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Abstract

Astrocytes, a class of morphologically and functionally diverse primary homeostatic neuroglia, are key keepers of neural tissue homeostasis and fundamental contributors to brain defence in pathological contexts. Failure of astroglial support and defence facilitate the evolution of neurological diseases, which often results in aberrant synaptic transmission, neurodegeneration, and death of neurones. In Alzheimer's disease (AD) astrocytes undergo complex and multifaceted metamorphoses ranging from atrophy with loss of function to reactive astrogliosis with hypertrophy. Astroglial asthenia underlies reduced homeostatic support and neuroprotection that may account for impaired synaptic transmission and neuronal demise. Reactive astrogliosis which mainly develops in astrocytes associated with senile plaque is prominent at the early to moderate stages of AD manifested by mild cognitive impairment; down-regulation of astrogliosis (reflecting astroglial paralysis) is associated with late stages of the disease characterised by severe dementia. Cell-specific therapies aimed at boosting astroglial supportive and defensive capabilities and preventing astroglial paralysis may offer new directions in preventing, arresting or even curing AD-linked neurodegeneration.

Keywords: Astrocytes; Alzheimer's disease; Astroglial atrophy; Astrogliosis; Neurological diseases; Neurodegeneration

The epidemic of neurodegenerative diseases

The "epidemic" of neurodegenerative diseases prophesied by Robert Katzman in 1976 [63] spreads through the aging world population with little prospective for therapeutic containment. Despite remarkable progress in understanding the biochemistry and genetics of neurodegenerative processes the genesis and evolution of the majority of sporadic cases remain obscure, whereas pharmacological options remain symptomatic [122]. The ultimate outcome of neurodegeneration is neural cell death, brain atrophy and loss of brain function. A direct link between the decrease in the size (i.e., atrophy) of the brain tissue and decrease in cognitive capabilities (i.e., dementia) was suggested by Thomas de Willis at the end of 17th century [156]. Aberrant processing of proteins lies at the core of neurodegeneration; compromised synthesis/degradation or clearance of proteins results in accumulation of intra- or extracellular "toxic" proteins [55,118]. Despite the multitude of specific pathological pathways idiosyncratic for certain disease (e.g. β-amyloid accumulation and abnormal tau phosphorylation in Alzheimer's disease (AD), α-synuclein accumulation in Parkinson's disease or expression of mutant glial fibrillary acidic protein in Alexander disease) all neurodegenerative processes share a common pathological phenotype - they all trigger cell death and destroy connectivity in the neural networks.

Extracellular depositions of β-amyloid and intracellular accumulation of misphosphorylated tau protein (both processes are, most likely, interrelated with indications for tau pathology being driven by β-amyloid accumulation) are common histological denominators of the AD brains. Occurrence of these lesions, however, varies and there is no obvious correlation between their densities and the severity of dementia. The concept that tissue depositions of pathological material are causative for neurodegeneration was proposed by Oskar Fischer in 1907 [39,40]. The specific role for β-amyloid in the AD (the amyloid cascade hypothesis), remains, however, disputed [61,87,23]. Amyloid plaques occur in several neurological diseases; they were initially discovered by Paul Blocq and Gheorghe Marinescu in post-mortem brains from elderly patients with chronic epilepsy [11]; amyloid depositions populate posttraumatic nervous tissues, tissues infected with prions and brains affected by Parkinson's or Huntington's disease. Similarly, tau pathology is characteristic of fronto-temporal dementia and prion infection. In recent years the new concept of tau astrogliopathy had emerged, after the discovery of multiple pathological phenotypes of astrocytes infested with tau and related to specific forms of age-associated dementia [71]. Even acute sleep deprivation for a single night causes accumulation of β-amyloid, which is seemingly unrelated to any predisposition to AD [125].

It is, however, almost beyond dispute that the gross histopathological signs of AD became apparent at the late stages of the disease. The AD begins with prolonged (10 - 15 years) asymptomatic phase, when the overall cognitive function remains (almost) intact, although pathological changes begin to accumulate. It is most probable that from the very beginning AD-pathology affects synaptic transmission. There is a close correlation between synaptic alterations and cognitive impairments in AD patients, and these synaptic alterations are often considered to occur at the very early (pre-plaque) stages of the disease [31,135,55,89]. Nervous tissue, affected by AD is characterised by compromised synaptic connectivity and neuronal hyperexcitability, which are indicative of dyshomeostasis of ions and neurotransmitters [64,84,42,19]. The brain unwiring in AD is also manifested in white matter damage, which is

observed from the early stages and correlates with cognitive deficit [14]. Finally, the AD alters metabolic homeostasis of the nervous system; region-specific hypometabolism underlies AD-specific diagnostic phenotype used for FDG-PET diagnostics [59]. All these features indicate that AD (similarly to other neurodegenerative diseases) is a chronic homeostatic failure of the brain tissue, which, naturally, has to be associated with the failure of the homeostatic neuroglia.

Astrocytes provide homeostatic support and neuroprotection

The human brain evolved for over ~500 million years from the diffuse nervous system that appeared in the most primitive multicellular organisms. Evolution of nervous system progressed through an increase in the complexity of nervous tissue with a parallel increase in heterogeneity and specialisation of neural cells. Emergence of the central nervous system (CNS) with intricate synaptic web required sophisticated homeostatic support and thus much specialisation has occurred among neural cells, which were fundamentally divided into neurones, representing the executive arm and neuroglia, representing the housekeeping branch [111,143]. This division of responsibilities reflected the perfection of fast neuronal signalling (action potentials and synaptic transmission) that requires much energy and high level of protein expression with an inevitable loss of neuronal abilities to control tissue homeostasis. This task was transferred to neuroglia, which indeed is responsible for each and every homeostatic cascade operating in the nervous system. Conceptually, the term neuroglia covers cells responsible for homeostasis and defence of neural tissue. Neuroglia include several types of heterogeneous (both morphologically and functionally) cells of neural (astroglia, oligodendroglia, radial glia, NG2 cells, peripheral glia and enteric glia) and non-neural (microglia) origin (for overview and references see [67,139,48,144,147,66,129,69]).

