidative metabolism, which may limit their ability to supply neurones with metabolic substrates [78]. Furthermore, there are indications that ageing reduces astroglial ability to produce lactate and hence to operate lactate shuttle [63].

Ageing astrocytes and mitochondria

Decline in mitochondrial function in ageing is well documented [142; 159], and the mitochondrial, as well as closely associated ROS, theories of ageing is generally accepted [192]. Mitochondrial ageing is associated with an increase in mutations of mtRNA and overall decline in mitochondrial energy production capacity. Mitochondrial failure obviously has grave repercussions for energy-demanding neural tissue. Data emerging in recent years indicate that astrocytes contribute to disposal of neuronal mitochondria. The latter are translocated into astroglial cells where they are disposed off through mitophagy. This transcellular mitochondrial degradation has been named "transmitophagy" [39]. Not only astrocytes degrade neuronal damaged mitochondria, but they supply neurones with functional mitochondria which assist energy production in stressed neurones for example in conditions of ischaemic insult

[66]. Age-dependent astroglial dystrophy and decline in astroglial homoeostatic capabilities may supposedly, affect this pathway thus diminishing neuroprotection.

Cholesterol synthesis

The brain relies entirely on cholesterol synthesised *in situ*, because blood-brain barrier does not permit cholesterol transport [42]. Astrocytes are the source of cholesterol needed for synaptogenesis and morphological plasticity of neurones and astroglial synthesis of cholesterol seems to be the limiting factor regulating synapse formation [113]. In ageing the main cholesterol synthesising enzyme HMG-CoA reductase or Hmgcr, is down-regulated; at the same time the level of the receptors for cholesterol transport in astrocytes is increasing [16], indicating disrupted cholesterol homoeostasis that may impair synaptic plasticity [141].

Age-dependent decline of astroglial defensive capabilities

Astrocytes are vital elements of the brain defensive system through both homoeostatic support and astroglial reactivity [143]. In particular, astroglial cells are fundamental for the brain antioxidant system utilising glutathione and ascorbic acid [107]. Glutathione synthesis in neurones requires cysteine or glutamylcysteine as obligatory precursors; both of which are shuttled from astrocytes. Astrocytes (in contrast to neurones) specifically express the Sxc^- glutamate/cystine exchanger [22] and thus can accumulate cystine; this latter is reduced to cysteine or converted into glutamylcysteine which can be utilised for gluthatione synthesis. Astrocytes secrete cysteine and gluthatione which is, when outside of the cell, converted into glutamylcysteine. Cysteine and glutamylcysteine are taken up by neurones; with cysteine in particular being transported by EAAT2/3 transporters [30]. Thus, without astrocytes neuronal glutathione production suffers because of the luck of substrate. Removal of astrocytes from neuronal-astroglial co-cultures substantially reduces neuronal gluthatione production, which facilitates neuronal oxidative damage. It was estimated that in co-culture an individual astrocyte was able to provide antioxidative protection to ~ 20 neurones [41]. Ageing is associated with decrease in the brain levels of glutathione most likely because of decreased production in astrocytes [48; 106]. 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 and oxidative damage [101].

Reactive astrogliosis, as has been alluded above, is a specific defensive programme of astroglia, while deficits in reactive astroglial response are known to exacerbate neuropathology [143]. It has been suggested that age-dependent decrease in reactive capabilities of astrocytes and microglia, the glial paralysis, facilitates the development of neurodegenerative diseases and Alzheimer disease in particular [202]. Early stages of Alzheimer's disease, associated with mild cognitive impairment, are characterised with an increased astrogliosis [27; 160]. Conversely, advanced stages manifested by dementia are associated with decrease in astroglial reactivity: in general, demented brains have less reactive astrocytes [111; 160]. In depth analysis of nervous tissue of patients with Alzheimer disease pathology associated with cognitive preservation or with dementias revealed, in the latter group, idiosyncratic reactive astrocytes with thick and long processes and with an increased level of expression of glutamate

transporter EAAT2 [91]. Ageing also disrupts reactive astrogliotic response to the traumatic brain injury; furthermore, aged astrocytes are much more susceptible to trauma induced rapid degeneration ultimately ending in clasmatodendrosis [47]. Finally, aged astrocytes whether in homoeostatic or in reactive state, fail to support remyelination further exacerbating the course of pathology in old age [156].

To summarise, ageing significantly decreases defensive and neuroprotective capacity of astrocytes thus increasing brain vulnerability to acute lesions and chronic pathologies including neurodegeneration.

Lifestyle changes such as environmental stimulation and dieting promote astrocytes complexity and prolong cognitive ageing

Environmental stimulation, combined with physical activity, social interactions, and richness of visual and sensory stimuli, represents the most powerful non-invasive and non-pharmacological approach to mitigate neurological conditions associated with ageing. Keeping people physically, mentally and socially fit prolongs cognitive longevity, while the brain subjecting to enriched environment undergoes a series of molecular, cellular, and structural changes conferring a greater capacity to withstand cognitive decline imposed by the normal ageing and age-associated diseases [36; 102; 119; 120]. Furthermore, polytherapies, which include physical exercise and intellectual engagement, have demonstrated positive results in restoring cognitive abilities and ameliorate psychiatric symptoms in patients with Alzheimer's disease associated cognitive deficits [20; 115].

