Chapter 8
Neuroglia in Ageing

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Abstract 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. While the exact mechanisms of ageing of the nervous system remain elusive, it is without doubt that morpho-functional changes in a variety of neuroglial cells contribute to this process. The age-dependent changes in neuroglia are characterised by a progressive loss of function. This reduces glial ability to homeostatically nurture, protect and regenerate the nervous tissue. Such neuroglial paralysis also facilitates neurodegenerative processes. Ageing of neuroglia is variable and can be affected by environmental factors and comorbidities.

Keywords Ageing · Astrocyte · Microglia · NG2 cells · Oligodendrocytes

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8.1 Mechanisms of Ageing

Ageing reduces the functional capacity of all organs and systems ultimately weakening the whole organism, reducing its adaptability, wearing its defensive systems and bringing it to death through age-dependent diseases. The nervous system similarly undergoes senescence that often impairs upon cognitive abilities, affects learning capabilities and enfeebles memory. Nonetheless, brain sustains ageing with surprising tenacity; the cognitive functions attain the summit in middle age and often sustain into old age, while the decline in other systems (including skeleto-muscular, cardiovascular or endocrine) progresses much faster. What are the mechanisms of ageing and which molecular and cellular processes underlie the ageing of the brain remains a matter of intense polemics. Indeed, there are in excess of 300 theories of ageing, which highlight various pathways, many of which do contribute to this process [52].

Probably, the very first coherent theory of ageing was proposed by August Weismann [99], who considered ageing as a very natural process favoured by natural selection to prevent competition of species with their own progeny. According to this view, mechanisms of ageing could be many and they can be different in different organisms. The only common future is guaranteed termination of individual life after a presumed fulfilment of the reproductive duties. One of the widely considered pathways is the activation of endoplasmic reticulum stress/unfolded protein responses that positively correlate with longevity and negatively with fertility [86]. Another relatively old group of theories regards ageing as a result of mechanochemical deterioration of molecules and basic systems such as organelles or cell membranes; this was initially proposed as 'hysteresis of colloids' [72, 73]. The mitochondrial or free radical theory of ageing links the damage of biological systems to reactive oxygen species and regards mitochondria as the age-defining clock [29, 30]. By reducing caloric intake this may resist the rate of ageing [48]. The gene regulation theories assume that changes in gene expression define ageing process [39], while the telomere theory postulates that it is the telomere DNA localised at the end of chromosomes which determines the life span: the shortening of telomeres eventually brings to the arrest of cell replication and death [28], The inflammatory theory of ageing became popular in recent years leading to the concept of 'inflammaging' [21]. Additionally, several theories look into the role of signalling systems both at organism (e.g., neuroendocrine or immune theories of ageing [20]) and cellular (e.g., calcium theory of ageing [40, 45, 89]) levels.

8.2 Ageing of the Brain

The maintenance of cognitive capacity of the brain over most of the human's lifespan results, most likely, from prominent neuroplasticity, remarkably long development and high degree of homeostatic and protective capabilities of neuroglia. The human brain is optimised for learning, with numerous mechanisms from adult neurogenesis

(which supplies the hippocampus with new neurones [54]) and adult myelination (which lasts well into the fourth decade of human life [1, 98], while oligodendroglial progenitors are present throughout the brain across the whole lifespan and probably contribute to late-life regenerative myelination), to the highly sophisticated glymphatic system that purges the brain from toxic waste products [36], thus maintaining neural environment. Ageing affects cognition components in a rather distinct way. The age-dependent decline mainly affects the real-time processing and formation of new memories and behaviours, whereas the capacity to analyse semantic and long-term memories suffer much less [18, 35]. For example, a group of young adults were significantly better than the group of old people in recalling a list of words. However the ability of elders to use complex processing activities was indistinguishable from the youngsters [47]. This benign or physiological brain ageing is not granted to all, and age-dependent pathologies, most notably of neurodegenerative nature, affect a substantial part of population.