The homeostasis of the CNS is mainly controlled by astrocytes, which are arguably the most diverse glial cells in the brain and in the spinal cord (Fig. 1). Astrocytes are defined as a class of neural cells, which sustain homeostasis and provide for neuroprotection and defence of the CNS tissue [144]. Astroglial cells account (depending on the brain region) for 20 - 40% of all neuroglial cells [140]. Astrocytes demonstrate remarkable adaptive plasticity that defines the functional maintenance of the CNS in development and ageing. Astrocytes maintain homeostasis of the CNS at all levels of organisation, from molecular to organ. Astroglia contribute to ionostasis of the CNS tissue by regulating fluxes of major ions; astrocytes control the turnover of major neurotransmitters through dedicated systems responsible for their uptake and for release of neurotransmitter precursors. Protoplasmic astrocytes divide (through the process known as tiling) the grey matter into relatively independent “neurovascular units”; the territorial domain of a single astrocyte (which overlaps in adult healthy brain with neighbouring astrocytes only at the level of very distal processes) integrates all neuronal elements and establishes direct link (through perivascular process and the endfoot) with capillaries [57,56,20,94]. The morphology of astrocytic processes defines their function. The processes can be classified into (i) astrocytic branches of several orders that contain organelles including endoplasmic reticulum Ca^{2+} stores, (ii) organelle-free perisynaptic leaflets that form astroglial cradle and (iii) endfeet tiling the blood vessels [68,101,144]. Astrocytic branches are responsible for amplification and propagation of Ca^{2+} signals within an astrocyte and possibly beyond [123]. Astrocytic perisynaptic leaflets are rich in glutamate transporters as well as

other homeostatic transporters and selectively approach dendritic spines, providing less coverage of presynaptic boutons and little coverage of dendritic shaft [44]. Endfeet intimately interact with cells of blood vessels supporting the blood-brain-barrier [130]. Using perivascular processes and endfeet astrocytes adjust local blood flow to the level of neuronal activity [90,165,131,38]. Astrocytes also act as an energy source (being the main if not the only producer of glycogen in the CNS) by providing neurones with lactate in an activity-dependent manner [104,80]. Furthermore, astrocytes synthesize glutamate *de novo* and supply neurones with glutamine, which is the main precursor for glutamate and GABA [54]. Astroglial leaflets cover central synapses by a synaptic cradle and control neuronal excitability and synaptic transmission in many ways from assisting synaptogenesis and synaptic maintenance to regulating extracellular concentration of ions and major neurotransmitters (such as glutamate, GABA monoamines and adenosine) [33,12,142,95,91]. Astrocytes are central elements for cellular homeostasis of the CNS being responsible for embryonic neurogenesis (which relies on radial glia) and for neurogenesis in the adult CNS (that occurs from radial astrocytes of the neurogenic niches, [36,13]). Finally, astrocytes are fundamental to brain defence through evolutionary conserved and complex programmes controlling reactive astrogliosis launched in response to polyaethiological insults [103,128,151,102].

Principles and classification of astrogliopathology

Neurological disorders are, in essence, the failures of homeostasis, and neuroglia, being the homeostatic neural cells, are central to all types of neuropathologies. Whatever the aetiology, neurological diseases are, to a great extent, pathologies of neuroglia, the compromised function of which determines the survival or death of neurones thus defining the progression and the outcome of neurological diseases. The central role for neuroglia in neuropathology begun to be appreciated only recently when numerous experimental finding questioned the neuronocentric views [103,151,3,150,30,146,17,163,45,16,137,113].

The pathological potential of neuroglia was contemplated already by Rudolf Virchow who prophesied that “This very interstitial tissue of the brain and spinal marrow (*i.e. neuroglia - authors*) is one of the most frequent seats of morbid change” [153]. A fundamental contribution of neuroglia to neuropathology was also considered by Alois Alzheimer, Franz Nissl, Santiago Ramon-y-Cajal, Rio-Hortega and William Lloyd Andriezen, the latter reflecting that astrocytes “exhibit a morbid hypertrophy in pathological conditions” [2]. It is now the fact universally acknowledged that many neurological diseases are associated with the astrocytic hypertrophy and activation of microglia often referred to as reactive gliosis. For many years the reactive gliosis was considered as a general non-specific pathological reaction often regarded as a basis for neuroinflammation that is chiefly involved in exacerbating neuronal damage. This oversimplification is, however, very much detached from reality. The main function of neuroglia is the preservation of the nervous tissue, and as such multiple molecular cascades expressed in glia are genuinely neuroprotective. Astrocytes, for example, are primarily responsible for homeostasis of ions and neurotransmitters thus fencing against excitotoxic damage. Oligodendrocytes maintain axonal survival and the death of the former spells the imminent death on the latter. Microglia release multiple trophic factors ensuring neuronal survival. The reactive changes instigated by a brain lesion, represented by astrogliosis and microglial activation are therefore genuinely

survivalistic. These glial responses are complex and multistaged and are, as a rule, neuroprotective [103]. Being driven to an extreme by the severity of the insult, glial cells can assume the role of natural killer and contribute to the neural cell death, and yet even neurotoxicity of glia has, at its core, neuroprotective significance. Indeed, astroglial scar limits the area of damage and astrocytes and microglia surrounding the lesioned area through the releasing of neurotoxic factors rapidly exterminate pathologically affected cells thus contributing to the final clean-up (by phagocytic microglia and astrocytes [86,154]) and ultimate protection of the undamaged neural circuits lying outside of the damaged area. Finally, neuroglia is primarily responsible for the recovery of homeostasis in post-lesioned nervous tissue through promoting vascularization, reforming the blood-brain barrier, stimulating synaptogenesis and accomplishing re-myelination [128,151,1,18]. Conceptually, disruption of glial protection is fatal for the nervous system. All in all, neurones cannot function correctly or survive in the absence of glia, whereas glial cells can survive and operate in the presence of dead or dying neurones.