Experimental data accumulated over recent decade demonstrate that environmental enrichment, physical activity or their combinations increase astroglial cells size, complexity, and expression of GFAP at the same time improving cognitive processes in animals (Table 4) thus suggesting astrocytic plasticity as a potential target and executor of environmental stimulation [43; 175]. Exposure to environmental enrichment and physical activity demonstrate a complex effect of astrocyte morphology in hippocampus: it boosts cell complexity by increasing length and number of primary branches and ramifications, which developed in parallel with an increase in the number of synaptic contacts [43; 212]. In aged 3xTg-AD mice model, environmental stimulus led to a significant increase of astroglial complexity, completely reversing morphological atrophy found early stages of AD and potentially related to synapse deficiency and cognitive abnormalities [164]. In the same study, environmental enrichment increased the size and complexity of astrocytes from healthy control mice. In another AD model, in the PDAPP-J20 transgenic mice, enriched environment from 5 to 8 months old increased the complexity of atrophic astrocytes located away from senile plaques and decreased the complexity (i.e. decreased reactivity) of plaque-associated astroglial cells [10]. Similarly, enriched environment increased astroglial complexity and improved cognitive performance even in advanced ages, when compared to animals housed in standard conditions [175]. Finally, enriched environment and physical exercise significantly improve the proliferative capacity of radial stem astrocytes thus rescuing neurogenesis in both normal ageing and in neurodegeneration [163].

Another important lifestyle factor impacting upon cognitive longevity and affecting morphological and functional properties of astroglia is associated with food intake

and dieting. Caloric restriction received particular attention as limiting food intake while preserving healthy nutrition is known to prolong life span of many species from worms to primates and improve health [112]. Exposure of mice to classically restricted diet boost astroglial complexity by increasing the volume of peripheral processes thus increasing synaptic coverage. This in turn increased the efficacy of astrocytic K^+ buffering and glutamate uptake and limited glutamate spillover which improved synaptic plasticity [153].

To conclude, changes in lifestyle that include healthy and moderate diet, physical activity, increased intellectual engagement and social exposure all improve cognitive longevity; these interventions also improved astroglial homoeostatic capacity that may increase neural reserve and neural compensation thus contributing to the healthy ageing.

Conclusions

The cognitive longevity is directly determined by the cognitive reserve, which originates from life-long interaction of the environment and genome. Experiences of life, intellectual engagement and physical exercise, caloric regime and healthy diet – all these factors define the brain ageing, the ability of the brain to withstand pathological attacks and its ability to counteract neurodegeneration which frequently develops at advanced age. Astrocytes, being the primary homoeostatic and defensive elements of the brain contribute to the maintenances of cognitive reserve through multiple mechanisms. Brain ageing is accompanied with progressive decrease in astroglial functional capacity; when the loss of function integrates the probability of pathological switch in the ageing process is increasing. Astroglial function can be maintained through the adoption of ageing friendly lifestyle; with "cerebral gymnastics" promoted by Ramon y Cajal [155] playing a specific role in cognitive preservation of the human brain. To conclude this essay we shall again quote the author of the "Old age" describing the high activity of old people, which, without doubt, is a key factor in keeping the brain alert and well throughout the lifespan given by nature to mankind.

"*it is satisfactory to note how many of the very aged are in good possession of their mental faculties, taking a keen interest in passing events, forming a clear judgment upon them, and full of thought for the present and future welfare of others. It is no less satisfactory to find that the active, even severe and long-continued, functional activity of the matured brain seems in no way to impair its enduring qualities, and that good, earnest, useful employment of body and mind are not only compatible with, but even conducive to, longevity.*"[1]

[1] George Murray Humphry, 1889, The Old Age. The results of information received respecting nearly nine hundred persons who had attained the age of eighty years, including seventy-four centenarians. MacMillan & Bowes, Cambridge, p. 24

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Figure legends

Figure 1. Classification of astrogliopathology (see text for further explanations).

Figure 2. Reconstructions of hippocampal protoplasmic astrocytes from young, adult and old mice (own observations).

Figure 3. Age-dependent remodelling of astroglial profiles in different brain areas.

Confocal images showing glial fibrillary acidic protein-GFAP (A to F), s100B (G to L) and glutamine synthetase-GS (M to R) immunolabelled astrocytes in the dentate gyrus and CA1 hippocampal areas as well as in the entorhinal cortex of mice at 3 and 24 months. Modified from [166].

Figure 4. Age-dependent shrinkage of astroglial peripheral processes opens diffusional channels in the neuropil thus providing a mechanistic explanation of an increased diffusivity of the grey matter observed in old humans by diffusion tensor imaging.

Figure 5. 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 μM glutamate (left column), 10 μM NMDA (middle column) and 10 μM ATP/αβmeATP (a potent and stable agonist at $P2X_1$, $P2X_3$, $P2X_{2/3}$, $P2X_{1/5}$ and $P2X_{4/6}$ receptors. It is also a weak partial agonist at human and mouse $P2X_4$ receptors, but an antagonist at the rat $P2X_4$ 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 μM D-AP5, an NMDA antagonist, and 30 μM CNQX, an AMPA receptor antagonist; ATP-evoked currents were inhibited by 10 μM 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 ± 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.

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Figure 6. Loss of function in aged astrocytes.