Age is the main risk factor for neurodegenerative diseases, which are often considered as a natural outcome of senescence process. However, there is a fundamental difference between physiological ageing and neurodegeneration. The latter reflects massive neuronal death and atrophy of the brain tissue, whereas the former is not associated with a substantial neuronal loss. The overall number of neurones is not significantly affected in physiological ageing in rodents, primates and humans [6, 12, 19, 100]. Likewise, the number and density of synapses are not significantly affected by ageing [23, 80], albeit synaptic size is reduced [56].

Factors which determine the fateful difference between physiological and pathological ageing are many. These are represented by genetic factors (the best example being familial Alzheimer's disease or Huntington disease), the environment and life style (including diet, education, mental or physical activity) and the associated pathology (such as vascular disorders and ischaemic lesions). Another fundamental factor that defines the degree of cognitive deficit of ageing and age-dependent neuropathologies is known as the cognitive reserve. The cognitive reserve is an intrinsic quality of an individual brain that determines the neurological deficit when a similar brain damage results in very different cognitive outcomes in different subjects [82, 103]. The cognitive reserve in turn is defined by (i) neuronal reserve, which is the status of neuronal networks acquired during the life span through learning and cognitive load and (ii) neuronal compensation that reflects the defensive, plastic and regenerative capacities of the individual brain. To a large extent, the neuronal compensation is defined by neuroglia, which is responsible for neuroprotection, regeneration and post-lesion remodelling of the neural circuitry. The role of neuroglia is therefore fundamental in defining physiological versus pathological senescence; the failure of glial cells to protect and sustain the neuronal networks, the neural tissue and the CNS as an organ facilitates the progression from physiological to pathological brain ageing [93].

8.3 Astroglia in Physiological Ageing

8.3.1 Morphology and Gene Profiling

Age-dependent changes in astroglial morphology and gene expression are complex and region specific. Total number of astrocytes in physiologically aged human brain does not seem to change significantly, even in centenarians [19, 59]. When it comes to astroglial morphological profiles and expression of glial fibrillary acidic protein (GFAP), which are indicative of astroglial reactivity, the data remain quite controversial. Both a decrease [7] and an increase [14] in the number of GFAP-positive astrocytes, in particular in hypothalamic areas [27], as well as astroglial atrophy and astroglial hypertrophy were observed. The volume of astroglial territorial domains has been found to almost double in 21-month-old mice when compared to 5-month-old animals [26]. Increase of GFAP expression and hypertrophy of GFAP-positive astrocytes have been described in the hippocampus of aged rodents [7, 34, 49] and humans [10, 55]. Ageing had a distinct effect on different subpopulation of astrocytes in a region-dependent manner (Fig. 8.1, [69]). The densities of GFAP-positive astrocytes in the CA1 region and dentate gyrus of the hippocampus of old (24-month-old) mice demonstrated prominent hypertrophy when compared to young (3-month-old) or adult (9-month-old) controls. To the contrary, GFAP-positive profiles of astrocytes in the entorhinal cortex of old animals were atrophic when compared to the young or adult mice. Ageing results in a substantial decrease in the number and complexity of processes of astrocytes in the entorhinal cortex. The astrocytes immunoreactive to