Pathological metamorphoses of astrocytes are complex, disease- and disease-stage specific and may change substantially in the course of pathological evolution. Conceptually, astrogliopathological phenotypes (see Glossary) are classified into three major groups: (i) reactive astrogliosis (ii) astrodegeneration with astroglial atrophy and loss of function and (iii) pathological remodelling [103,151]. The latter two groups of the non-reactive pathological transformation of astrocytes can be summarily identified as astrocytopathies to distinguish from reactive astrogliosis [37]. Astrocytes in the CNS are organised in the forms of syncytia confined to specific anatomical structures [46,47]. Astroglial syncytia are formed by intercellular gap junctions, which are composed from connexons (astrocytes express connexins 43, 30 and 26; with predominant presence of Cx43) permeable for ions and small (<1000 Da) molecules. Gap junctions participate in regulation of astrocyte electric properties [79], redistribution of K^+ ("spatial K^+ buffering" - [155]), diffusional transport of glucose [116] and propagation of Ca^{2+} waves [123,148]. Pathological changes in astrocytes frequently affect gap junctional connectivity within astroglial networks; which may define various aspects of neuropathological progression, for example, in epilepsy [9,106] or neurodegeneration [162].

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Glossary of astrogliopathology:

Reactive astrogliosis, reactive astrocytes:
There is no universally agreed definition of the reactive astrogliosis [34]. We define reactive astrogliosis as an evolutionary conserved defensive response of astrocytes to pathological lesions caused by endo- or exogeneous agents. Reactive astrogliosis leads to substantial changes in gene expression, resulting in a remodelling of astroglial morphology, biochemistry, and function, thus producing reactive astrocytes with multiple phenotypes. These reactive phenotypes are disease- and context- (for example ageing) specific, while reactive astrocytes could be either neuroprotective or neurotoxic, again depending on the pathology and severity of lesion. Morphology of reactive astrocytes is characterised by hypertrophy of somata and primary processes and significant up-regulation of intermediate filament proteins GFAP and vimentin (see [34,102,103,151] for further details).

Astroglial atrophy:
Defined as decrease in surface area and volume of astroglial morphological profiles; astroglial atrophy is manifested in specific diminution of peripheral and perisynaptic processes, which decrease synaptic coverage and synaptic homeostatic support. Astroglia atrophy has been reported in stress, depression, Alzheimer's disease and epilepsy [106,164,152,93].

Loss of function:
Decrease in expression or activity of astroglial homoeostatic molecules or pathways (for example decrease in glutamate transporters activity), which reduce glial homeostatic support this instigating or exacerbating neuropathology.

Astroglial pathological remodelling:
Development of specific astroglial phenotype, which drives neuropathology. Examples are expression of mutant sporadically mutated GFAP in Alexander disease, which affect development of white matter and causes severe leicomalacia [82] or changes in glutamate/ammonium handling in hepatic encephalopathy, which affects neurotransmission and neuronal excitability thus causing psychotic symptoms and ultimately brain oedema [146].

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Reactive astrogliosis is the most characterised response of astrocytes to pathological lesions [102]. Reactive astrogliosis can be defined as an evolutionary conserved defensive reprogramming of astroglia aimed at: (i) increased neuroprotection and trophic support of nervous tissue; (ii) isolation of the lesioned area; (iii) reconstruction of the damaged blood-brain barrier; and (iv) providing for post-lesion regeneration of brain circuits [102,103,128]. Astroglial reactivity is heterogeneous and disease-specific; reactive phenotypes may demonstrate both neuroprotective and neurotoxic features [76], although generally suppression of astrogliotic response exacerbates neuropathology [103].

Pathological remodelling of astrocytes reflects the emergence of abnormal astroglial phenotypes which either cause or drive neuropathological changes. Examples of pathological remodelling of astrocytes include for example leukodystrophies, such as Alexander disease, megalencephalic leukoencephalopathy with subcortical cysts or vanishing white matter syndrome. In all these pathologies pathologically modified astrocytes trigger white matter lesions [74]. Pathological remodelling of astrocytes was suggested as a contributing factor to mesial temporal lobe epilepsy. In this condition astrocytes acquire aberrant morphology, decrease gap junctional coupling and reduce K^+ buffering capability [9]. Yet another example of pathological remodelling of astrocytes is observed following infection with *Toxoplasma gondii.* Infiltration of astrocytes with *T. gondii* results in abnormal elevation of synthesis and release kynurenic acid that, through inhibition of NMDA and acetylcholine receptors, affects neurotransmission which may be linked to an increased risk of schizophrenia [120]. In AD astrocytes start to express GAD67 that also affects the balance of excitation and inhibition in the neuronal network [43,157].

Astrodegeneration appears as a decrease in astroglial density, is often accompanied by morphological atrophy, and invariably is associated with a loss of function, i.e. in decreased homeostatic, supportive and neuroprotective capabilities, which all constitute astroglial asthenia. Astroglial atrophy and asthenia are observed in a wide range of neurological disorders including neuropsychiatric diseases, addictive disorders, epilepsy and neurodegeneration. Reduction of astroglial numbers, astroglial morphological atrophy with decreased expression of GFAP as well as deficient glutamate uptake are detected in major neuropsychiatric diseases such as bipolar disease, major depression and schizophrenia [28,29,83,92,108-110,149,119]. Deficient astroglial support leads to abnormal neurotransmission and oxidative or excitotoxic stress which contribute to psychotic symptomatology. Similar morphological atrophy of astrocytes associated with decreased expression of glutamate transporters and deficient glutamate uptake were identified in the nucleus accumbens of cocaine-addicted rats [121]. Significant atrophy of astrocytes which may underlie aberrant K^+ buffering and glutamate homeostasis has been detected in experimental epilepsy [106]. Finally, as will be discussed below, astroglial atrophy is often seen in various neurodegenerative disorders.