Tables

Table 1. Astroglial markers in aged brain

Specie/Age/brain region	Experimental techniques	Main findings	References
Humans/12 – 98 years/Hippocampus, entorhinal cortex	Immunoblot for GFAP	GFAP protein significantly increased in older (> 65 years) subjects without any signs of neurodegenerative pathology in hippocampal formation and in entorhinal cortex.	[37]
Rats (Fisher-344), human post-mortem tussie/6 and 24 months for rat, 25 – 59 and 60 – 79 years for humans/hippocampus, frontal and temporal cortex.	RNA blot hybridisation for GFAP and GS.	GFAP, but not glutamine synthetase mRNA increased with age in all ages; increase in GFAP was the same in physiological or neurodegenerative human brain samples.	[130]
Rats, female (Brown Norway)/ 5 and 25 months/ circumventricular organ, nucleus of the solitary tract	GFAP immunohistochemistry	GFAP intensity was increased in subfornical organ, area postrema and nucleus tarctus solitarii of old rats; GFAP intensity in organum vasculosum of the lamina terminalis did not change with age.	[62]
Mice (C57BL/6J)/ 6, 12 and 29 months/cerebral cortex, hippocampus, and cerebellum	RNA gel-blot, solution hybridization assay for GFAP and GS	Significant increase in GFAP mRNA and no changes for glutamine synthetase mRNA were detected in all brain areas	[60]

		analysed.	
Mice, female (C57BL/6J)/ 5, 18,23-26 months/ corpus callosum, fimbria, stria terminalis, and optic nerve,thalamus, hypothalamus	*in situ* hybridization and immunocytochemistry	The two-fold increase in GFAP protein was observed in all areas of old animals.	[92]
Senescence-accelerated-prone mice (SAMP8)/3,16 months	GFAP, GS, S100B immunohistochemistry, western blot and RT-PCR	In old mice GFAP has been increased at both mRNA and protein levels; glutamine synthetase and protein S100B did not change with age.	[220]

Abbreviations:
GFAP – glial fibrillary acidic protein
GS – glutamine synthetase

Table 2. Numbers and morphological appearance of aged astrocytes

Specie/Age/brain region	Experimental techniques	Main findings	References
Rhesus macaques/ 4 groups: juvenile (5 month – 2 years), adolescent (3 – 5 years), adult (7 – 12 years) and geriatrics (> 20 years)/ Frontal lobe	Immunocutochemistry, morphometry, neurolucida, Sholl analysis	Astroglial density does not change with age; astroglial complexity increases from juvenile to adult animals and decreases in ageing.	[158]
Rat (Sprague-Dawley and Fisher-344)/ 1 – 18 months (SD) 1 – 30 month (F-344)/ Cerebrum and cerebellum	GFAP immunohistochemistry, morphometry	Perimeter and surface area of GFAP-positive astrocytes significantly increased during attaining the adulthood; at advanced age an increase was much smaller.	[14]
Rat (Fisher-344)/ 3 and 25 months /Dentate gyrus of the hippocampus	Electron microscopy	Increase in astroglial processes profile (41% in numbers and 43% in volume fraction) were detected in old specimens; the mean number of astrocytes per square area did not change with age.	[58]
Rat (Fisher-344)/2-3 and 24-25 months/Hippocampus	Cajal gold chloride stain	Hypertrophic astrocytes were located near the areas of neurodegeneration and neuronal loss.	[98]
Rat/3 to 29.6 months/Cerebral cortex	Electron microscopy	No age-dependent morphological changes in astrocytes were observed, save an increase in	[200]

		membrane-bound inclusions.	
Mice/2 weeks, 8 weeks, 18 weeks, 40-42 weeks and 50-59 weeks/Hippocampus CA1	GFAP immunohistochemistry	Significant increase in the number of GFAP-positive cells was observed in the CA1 area of old mice.	[67]
Rats, male (Wistar)/ 3 and 22 months/hippocampus	GFAP immunohistochemistry; GFAP Western blot LPS infusion for 4 weeks intraventricularly	GFAP protein increased in aged mice by 108%; in LPS-treated by 129%. The density of GFAP-positive astrocytes was significantly decreased in old rats, they demonstrate atrophic morphology with shorter processes; some processes show signs of clasmatodendrosis. To the contrary LPS triggered hypertrophy of GFAP profiles.	[29]
Humans (18 females, 13 males)/18-93 years/Neocortex	Stereological cell count	The number of astrocytes was stable throughout the life span.	[145]
Humans (23 females) 65-105 years/Neocortex	Stereological cell count	The trend for reduction of astrocyte numbers in the oldest subjects was noted; although it did not reach statistical significance	[50]
Mice, females (C57Bl/6NNIA, B6)/3-4, 13-14, 20-24 months/hippocampus	GFAP immunolabelling, Stereological cell count	Ageing increased numbers of GFAP-positive astrocytes by 20%.	[123]
Mice/males (C57BL/6J)/ 4-5 s, 13-14, 27-28 months/hippocampus	Stereological cell count	No significant differences in astrocytes numbers at different ages were found	[104]
Rats, males (Sprague-Dawley) 12,	GFAP immunohistochemistry;	Ageing was associated with an	[3]