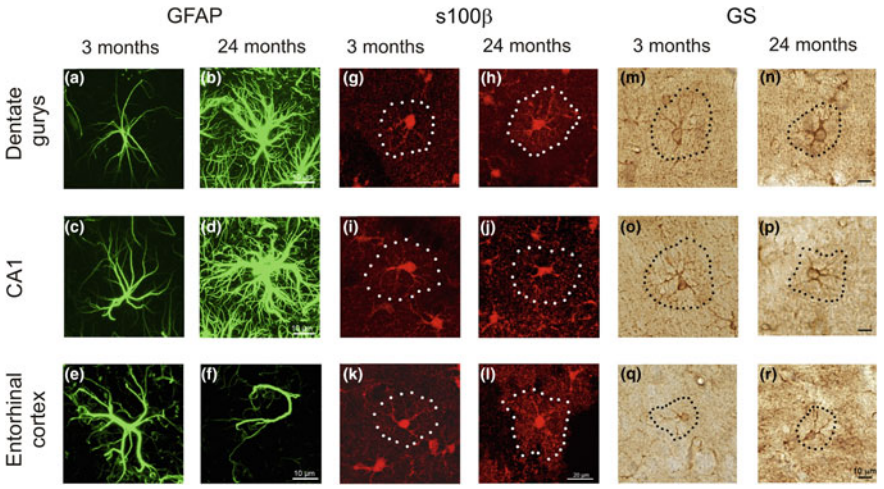


Fig. 8.1 Age-dependent remodelling of astroglial profiles in different brain areas. Confocal images showing glial fibrillary acidic protein—GFAP (a to f), s100β (g to l) and glutamine synthetase—GS (m to r) immunolabelled astrocytes in the dentate gyrus and CA1 hippocampal areas as well as in the entorhinal cortex of mice at 3 and 24 months. Modified from [69]

s100β protein were hypertrophic in the aged dentate gyrus but not in the CA1 region of the hippocampus as well as in the entorhinal cortex, whereas the profiles of a subpopulation of astrocytes labelled with glutamine synthetase were atrophic in the hippocampus with no changes in the entorhinal cortex [69]. Glutamine synthetase is a central enzyme necessary for operation of glutamine–glutamate/GABA shuttle, as well as for ammonium detoxification [70]. Suppression of expression of this enzyme may therefore affect neurotransmission and promote astroglial synthesis of GABA, an inhibitory neurotransmitter [22]. In parallel, hypertrophy of GFAP-positive astrocytes may be connected with environmental stimulation and plasticity representing the neural compensation. Exposure to the enriched environment is known for its positive effects on learning and memory, which occur in parallel with an increase in GFAP-positive astroglial profiles [68, 75].

The transcriptomic analysis of aged astrocytes similarly found a complex modification in genes expression. For example, astroglial cells from the cerebral cortex of aged mice demonstrated an increase in genes related to immune response with a decrease in expression of GFAP and genes related to neuroprotection and neuronal support [58]. Comparison of RNA-Seq from old and young astrocytes in the motor and visual cortices, hypothalamus and cerebellum revealed region specificity, with more significant changes in astroglia from the hippocampus and cerebellum [4], where astrocytes increased an expression of proinflammatory genes, genes encoding GFAP and Serpin3n, and genes linked to synaptic elimination such as complement component 3 and 4b [4]. Very similar results have been obtained in analysing the gene expression profiles of astrocytes from the hippocampus, cortex and striatum. Ageing affected hippocampal and striatal astrocytes the most with up-regulation of inflammatory genes and genes related to synaptic elimination [8]. Analysis of the gene expression profiles of different brain cells from ten brain regions of post-mortem tissue of humans, aged between 16 and 102 years, found that changes in astrocytes and oligodendrocytes were more prominent and complex compared to other cell types [81]; in particular, no age-dependent changes in neuronal gene expression pattern were identified. Again, these results indicate that functional preservation of neuroglia is critical for maintaining the ageing brain.

8.3.2 Astroglial Function

Although the data on physiology of aged astrocytes are rather limited, there are some hints for age-dependent remodelling of signalling and homeostatic profiles of astroglial cells. The resting membrane potential (around −80 mV) and membrane input resistance of astrocytes in cortical slices (from animals aged between 1 and 21 months) does not change much in ageing; if anything the input resistance is somewhat smaller in young adult mice (3–6-month-old—Fig. 8.2, [44]). Astrocytes from older mice express major types of receptors and are capable to generate ionotropic receptor-driven glial "postsynaptic" currents in response to neuronal activity [24, 44]. The density of ionotropic glutamate (AMPA and NMDA) receptors and P2X