Astrocytic atrophy may have a complex nature and affect astrocytic branches, leaflets and endfeet differently. Since these astrocytic processes have distinct functions, the effect on the neuronal network will be different. Atrophy of branches arguably affects astroglial Ca^{2+} signalling possibly inducing further morphological changes [132]. Perisynaptic leaflets are highly plastic and their changes accompany (or even drive) synaptic plasticity [105]. Hence, changes in perisynaptic leaflets can potentially contribute to pathological processes. Finally, atrophy of endfeet can potentially damage the neuron-glia-vascular unit, and affect the blood-brain barrier thus promoting neurodegeneration [126,133,78].

Morphological atrophy and functional impairment of astrocytes in neurodegenerative disorders

Degeneration of astroglia resulting in astroglial atrophy or death has been recently documented for several classes of neurological diseases associated with disruption in neural connectivity including excitotoxic neurodegeneration (Wernicke encephalopathy) chronic neurodegeneration (amyotrophic lateral sclerosis and Alzheimer's disease, thalamic dementia, fronto-temporal dementia) and psychiatric disorders such as schizophrenia, bipolar disorder and major depression, which all affect cognition. These dystrophic changes in astroglia often precede neurological symptoms or are key pathogenic factors.

In acute excitotoxic neurodegeneration of Wernicke type massive neuronal death results from functional astroglial degeneration manifested by down-regulation of expression of astroglial glutamate transporters. This compromises the ability of astrocytes to remove the excess of glutamate and to regulate glutamatergic transmission, which in turn results in severe excitotoxicity that underlies rapid development of severe dementia with prominent psychotic components [51,52].

In amyotrophic lateral sclerosis (ALS) astroglial degeneration precedes the development of neuronal death and clinical symptoms in transgenic mice model of the disease, in which cells express ALS-associated mutant human gene for superoxide

dismutase 1 (hSOD1^{G93A}). At the later stage of the disease neuronal death triggers reactive astrogliosis and yet atrophic astrocytes remain in the tissue [114,115,160,4]. Astrocytes selectively expressing SOD1 gene acquire vulnerability to glutamate excitotoxicity that may underlie their early degeneration. These degenerated astrocytes lose their ability to effectively control glutamate homeostasis through down-regulation of glutamate transporters expression that further exacerbates excitotoxicity and contributes to neuronal death [115]. Critically, specific silencing of SOD1 gene in astrocytes delays the progression of ALS symptoms in the mouse model [159].

Dystrophic changes in astrocytes have been observed in several types of neurodegenereative pathologies including fronto-temporal dementia and Pick's disease, some studies mentioned the direct correlation between the degree of glial atrophy and the severity of dementia [15,65]. In Huntington disease functional astro-degeneration is manifested by a decrease in expression of glutamate transporters and hence in exacerbated glutamate excitotoxicity [35]; similarly, astrocytes demonstrate deficits in K^+ buffering [136]. In addition astrocytes in HD reduce production and release of glutathione and ascorbic acid that act as major scavengers of reactive oxygen species (ROS) in CNS; this further reduces astroglial neuroprotection.

Astroglial atrophy in Alzheimer's disease

Astroglial changes in AD are highly heterogeneous in different brain regions and are represented by both astrogliosis with astroglial hypertrophy and astroglial atrophy (Table 1 and [3,141,145]). When analysing longitudinal changes in expression of GFAP, a classic marker for astrogliosis, in a triple transgenic mouse model of AD a decrease in the morphological presence of GFAP profiles and an overall decrease in GFAP expression was observed in early pre-plaque stages of the disease (Fig. 3 and [73,97,98,161]). This decrease in GFAP-positive astroglial profiles appear very early (at ~ 1 month of age) in the entorhinal cortex, somewhat later (~ 3 months of age) in the prefrontal cortex and even later (~ 9 - 12 months) in the hippocampus. Morphological atrophy of astrocytes was also confirmed when analysing profiles labelled with antibodies against glutamine synthetase and protein s100b [98,161]; this staining reveals full extent of the cells (in contrast to GFAP which stains only primary processes). Emergence of senile plaques triggers astrogliosis, which, however, similarly differs between brain regions. In hippocampus β-amyloid depositions and β-amyloid plaques are surrounded by hypertrophic astrocytes [97], whereas little, if any, signs of astrogliosis were found in entorhinal and prefrontal cortices [73,161]. The GFAP-hypertrophic astrocytes in hippocampus were associated with β-amyloid depositions/plaques, while distantly to the plaques GFAP profiles remain atrophic [97]. Similar astroglial atrophy has been characterised in other models of AD including PDAPP-J20 transgenic mice, 5xTG-AD mice and Swiss 3 mouse AD model (Table 1, [58,107,32,8]). Astroglial atrophy was found not only in experimental model animals but in post-mortem tissues of patients with advanced (Braak V-VI) stages of the disease (Rodriguez and Verkhratsky, personal observations). Similarly, morphological atrophy was observed in astrocytes derived from induced pluripotent stem cells obtained from patients with both familial and sporadic forms of AD (Fig. 4; [60]). Enriched environment as well as physical activity prevents astroglial atrophy in an AD mouse model and ameliorates the symptoms, confirming the causal link between astrocyte remodelling and dementia [7,112].