24 months/Frontal cortex, hippocampus		increase in number of GFAP-positive astrocytes and an increase in size of GFAP-positive profiles	
Resus monkey/ males- 9-10, 14-17; females 22-29 years/ cortex, putamen, globus pallidus, hippocampus	GFAP immunohistochemistry; unbiased stereology	Astrocytes in old animals do not show age-dependent changes.	[83]
Mice, female (albino Swiss)/ 6, 20 months/hippocampus	GFAP immunohistochemistry, behavioural tests	Number of astrocytes increased with aging in the molecular layer and in the polymorphic layer, it remained unchanged in the granular layer. Astrocytes in molecular layer show hypertrophy of GFAP-positive profiles.	[44]
Mice, males, females (C57BL/6)/3 months, 1, 2 years/Olfactory bulb	GFAP immunohistochemistry	Olfactory bulb astrocytes increase in complexity between 3 months and 1 year; while no change in astroglial morphology or numbers was detected in aged animals.	[90]
Humans/ 28 weeks of gestation – 88 years/Subsntatia nigra	Nissl staining, GFAP, s100B immunohistochemsitry	No significant change in total number of glial cells was observed in old age. Similarly no major changes in astrocyte morphology were detected.	[82]
Mice (C57BL6)/ 0.6, 6, 19, 24 months/Hippocampus	Golgi staining; caloric restriction	No significant changes in astrocytes size in old age have been detected. Somata of astrocytes in calorically restricted	[28]

		animals were smaller than in *ad libidum* fed mice.	
Mice(Swiss)/ 6, 20 months/Hippocampus	GFAP immunohistochemsitry, Behavioural tests, 3D astrocytes morphometry	Both ageing and environmental deprivation reduced complexity of astrocytes; environmental enrichment in contrast increases astroglial complexity in all ages.	[43]
Rats (Wistar)/3, 6-12, 18-25 months/Retina	GFAP, S100B immunohistochemistry, TUNEL assay	The number of astrocytes decreases in old age due to an age-dependent increase in astroglial apoptosis	[108]
Mice, males (SV129/C57BL6)/ 3, 9, 18, 24 months/Hippocampus, entorhinal cortex	GFAP, GS, S100B immunohistochemsitry	GFAP profiles in old mice showed hypertrophy in CA1 region and in DG. In contrast in EC GFAP-positive profiles show substantial decrease in size and complexity. GD-positive profiles were smaller in old hippocampus and were unchanged in EC. Finally, S100B profiles were larger in old EC, displayed moderate changes in DG and no changes in CA1.	[166]

Table 3. Physiology of aged astrocytes

Specie/Age/Brain region	Experimental techniques	Main findings	References
Mice/8-14, 48 - 80 weeks (~2.5, 20 months) old/Cerebellum, Bergmann glia	*In vivo* transcranial confocal microscopy, Oregon Green BAPTA-1 $[Ca^{2+}]_i$ recordings	Old Bergmann glial cells demonstrated much higher (almost 20 times) spontaneous $[Ca^{2+}]_i$ activity	[110]
GFAP-EGFP mice/4,10,21 months/Cortex	*In situ* recording from astrocytes in acute cortical slices, whole-cell voltage-clamp, two-photon microscopy, Oregon Green BAPTA-1 $[Ca^{2+}]_i$ recordings, Enriched environment together with physical exercise, calorie restriction (15%).	Old astrocytes demonstrated decreases P2X receptors mediated miniature EPSCs and suppressed purinergic Ca^{2+} signalling. Exposure to enriched environment or co calorie restrictive diet rescued purinergic signalling	[96]
GFAP-EGFP mice/1,3,6,9,12,18-21 months/Somato-sensory cortex	*In situ* recording from astrocytes in acute cortical slices, whole-cell voltage-clamp, fura-2 /monochromator based $[Ca^{2+}]_i$ recordings	The density of P2X, NMDA and AMPA ionotropic receptor currents, as well as the density of glutamate transporter currants showed bell-shaped age dependence. All densities peaked at 3 – 6 months, and then steeply declined and stayed unchanged in old age. Neurotransmitter-evoked astroglial Ca^{2+} signalling showed same age dependence.	[97]
Mice (C57BL/6)/05, 5, 12, 20 months/Somato-sensory cortex, hippocampus	*In situ* recording from astrocytes in acute cortical slices, whole-cell voltage-clamp, fluo-4	No major changes in astroglial basic electrophysiological parameters as well as in astroglial	[59]

	/monochromator based $[Ca^{2+}]_i$ recordings	Ca^{2+} signalling have been detected.	
Humans, males and females/28 – 59 years old/ excess tissue for temporal lobe drug-resistant epilepsy surgery	*In situ* recording from astrocytes in acute cortical slices, whole-cell voltage-clamp, fluo-4 /monochromator based $[Ca^{2+}]_i$ recordings	No major differences in electrophysiological parameters or Ca^{2+} signals were noted in aged tissues.	[127]
Rats (Sprague–Dawley)/3-6,24-27 months/Hippocampus	GLAST/GLT-1 immunoblotting; d- $[^3H]$aspartate uptake (measure of glutamate transport) *In situ* extracellular recordings from acute slices	Protein levels of both astroglial transporters GLAST and GLT-1 are significantly decreased in old age. This facilitates glutamate spillover, activation of extarsynaptic NMDA and mGluR receptors, which modulate synaptic plasticity.	[154]
Rats (Fisher F-344)/3, 24 months/Hippocampus	qPCR, immunoblotting, Morris water maze	mRNA for GLT-1 was decreased in aged rats which correlated with memory impairment; treatment with Riluzole significantly increased GLT-1 expression and improved memory.	[23]