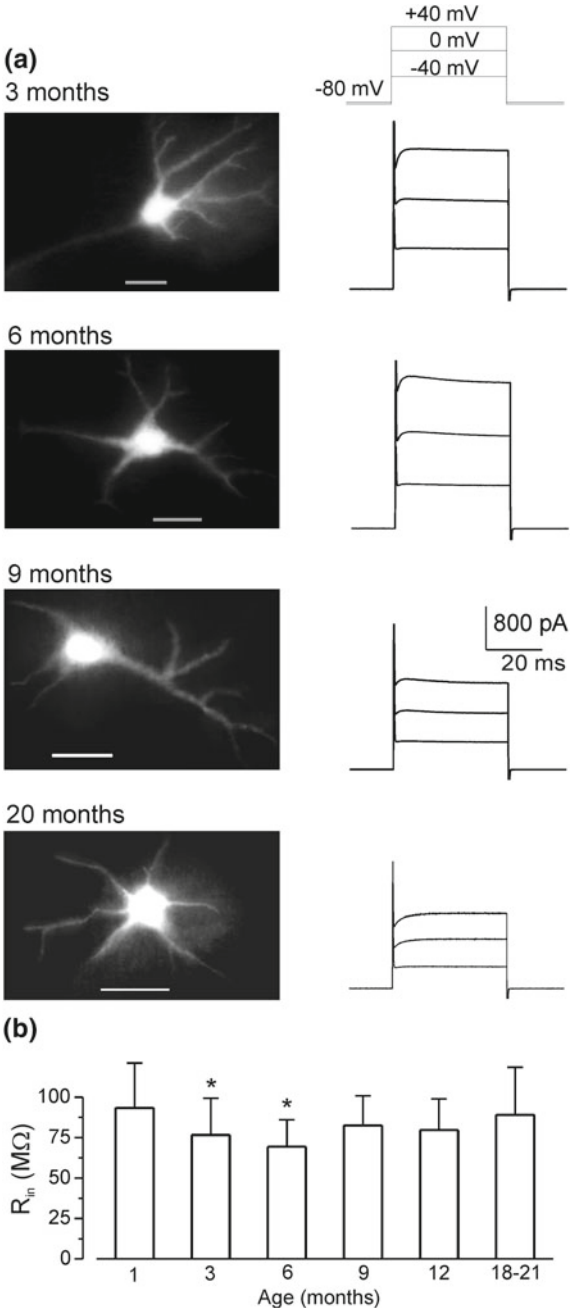


Fig. 8.2 Basic electrophysiological properties of cortical astrocytes in brain slices from mice of different ages. **a** Experiments were performed on mice expressing EGFP under control of human GFAP promoter. Representative EGFP images (*left column*) of cortical layer II astrocytes of different age groups and corresponding whole-cell currents (*right column*) evoked by depolarizing steps from the holding potential of −80 mV; the voltage protocol is shown on the top. **b** Age-related changes in the input resistance of cortical astrocytes. Data are presented as mean ± SD for 15 cells in each age group; decrease in the astrocyte R_{in} measured in 3 and 6 months in comparison to 1 month was statistically significant with $P < 0.05$ (one-way ANOVA). Reproduced with permission from [44]

purinoceptors, as well as the density of plasmalemmal glutamate transporter currents demonstrate bell-shaped age dependency (Fig. 8.3). Ionic currents generated by the above receptors and transporters are maximal in young adult (3- to 6-month-old) animals; at 9–21 months of age these currents are much smaller, although they are similar to currents recorded in 1-month-old animals [44].