It has to be noted, however, that animal models of AD only partially reproduce the human disease. For example, while 3xTG-AD mouse develops both plaque and tangle pathologies accompanied by some cognitive deficits, there is no massive neuronal loss which is a hallmark of AD progression in patients [10]. In this regard, new stem cell technologies that enable the generation of astrocytes from the disease-specific inducible pluripotent stem cells (iPSCs) [117], or by direct reprogramming of patient-specific fibroblasts [21] present a great promise. Although the field is still in its infancy, current technologies allow generation of relatively pure populations of cells exhibiting molecular and functional properties (electrophysiological signatures, generation of spontaneous calcium signals, glutamate uptake, support of synapse formation, etc.) similar to those of adult human astrocytes. The disease-specific astrocytes also recapitulate some important pathological aspects of AD. Thus, astrocytes derived from the familial AD and sporadic AD patients exhibited a less complex morphological appearance, decreased heterogeneity, overall atrophic profiles and abnormal localisation of key functional astroglial markers resembling changes occurring during glial paralysis at the early stages of the disease [60]. Astrocytes derived from familial AD patients also demonstrated increased β-amyloid production, deregulated Ca^{2+} homeostasis, altered cytokine release, increased ROS, decreased lactate production and compromised neuroprotection and neuronal support [85,96]. Astrocytes differentiated from isogenic APOE4 astrocytes were impaired in their ability to clear extracellular β-amyloid and displayed aberrant cholesterol accumulation [77]. A new human AD "triculture" model using neurones, astrocytes, and microglia in a 3D microfluidic platform has been successfully used to study neuroinflammatory responses [100]. These studies demonstrate the substantial potential of iPSC-based human astrocyte models to reveal the cellular mechanisms of AD. Undoubtedly, these technologies will improve our understanding of the molecular mechanisms controlling astroglial response and also help to develop astrocyte-specific therapies against AD.

Reactive astrogliosis in AD: does it always signal neuroinflammation?

Reactive astrogliosis is another hallmark of AD; increased expression of GFAP, vimentin or S100B protein as well as astroglial hypertrophy has been observed in post-mortem tissues from AD patients [6,49,81,88] as well as in brain samples of AD animal models [97,145,62,99]. In AD brains reactive astrocytes are mainly associated with senile plaques [72,97,99]. The idea that chronic neuroinflammation is directly responsible for the progression of idiopathic AD from the very early stages, and may even have an etiological significance, become quite popular in recent years [53]. It is also generally assumed that this chronic neuroinflammation results from activation of microglia and reactive astrogliosis. This statement however, needs clarification, as indeed reactive gliosis is far from being a straight pathological reaction ultimately resulting in damage to the brain tissue. The reactions of neuroglia to brain lesion (reflected by both reactive astrogliosis and activation of microglia) essentially represent a defensive response aimed at counteracting pathology and remodelling post-lesioned circuitry. Neither astrogliosis, nor microglial activation develops in all-or-none fashion; to the contrary they represent a continuum of phenotypic remodelling, fundamentally associated with neuroprotection. There are many stages and degrees in astrogliosis [103,127], and some of these are fully reversible. Limited brain lesions trigger astroglial hypertrophy and biochemical remodelling without

affecting microdomain organisation (anisomorphic astrogliosis); and only severe lesions disrupt astroglial territorial maps, arrange astrocytes in palisades and trigger scar formation [127,128]. Even the scar formation is in essence defensive and survivalistic reaction aimed at isolation of lesioned area from the healthy tissue. Likewise, activation of microglia is a multistage process with a multitude of activated phenotypes, many of which have a neuroprotective role [50,66]. In AD, both reactive astrogliosis and activation of microglia are directly associated with plaque formation; activated glial cells become elements of plaques. Importantly, activated astrocytes surrounding the plaques retain their domain organisation and do not express severe astrogliotic features; similarly, microglial cells do not turn in all-devouring macrophages, but retain intermediate activated phenotype. Suppression of astroglial reactivity, contrary to the inflammatory hypothesis, exacerbates β-amyloid load and reduces neuroprotection [72].

Astroglial atrophy contributes to synaptic dysfunction and cognitive deficits

Atrophic changes in astrocytes, characterised in several AD animal models as well as in stem-cells derived astrocytes appear as shrinkage of astroglial territories, with a decrease in astroglial coverage of synaptic contacts and other neuronal structures with the ultimate decline in astroglial homeostatic support. Astroglial atrophy and loss of function may contribute to early cognitive deficits through dwindling synaptic support and synaptic malfunction. Decreased astroglial synaptic coverage may also result in neurotransmitter spillover with subsequent hyperexcitability of neuronal networks often observed in neurodegeneration. In addition enhanced glutamate spillover may increase the recruitment of extrasynaptic NR2B subunit-containing NMDA receptors associated with long-term depression, LTD [5]. Thus decreased astroglial synaptic coverage with a consequent reduction in astroglial glutamate uptake may impair upon synaptic plasticity shifting it towards depression, which can in turn negatively affect memory [138,27].

Notably, K^+ clearance and glutamate uptake by astrocytes are tightly linked [75]. During synaptic transmission most of K^+ entering the synaptic cleft is released through postsynaptic AMPA and NMDA receptors [24]. Accumulation of K^+ in the synaptic cleft depolarises presynaptic terminal causing activity-dependent facilitation of glutamate release [124,26]. Reduction of glutamate uptake can enhance recruitment of postsynaptic AMPA/NMDA receptors and causes further K^+ release. This instigates positive feedback, which can potentially lead to an uncontrolled increase in excitability of the synaptic network, excitotoxity and neuronal death. In physiological conditions, these processes are tightly controlled by astroglial glutamate uptake and K^+ clearance. However, when glutamate uptake is impaired (due to the reduction of transporter expression or withdrawal of perisynaptic leaflets) the resulting vicious circle may contribute to neurodegeneration.

Furthermore limited astroglial support may instigate early extinction of synapses [166]. Dysfunctional synaptic transmission and loss of synapses are indeed the very first morphological changes in AD, which mount years before the occurrence of specific clinical presentation [134,25,70]. Astroglial asthenia may also suppress regenerative synaptogenesis; while the loss of astroglial transporters may contribute to excitotoxicity through impaired glutamate and K^+ buffering [166,152].

Failure of astroglial reactivity paves the way to dementia?