Table 4. Enriched environment, astrocytes and cognitive performance

Specie	Brain Area	Housing Conditions	Astroglial morphology	Neurobehavioural Findings	Reference
Mice 3xTG-AD model	Hippocampus (DG)	Enriched environment exposure for 9 months from 3 to 12 months of age	Increased surface and volume of the GFAP positive astrocytes in healthy animals. Reverted astrocytic atrophic changes induced by AD-like pathology	Not tested	[164]
Mice	Hippocampus	Enriched environment exposure for 2 months	Increased Astrocyte ramification and increased number and length of primary processes	Not tested	[212]
Mice, APP AD	Hippocampus	Enriched environment exposure for 3 months from 5 to 8 months of age	Increased volume and ramification of β-amyloid plaque-associate astrocytes thus rescuing pathological changes in hippocampal astrocytes in APP transgenic mice	Not tested	[10]
Mice	Hippocampus	Enriched environment exposure for 6 and 20 months	Increased astrocyte complexity and morphological diversity	Improved contextual and item memory	[43]

Rat	Hippocampus (DG)	Enriched environment exposure for 28 days	Increased astrocyte complexity	Not tested	[174]
Rat	Hippocampus	Enriched environment exposure for 2 months	Increased astrocyte complexity	Improved spatial memory	[175]