Astrocytes are endowed with specific type of excitability, known as ionic excitability, which is associated with spatially and temporally organised fluctuations in the cytosolic concentration of several ions, including Ca^{2+}, Na^{+}, Cl^{-} and possibly K^{+} and H^{+} [95, 97]. Intracellular Ca^{2+} and Na^{+} signalling are of particular importance [95, 96] being involved in regulation of numerous astroglial physiological processes such as secretion [94] or homeostatic transport [41, 71]. Neurotransmitter or synaptically induced astroglial Ca^{2+} signals are age dependent. For example, Ca^{2+} signals are the largest in young adult mice and are relatively small in old and very young animals (Fig. 8.4; [44]). This dependence may be reflected in the functional expression of astrocytic receptors. Most likely an increase in the density of receptors, as well as in the density of plasmalemmal glutamate transporters and in the amplitude of Ca^{2+} signals, occur in the period of maximal environmental stimulation associated

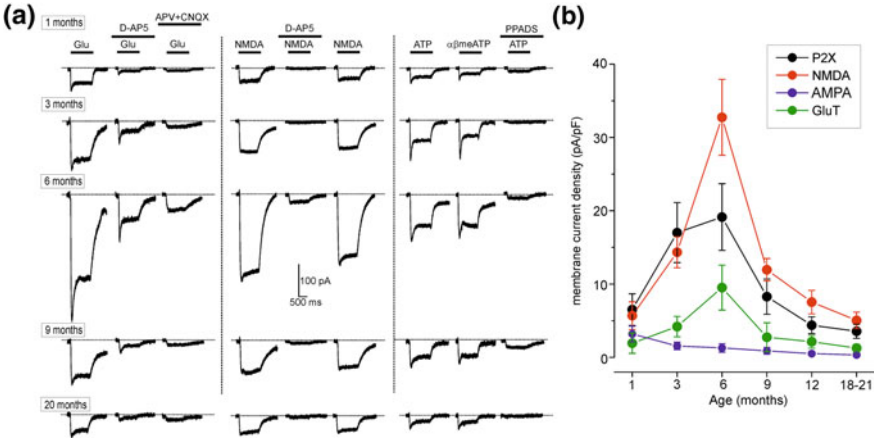
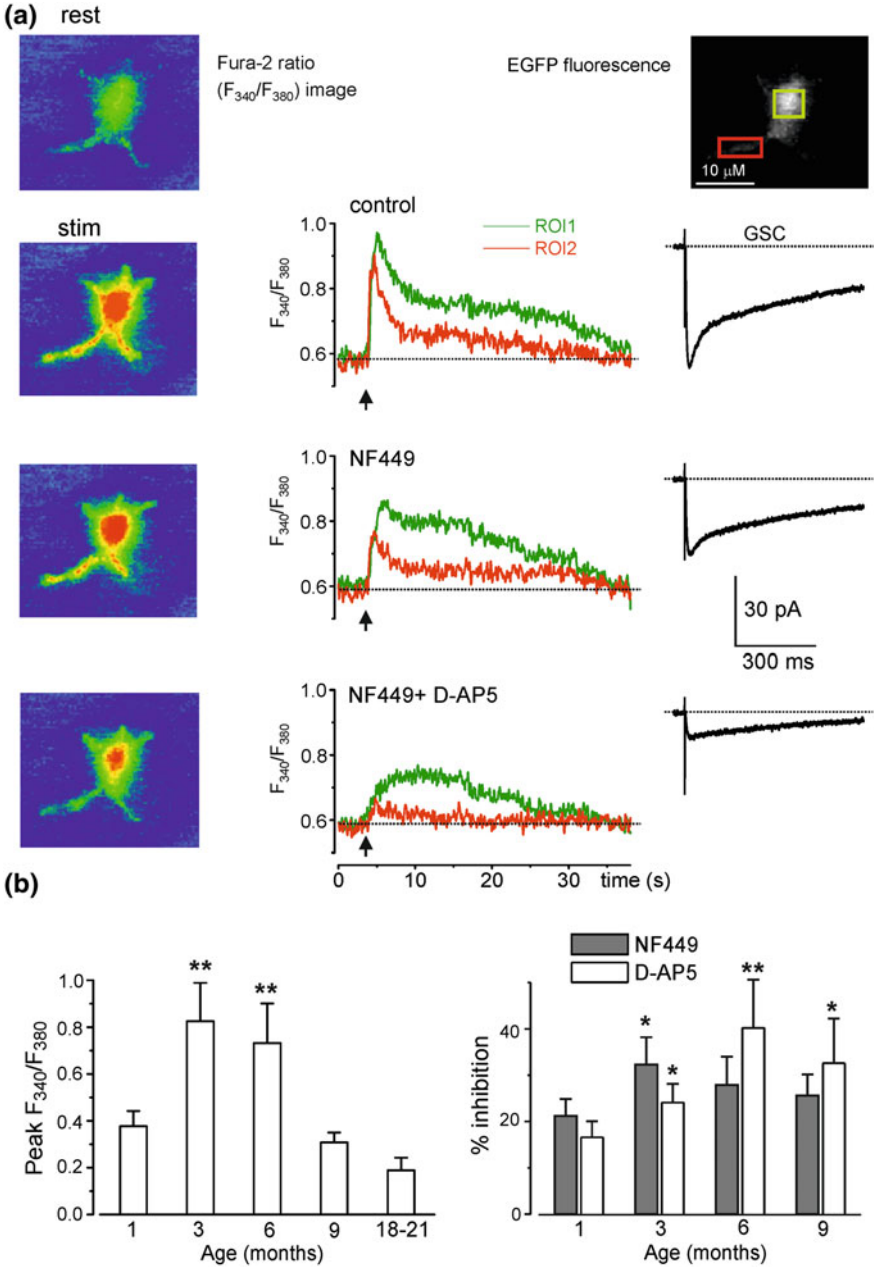