As has been alluded above, astrogliosis represents a powerful defensive program, which arguably contains various pathological processes including β-amyloid pathology and AD. Indeed there is compelling evidence demonstrating the neuroprotective potential of reactive astrocytes in AD. In the Tg2576 mice model (that bears APPSwe mutation) astrogliosis became prominent rather early and this correlates with the relatively slow development of AD. Furthermore, senile plaques in these animals resemble human β-amyloid deposits being represented by fleecy, granular, cored and diffused amyloid plaques [158]. In 3xTg-AD animals reactive astrocytes are positioned around senile plaques and close to perivascular β-amyloid deposits [97,98]. Conversely, in entorhinal and prefrontal cortices, the emergence of extracellular β-amyloid deposits does not initiate astrogliosis [73,161] which may reflect a failure of astroglial neuroprotection. This coincides with (and arguably underlines) high vulnerability of both regions to AD pathology; suggesting that paralysis of astroglial defence exacerbates AD-like pathology [141].

This notion also has been indirectly confirmed by the *in vivo* brain imaging of reactive astroglia in AD patients. Astrogliosis was assessed by positron-emission tomography detection of ^{11}C-deuterium-L-deprenyl (^{11}C-DED); deprenyl is a specific inhibitor of monoaminoxidase-B (MAO-B) localised predominantly in astrocytes. An increase in ^{11}C-DED signal hence is considered to reflect astroglial hypertrophy [41]. When using a multi-tracer PET detecting ^{11}C-PIB (marker of fibrillar β-amyloid), ^{18}F-FDG (marker of cerebral glucose metabolism) and ^{11}C-DED (marker of astrogliosis) the highest binding of ^{11}C-DED (which reflects prominent astrogliosis) was observed in patients with mild cognitive impairment (MCI) and high levels of fibrillar amyloid plaques in the brain (PIB+) reflecting prodromal AD [22]. The decrease in astroglial reactivity parallels the switch from MCI to full blown AD with senile dementia again demonstrating the neuroprotective role of astrogliotic remodelling [141].

Conclusions: the need for new astrocentric therapies?

In recent decades astrocytes emerged as a fundamental elements in pathophysiology of numerous neurological and neuropsychiatric diseases. Pathomorphological examination of astroglia has become a standard in describing the histology of the diseased brain. Pathophysiology of astroglia is a complex and multifactorial combination of degenerative and reactive remodelling, which can support neuroprotection or project neurotoxicity. Failure of astrocytes to support homeostasis of neural tissue and to protect this tissue against insults is arguably critical for determining the pathological evolution and ultimately neurological deficits. In the context of Alzheimer's disease astrocytes undergo atrophy with loss of function which may stipulate impairments of synaptic connectivity as well as contribute to neuronal death due to deficient neuroprotective support. At the same time, reactive astrocytes surround senile plaques in the AD brains; while initial stages of the disease are characterised by prominent astrogliosis. Exhaustion of astroglial defensive capacities and down-regulation of astrogliosis coincides with (and again may be instrumental for) the switch from mild cognitive impairment (characteristic for early to moderate AD stages) to senile dementia (which reflects late stages of the disease). Cell-specific therapies aimed at boosting astroglial supportive and defensive

capabilities and preventing astroglial paralysis may offer new directions in preventing, arresting or even curing AD-linked neurodegeneration.

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Figure legends

Figure 1. Diversity of astrocytes.

Figure 2. Classification of astrogliopathology. Modified from [103].

Figure 3. Astroglia atrophy in hippocampal (a) and cortical (B) regions of the wild type (WT) and triple transgenic AD model mouse (3xTG-AD). The images show GFAP-positive profiles of astroglial cells.
Collated from [73,97,161].

Figure 4. Astrocytes derived from iPSCs isolated from familial AD patient carrying *PSEN1* M146L mutation and sporadic AD patient carrying *ApoE4*$^{+/+}$ demonstrate significant atrophy when compared to those from healthy person.

(A) Morphological appearance (arborised, polarised and fibroblast-like) of astrocytes derived from iPSCs of healthy controls, familial and sporadic AD (FAD and SAD respectively). (B). Exemplar 3D IsoSurface renders constructed from serial confocal z-stacks display clear differences in cell size and overall morphology Scale bar = 10 μm. Quantification of cells using these renders by way of surface area (C), cell volume (D) and SA:Vol ratio (E) reveal significant differences in all aspects of cellular morphology between healthy and diseased astrocytes. Quantification of mean fluorescence intensity per immunoreactive cell reveals no significant difference in GFAP staining intensities between AD- and control astrocytes (F) but S100B, EAAT1 and GS intensities are reduced in both FAD and SAD cells (G, H and I, respectively). Asterisks on graph; *** $p<0.001$, ** $p<0.005$, * $p<0.05$.
Reproduced from [60].

Table 1. Astrocytes in AD: Atrophy and loss of function

Model/Age/Preparation	Properties	Loss of function	Reference
5xFAD/12 - 14 months/ Primary culture		Reduced β-amyloid$_{42}$ uptake; reduced neuroprotection reduced ability to promote neuronal growth	[58]
3xTG/ 4 Month/Fixed brain slices/Immunocytochemistry	Reduced morphological profiles and arborization	Treatment with L-norvaline rescued astroglial atrophy, increased dendritic spines densities and improved memory deficits	[107]
Swiss mice/ 3 month/Intracerebroventricular injection of 10 pM β-amyloid oligomers/immunocytochemistry	Morphological atrophy of astrocytes; ~23% reduction in a number of processes and ~40% reduction in surface area.	Decreased neuroprotection; increase in synaptic loss; both effects ameliorated by intracerebroventricular injection of 10 ng transforming growth factor-β1	[32]
PDAPP-J20 mice/5 months/Immunocytochemistry	Decreased volume and complexity of hippocampal astrocytes		[8]
3xTG AD mice/1 to 24 months/Immunocytochemistry	Decreased volume, surface area and complexity of astrocytes in entorhinal and prefrontal cortices and in the hippocampus.	Loss of homoeostatic support	[73,97,98,161]

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Responses to reviewer's comments

Reviewer #1:

In their manuscript, the authors acknowledged the growing evidence that glial cells are not only affected by neurodegenerative processes, but are causative for neurodegeneration in diseases such as Alzheimer's. The authors are well-known experts in the field. The composition of the manuscript is reasonable and the selected literature well-balanced. However, there are many minor points, mainly addressing spelling, grammar and style, some of which are listed below. Therefore, I recommend a thorough check of the text.