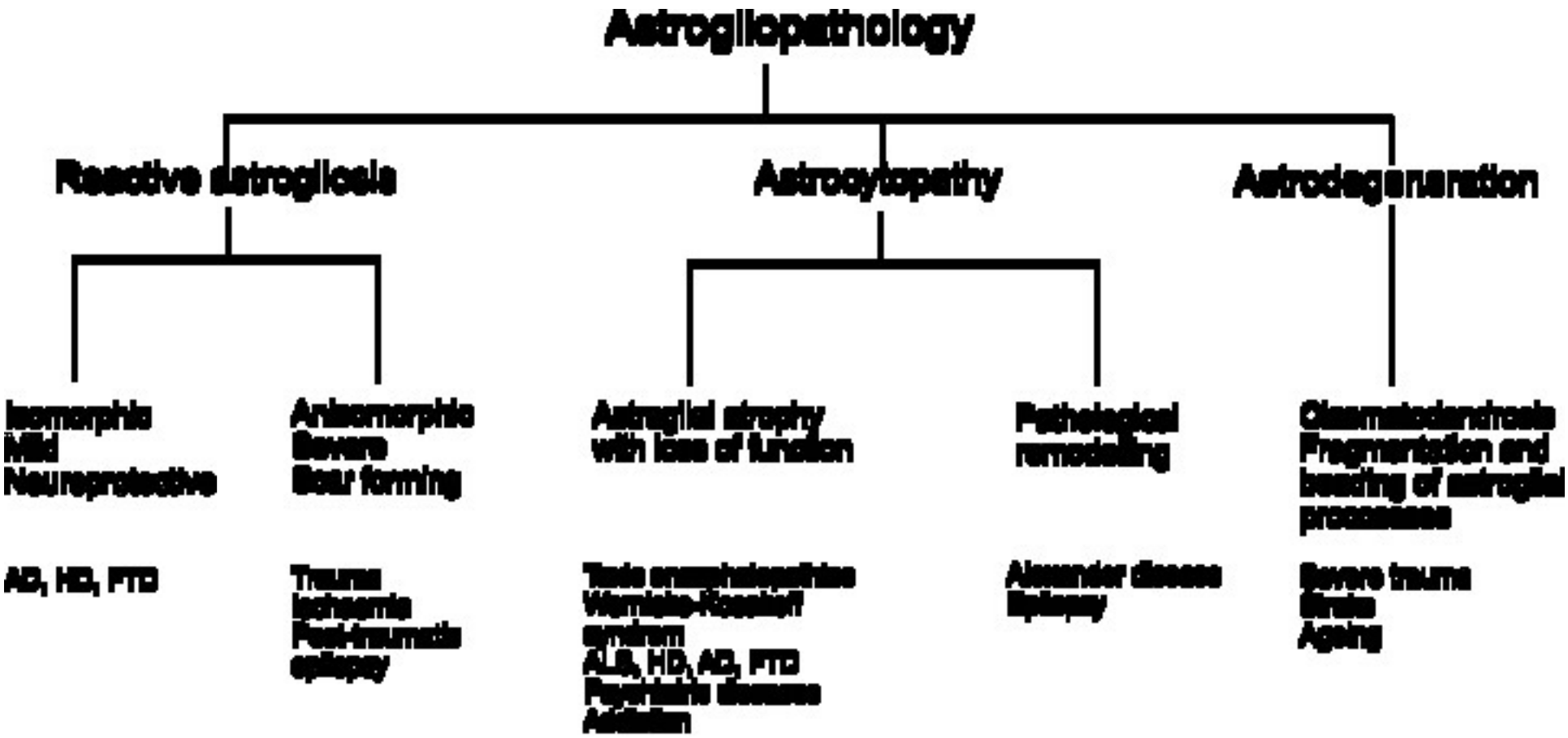


Fig. 1