Fig. 8.3 Ageing affects the density of plasmalemmal glutamate transporters and ionotropic receptor-mediated currents in acutely isolated single cortical astrocytes. **a** Representative whole-cell currents elicited in the acutely isolated astrocytes by application of 100 μM glutamate (left column), 10 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 is also a weak partial agonist at human and mouse P2X4 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


(a)
rest
Fura-2 ratio
(F340/F380) image
EGFP fluorescence
10 μM
stim
control
ROI1
ROI2
GSC
NF449
30 pA
300 ms
NF449+ D-AP5
F340/F380
time (s)
(b)
Peak F340/F380
Age (months)
NF449
D-AP5
% inhibition

◀**Fig. 8.4** Age-dependent changes in synaptically induced ionotropic Ca^{2+} signals in protoplasmic astrocytes in situ in cortical slices. **a** Cortical layer II astrocyte of 9-month-old mouse was loaded with the Ca^{2+} indicator Fura-2 in situ via patch pipette. Fluorescence images were recorded simultaneously with glial currents evoked by neuronal afferent stimulation in presence of a mixture of TBOA (GluT blocker) and CNQX in control, and after consecutive application of 10 nM NF-449 (selective antagonist of P2X receptors) and at that in a company of 30 μM D-AP5. Representative images (pseudo-colour, pipette image subtracted) and glial synaptic currents (GSC, right column) were recorded before (rest) and after stimulation as indicated. Ca^{2+} transients (middle column) are expressed as F_{340}/F_{380} ratio averaged over the corresponding regions of interest shown in the GFAP image of astrocyte (*top right*). **b** Age-related changes in the astrocytic Ca^{2+} signalling. *Left panel*, average peak amplitudes of $[Ca^{2+}]_i$ increases, induced by stimulation of neuronal afferents in cortical astrocytes of different ages. *Right panel*, average inhibitory effect of antagonists of P2X (NF449) and NMDA (D-AP5) receptors on the amplitudes of $[Ca^{2+}]_i$ increases responses in cortical astrocytes. Data are presented as mean ± SD for 3–4 cells for each age group; * $P < 0.05$, ** $P < 0.01$ one-way ANOVA compared to 1 month. Reproduced with permission from [44]

with intense learning; in younger and older ages synaptic activity is lower, which is reflected in a decrease in receptors expression.