Our reply: Thank you very much for constructive comments; we incorporated all your suggestions.

1) Many commas are missing.
Our reply: we proof read the text in particular from commas angle

2) P. 3, l. 18: Delete "n" in "Alzheimner's"
Our reply: Done
3) P. 3, l. 44: Delete one "single night" in "Even acute single night of sleep deprivation for a single night causes ..."
Our reply: Done
4) P. 5, l. 1: Change "reach" to "rich"
Our reply: Done
5) P. 6, l. 19: It is not clear what "disease- and disease-stage" means. The phrase appears to be incomplete.
Our reply: Thank you we meant "disease- and disease-stage specific"; teh text is amended
6) P. 6, l. 20: Reconsider the sentence and the numbering of "Conceptually, astrogliopathological phenotypes are classified into three major groups: (i) reactive astrogliosis (ii) astrodegeneration with astroglial atrophy and loss of function; (ii) pathological remodelling"
Our reply: Done
7) P. 8, l. 57: The parenthesis is not closed in "(Table 1, [53,92,29,7]."
Our reply: Done
8) P. 9, l. 4: Change "AD mice model" in "AD mouse models"
Our reply: Done
9) P. 9, l. 8: Delete "s" in "signals"
Our reply: Done
10) P. 9, l. 49: Change "stem cells derived astrocytes" in "stem cell-derived astrocytes"
Our reply: Done
11) P. 10, l. 50: Lower case "p" in "Positron-emission"

Our reply: Done
12) P. 11, l. 7: Change "In recent decade" either in "In the recent decade" or in "In recent decades"
Our reply: Done
13) P. 11, l. 9: Change "patomorhological" in "pathomorphological"
Our reply: Done
14) P. 11, l. 16: "Alzheimer" to "Alzheimer's"
Our reply: Done
15) Fig. 3, legend: Add "A" and "B" to the legend.
Our reply: Done
16) Fig. 4. Panel A is not correctly explained in the legend. "(A)" actually refers to panel B. Information referring to panel A is missing.
Our reply: Done
17) Fig. 4. "Scale bar = 10 um". there is no scale bar in the figure.
Our reply: it was actually - but too thin, and made it ticker.

Reviewer #2:
Verkhratsky and co-authors provide an up-to-date and timely review focusing on the changes in astrocytes in neurodegenerative (and Alzheimer's) disease, which will be very valuable to the broad readership of Pfluegers Archive. The historical views and broad introduction are informative and these days neglected by most authors. My comments to the abstract reflect my most important concerns; the authors should make an effort to better clarify the topic of astrocytopathy versus different from of astrogliosis that in are discussed throughout the text.

Our reply: Thank you very much for constructive comments; we incorporated your suggestions.

1. Abstract: several terms are used which are not clearly defined. Reactive gliosis is generally viewed as being accompanied by hypertrophy of astrocytes, the latter mostly referring to the increase in GFAP expression and increased thickness of primary processes. What then is meant by "hypotrophy "? The same is true for the terms "astrogliosis"/"astrogliotic response"/"astroglial hypothrophy / atrophy / paralysis". The abstract would greatly profit from a clear definition and separation of these terms. Alternatively, these terms should be omitted and more general summarizing statements be made in the abstract.

Our reply: We edited the abstract; obviously the word “hypotrophy” was a typo; astrogliosis is indeed characterised by hypertrophic changes.
As to terminology: (i) Astrogliosis;(ii) Astroglial atrophy and (iii) Pathological remodelling of astrocytes - this has been defined in several recent publications and are quite generally accepted (see consensus paper by Pekny M et al. (2016) Astrocytes: a central element in neurological diseases. Acta Neuropathol 131:323-345 doi:10.1007/s00401-015-1513-1; and recent essay by us Verkhratsky A, Zorec R, Parpura V (2017) Stratification of astrocytes in healthy and diseased brain. Brain Pathol 27:629-644 doi:10.1111/bpa.12537). To make this terminology easy for a reader we have now included a text box with a glossary of the terms.

2. There is a lot of evidence showing that astrocytes are not just "homeostatic" cells but actively contribute to brain communication.

Our reply: We fully agree that astrocytes communicate with other brain cells through numerous mechanisms; all their homeostatic cascades are “active” and do affect neurones and synapses. We have discussed these matters in depth in previous publications (e.g. Physiol Rev 2018).

3. Page3, line 59: what is meant by "compromised synaptic physiology"; isn't hyperexcitability always "aberrant"?

Our reply: we agree and we amended the text accordingly

4. Page 4, lines 1-7: white matter (oligodendrocyte) damage is probably not primarily associated with failure in homeostatic neuroglia, which, in the context of the review here, relates to astrocytes?

Our reply: Oligodenrocytes are as homeostatic as astrocytes in white matter - they support axons in many ways including for example supplying them with lactate. In this context we simply mentioned that oligodendrodegneration (which is rather prominent in AD) contributes to overall “unwiring” of the brain.

5. The term "homeostasis/homeostatic" is used quite often throughout the text. What exactly is meant by that? It is used as if "homeostasis" is the ultimate goal and ultimate condition to attain. Life as such is probably never in a "homeostatic" condition.

Our reply: this is of course very interesting and philosophical question; we as such are following the ideas of Claude Bernard, Walter Cannon and Joseph Barcroft. Failure in physiological homeostasis means disease and inability of the organism to maintain physiological homeostasis leads to extinction of biological entity; failure of homeostatic control of brain tissue signals its inability to perform - i.e. dementia in the broadest reading of it.