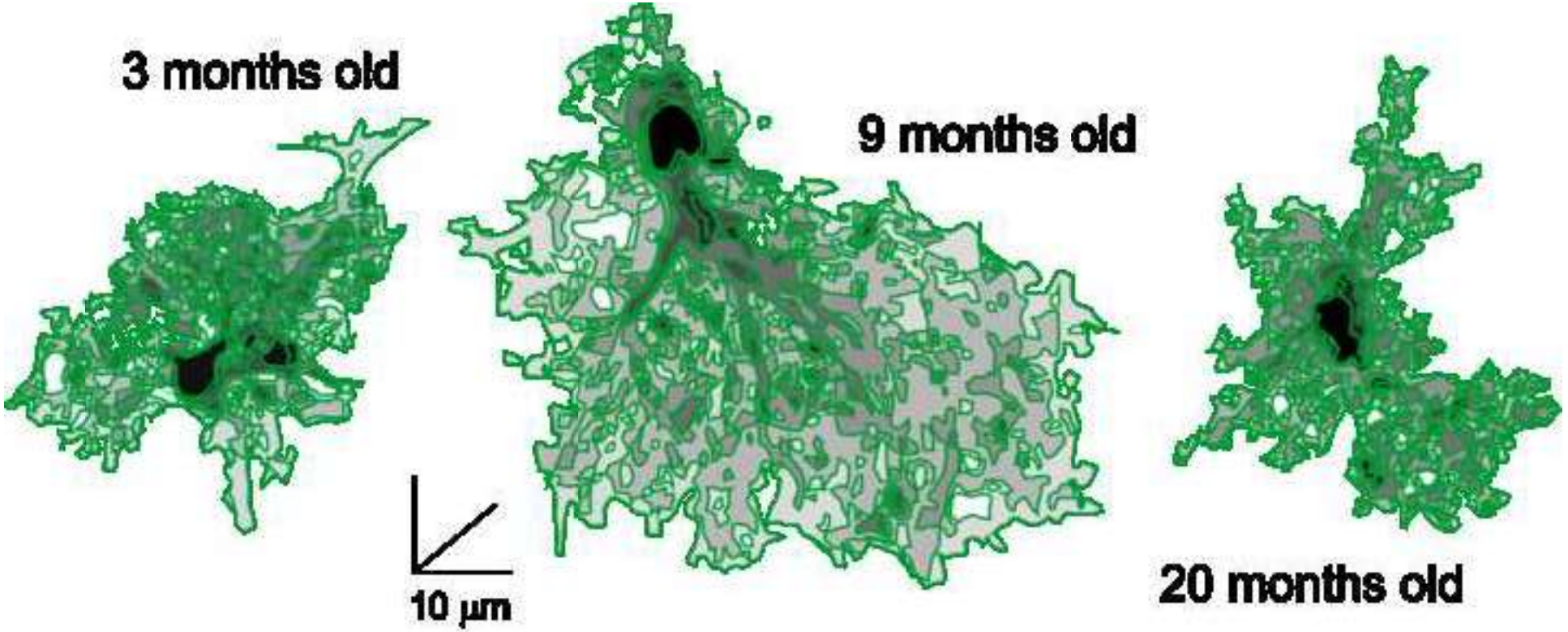
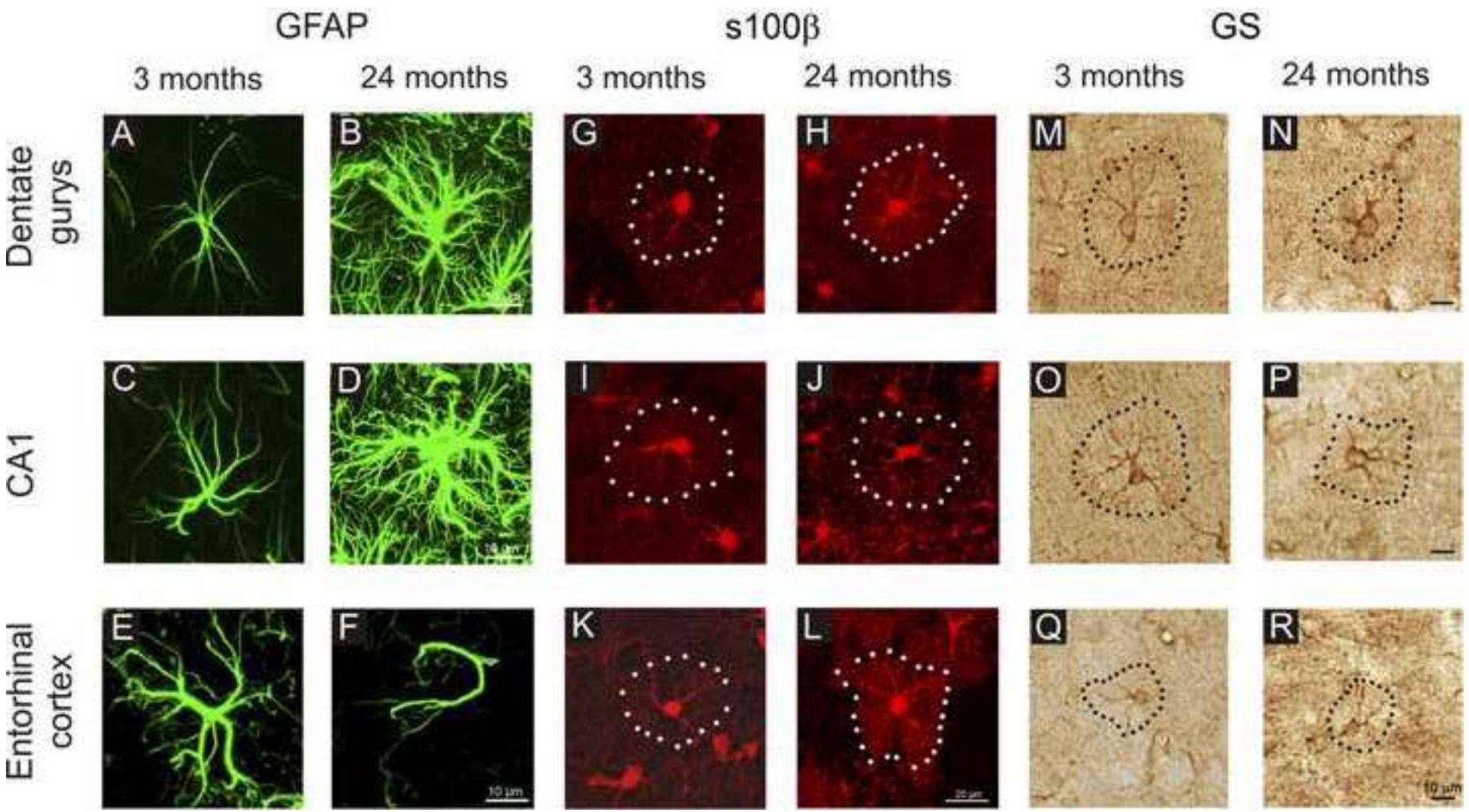