A decrease in astroglial gap junctional coupling was found in old (20–27-month-old mice) neocortical astroglial syncytia [63]; there were no changes at earlier ages (up to 14-month-old [9]). Astrocytes in older brains down-regulate expression of aquaporin 4 (AQP4). A decrease in the density of these channels in the perivascular endfeet affects clearance of the brain parenchyma through the glymphatic pathway [42]. This decrease in AQP4 in the endfeet may be linked to the deficits in vesicular trafficking, which is the key pathway in delivery of numerous molecules to specific locations at the plasmalemma [64]. Ageing affects astroglial metabolic pathways, as an age dependent increase in oxidative metabolism was reported in older astrocytes, which may limit their ability to supply neurones with metabolic substrates [37]. There is also evidence of age-dependent alterations in astroglial ability to produce lactate and hence to operate lactate shuttle [31]. Similarly, ageing is associated with an increase in the ratio of glutamate to glutamine in the brain that indicates some aberrations in the operation of the glutamate/GABA-glutamine shuttle [16, 32]. Ageing is also associated with a decrease in the brain levels of glutathione, mainly produced in astrocytes; this limits the ability of astroglia to resist the oxidative damage to the neural tissue [17, 50].

8.4 Oligodendroglia in Physiological Ageing

The human brain has a disproportionally large white matter when compared to other mammals and even high primates [76], as indeed the white matter occupies >50% of the human brain. Additionally, the level of myelination is well developed in the grey matter [43], further demonstrating the importance of connectome to the cognition and intelligence. The anatomical prevalence of the white matter in the human brain is also associated with very long development: myelination attains its peak at ~45–47 years

of age, with a subsequent slow and yet progressive age-dependent decline [1]. Normal ageing causes rather substantial shrinkage of the white matter which diminishes by ~11%; in comparison, the volume of the grey matter is decreased by only ~3% [33]. The highest degree of age-dependent alterations of the white matter is detected in the prefrontal cortex and associative tracts [66], which suffer early in Alzheimer's disease [15]. Incidentally, these brain regions emerge late in evolution and they are the slowest to develop, which instigated a 'last in, first out' hypothesis of the white matter ageing [66, 90]. Conceptually, changes in the white matter can be considered as a valuable marker of ageing [90], and moreover, accelerated degeneration of the white matter seems to indicate development of neurodegeneration and profound cognitive decline [65, 90].

Cells of the oligodendroglial lineage represented by oligodendrocytes and their precursors (also known as NG2 glia [13]) are, arguably, the most numerous glial cells in the human brain. Cells of the oligodendroglial lineage, in contrast to astrocytes, are highly vulnerable to excitotoxicity and to oxidative stress. The oligodendroglial precursors/NG2 cells, as well as more mature oligodendroglia, express several types of ionotropic glutamate receptors (including NMDA receptors) and P2X purinoceptors, which all can mediate excitotoxic Ca^{2+} overload and cause cell death [51, 53, 74, 92]. Furthermore, oligodendrocytes are highly vulnerable to oxidative damage, which is stipulated by a rather low content of antioxidants. In particular oligodendroglial cells contain two times less of glutathione compared to astrocytes, and yet they experience six times more of oxidative stress in physiological conditions [38, 87].

Ageing is associated with a significant decrease, by up to 30%, of the total number of oligodendrocytes [19, 59]. Rather surprisingly, in monkeys the number of oligodendroglial cells has been claimed to increase with age; for example, in the visual cortex of old monkeys the number of oligodendrocytes increased by 50% [62]. Notably, these oligodendrocytes also showed aberrant atrophic morphology and a deficiency in myelin production, which defined decreased CNS myelination in old primates [62]. The age-dependent myelin deficiencies are also associated with vasculature lesions in the white matter that add strain on oligodendrocytes and promote their degeneration [3, 101]. Ageing is also associated with a diminished capacity of remyelination supported by the NG2 glia. Notwithstanding the fact that the population of NG2-oligodendroglial precursors does not change numerically in the old brain, the capacity of NG2 cells to differentiate into mature oligodendrocytes is reduced. The NG2 cells in the old brain tend to retain their precursor status, so that the time of differentiation into mature myelinating phenotype is increased by almost two times [102]. All in all, age-dependent changes in the white matter are prominent and may be the leading cause of age-dependent cognitive decline.

8.5 Microglia in the Ageing Brain

Microglia in the ageing human brain undergoes rather idiosyncratic metamorphoses, which are not present in laboratory animals. Fundamentally, human microglia gradually degenerates, thus, reducing the defensive capabilities of the senescent nervous tissue.

In animals, the ageing process results in complex changes in microglial numbers and state. In old rats, microglial numbers decreased in the nigrostriatal system and cerebral cortex [77], and remained unchanged in the hippocampus [91]. In contrast, in old rhesus monkeys the densities of microglial cells increased, while these cells showed signs of increased phagocytosis [61]. In humans, ageing is associated with dystrophy and degeneration of microglia which resulted in deterioration of neuroprotective and defensive functions of these cells [84]. Morphological features of dystrophic aged microglia include deramification, spheroid formation, gnarling and fragmentation of processes [84]. The processes of aged microglial cells are shorter with less branching and reduced arborized area; the total number of microglia seems not to change with age [11]. Microglial dystrophy and a loss of function arguably increase the vulnerability of the old brain to neurodegeneration and may facilitate evolution of age-dependent cognitive disorders, including Alzheimer's disease [83]. The age-dependent microglial dystrophy can be associated with cytoskeleton abnormalities that underlie the cytorrhexis, rupturing of cells [88]. Microglial cells can accumulate tau [5] and the aged microglia (in marmosets) were reported to contain hyperphosphorylated tau [67]; this microgliatauopathy can be a factor that initiates microglial degeneration and dystrophy [67]. The prevalence of dystrophic microglia limits the neuroinflammatory capabilities of the old brain tissue, questioning the concept of inflammaging.

There is also evidence for age-dependent microglial activation in normal ageing, especially in rodents [57, 60] and in *Macaca nemestrina* monkeys [78]. There is an overall trend of hyperreactivity of microglia in aged mice [25, 46, 79], which is strikingly different to the dystrophy and a loss of function of human aged microglia, questioning the validity of rodents as an experimental models for brain ageing.

Aged human microglial cells are represented by two morphologically distinct classes identified as dystrophic or senescent microglia and dark microglia. The dystrophic microglial cells [85] are characterised by spherical swellings of processes, dilatation of the endoplasmic reticulum and abundance of lipofuscin deposits (that emerge from incomplete lysosomal degradation and endolysosomal stress and overload). The dystrophic microglial cells have been identified both in old brains and in high densities around senile plaques of Alzheimer's diseases patients [88]. Dystrophic microglial cells have fragmented processes and have a substantially diminished activation capacity [85, 88]. The dark microglia have been defined so because of the electron-dense cytoplasm and nucleoplasm, which in electron microscopy appear as dark as mitochondria [2]. The dark microglia are also characterised by ultrathin and highly ramified processes that frequently enwrap synaptic elements, axons and dendrites. This may indicate that dark microglial cells are involved in eliminating

synapses [2]. In addition, dark microglia have altered expression of classical marker IBA1 and they do not express the $P2Y_{12}$ purinoceptor, which is considered as a marker for healthy surveillance microglia. Dark microglial cells cumulate with ageing and even more so in age-dependent pathologies [2].

8.6 Conclusions

All types of neuroglial cells undergo age-dependent remodelling which seems to be critical to define a physiological or pathological outcome of ageing process. In general, the age-dependent changes in neuroglial cells are characterised by a progressive loss of function which limits neuroprotection and regenerative potential of the neural tissue. This process of neuroglial senescence, however, is variable and most likely individually tailored by the lifestyle, environmental stress and comorbidities. Neuroglial paralysis facilitates emergence of neurodegeneration and cognitive decline, and hence a neuroglial state represents a potential therapeutic target for age-associated neurological disorders.

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