Fig. 2



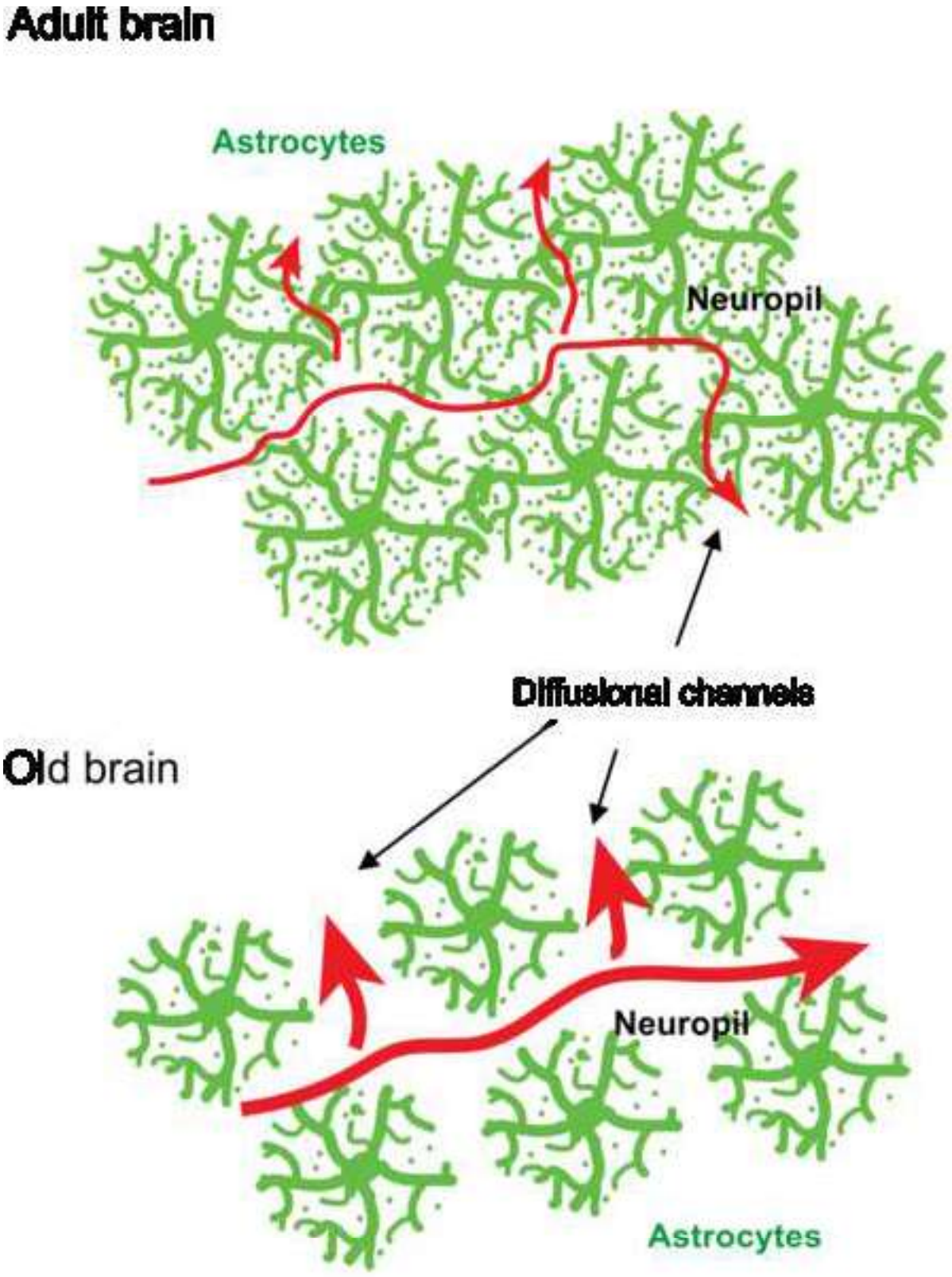


Fig. 4

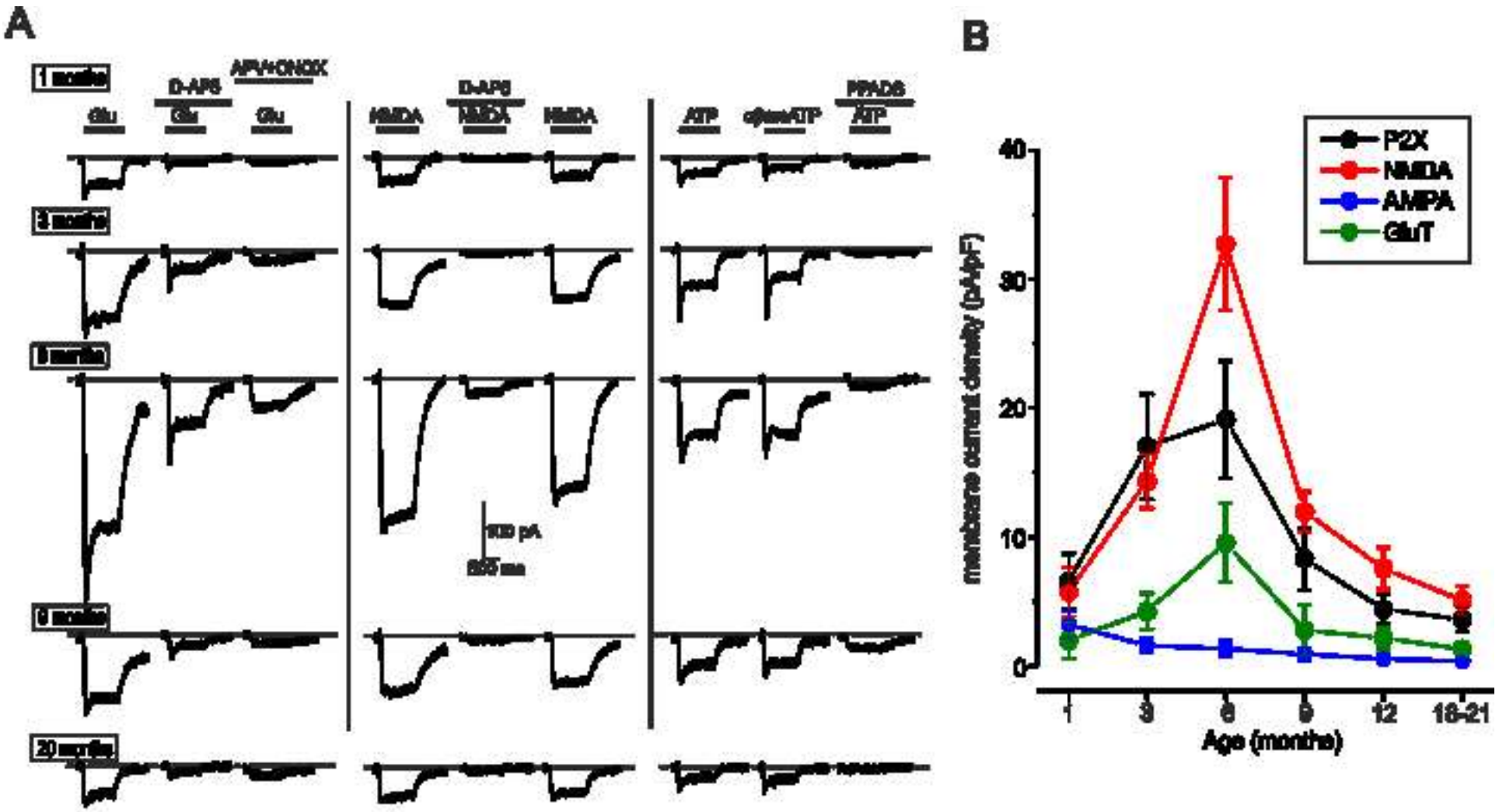


Fig. 5

Glutamate homeostasis

Ageing is associated with the decrease in the density of astrocytic glutamate transporters and a decrease in synaptic coverage by astroglial processes, which diminishes the efficacy of glutamate uptake, facilitates glutamate spillover and impairs on synaptic plasticity

Neurogenesis

Ageing is associated with progressive decline in neurogenesis throughout neurogenic niches due to a decrease and ultimate cessation of asymmetric division of radial stem astrocytes

Neuroglio-vascular unit and the blood-brain barrier

Ageing is associated with decrease in astroglial secretion of vasoactive factors, in decline in astroglio-vascular coupling and in the impairment of barrier functions of the blood-brain barrier

Glymphatic clearance

Ageing is associated with a decrease in AQP4 expression in the endfeet and almost 40% decline in the capacity of glymphatic clearance

Metabolic support

Lactate production is decreased in aged astrocytes, which limits operation of lactate shuttle thus diminishing neuronal metabolic support

Cholesterol synthesis

Cholesterol is one of the main synaptogenic factors secreted by astrocytes. Ageing is associated with a decrease in astroglial synthesis of cholesterol which limits morphological plasticity of neuronal networks

Astroglial defence

Ageing is associated with a decline in neuroprotective and defensive capabilities of astrocytes. Astrocytic production of glutathione is decreased in ageing thus compromising anti-oxidative protection. Ageing also diminishes astrocyte reactivity thus facilitating neurodegeneration.

Fig. 6