6. Page 5, line 28: There is no clear evidence that "neurological disorders are the failure of tissue homeostasis". This is a rather general statement and as such, debatable. What is meant by tissue homeostasis?

Our reply: This is how we define the disease: disease is failure of homeostatic systems which are unable anymore to maintain structural and functional stability. This definition is obviously open to debate, which we of course welcome. We agree however that the word “tissue” in inappropriate in this context and we amended the text accordingly.

7. Page 6, lines 16: Neurones can well survive without glia, this is the basis for primary neuronal cell cultures…

Our reply: well, purified cultures of central neurones (i.e. without astrocytes) do no thrive and form much less synapses; at the organism level ablation of glia is lethal for

neurones.

8. Pages 3-6: the text somewhat jumps back and forth, addressing properties of astrocytes and their arrangement in networks , as well as their physiological roles in healthy tissue and then mentioning changes in response to different pathologies. The review would greatly profit from a more organized separation of topics to discuss: e.g. first describe astrocytes and their function /organization in the healthy brain, and only then describe astrocytes under different pathological conditions.

Our reply: this is the matter of opinion: we feel that we did exactly what is proposed by the reviewer.

9. Page 6/7 and following: it is clearly debatable that reactive gliosis in a purely defensive programme. Reactive astrocytes reduce their "homeostatic" functions and have been reported to "de-differentiate", including a reduced expression of glutamate transporters, KIR-channels, NKA, gap junction coupling. Increased expression of GFAP should be mentioned, as well as changes in their morphology, retraction of processes …. This, however, is described only later (page 7), and referred to as "astrodegeneration". Again, it seems mandatory, that the different terms used ("reactive astrogliosis", "astrodegeneration", "astrotrophy",…) are better defined. Glial scar formation, involving reactive gliosis and proliferation of astrocytes must be separated from "hypertrophy" and "astrotrophy".

Our reply: we are somewhat confused by this logic. We of course agree that astroglial reactivity may produce neurotoxic phenotypes and we state that ("Being driven to an extreme by the severity of the insult, glial cells can assume the role of natural killer and contribute to the neural cell death, and yet even neurotoxicity of glia has, at its core, neuroprotective significance. "; our argument is rather simple - evolution never selects for negative mechanisms only for positive or neutral; hence astroglial neurotoxicity has at its core some beneficial effects - e.g. elimination of compromised neurones which may help faster resolution of pathology. The scar is obvioulsy defensive, this seems rather obvious - a making a wall between healthy and damaged tissues.; of course if driven out of balance neurotoxic phenotypes facilitates cell death and overall demise of tissue which we clearly acknowledged. We do not use term "astrotrophy"? The terms "atrophy" and loss of function which we employ are of very general usage in medical literature. Now we have introduced a glossary to clearly define these.

10. Page 9, lines38 ff: reactive astrocyte near lesions are characterized by aberrant calcium handling, which should be discussed.
Our reply: We fully agree with this; and yet in this paper we do not discuss much astroglial reactivity in AD; this has been covered by many excellent reviews; we specifically focus on astroglial atrophy, and hence this discussion is, in our opinion, outside of the scope.

11. Page 9, lines 54/55: the consequences of a retraction of astrocyte processes from synapses are not clear. Retraction of glial processes might also cause a widening of the ECS and a "dilution" of glutamate that escapes the synaptic cleft.

Our reply: In fact the main consequence of astroglial process retraction would be removal of glutamate transporters from the synapse. Indeed enhanced spillover obviously causes aberrant synaptic transmission - and weakens synaptic potentiation - this is exactly our logic.

12. Page 10, lines 8/9: reduction of glutamate uptake might be accompanied by de-sensitization of AMPA receptors.

Our reply: possibly yes however we are not aware about any data on this matter in the context of AD.

13. Page 10, line30: it is not clear from the text above, that astrogliosis is a "powerful defensive programme". The examples mentioned later show, at best, a correlation between astrocyte gliosis, amyloid deposition, and AD. At present, this seems rather like a "chicken and egg" problem, which should be acknowledged.

Our reply: we politely disagree; again this are all matters of opinion and writing style.

14. Fig. 1: the direct line between "specialized astrocytes" and "Human astrocytes" does not make sense.
Our reply: Thank you, we amended a figure accordingly

15. Fig. 2: should be revised and then could help in clarifying the different expressions used (see comments above). The term "astrogliopathology" somewhat bears the notion that astrocytes degenerate themselves. Reactive astrocytes are e.g. also observed during healthy ageing, is this pathological already? Again, please define what is mean by reactive astrogliosis and include all other terms used in the text.

Our reply: This figure shows classification of pathological changes which has been explained in detail in previous papers, which is of course fully acknowledged - this figure is take from Pekny et al., Acta Nauropathol, 2016. We do not call anywhere reactive astrocytes “pathological”; and yes astrocytes do degenerate by themselves in many neurological diseases; all this has been covered previously and we see no reason in repeating this here. We added the Glossary of terms to the text.

16. Fig. 3: apparently shows GFAP-labelling? Please state in the legend. GFAP does not delineate the entire morphology of the cells.

Our reply: Thank you; and we modified figure legend and the text accordingly.

17. Fig. 4: needs further explanation in the legend as well. These are cells derived from iPSCs?
Our reply: Yes and we edited the figure legend

18. General: the text is clearly written, but many typos and grammatical errors remain (e.g. page 3, lines 8/9, should read: "processes"; line 10: "A direct link…"; …). Please proofread carefully.

Our reply: we read the paper and amended many of those small typos - thank you.

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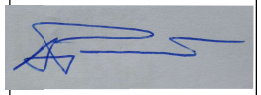
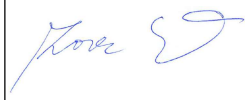
3rd author	Augustas	Pivoriunas	I agree to the proposed new authorship shown in section 4 /**and the addition/removal*of my name to the authorship list.**		State Research Institute Centre for Innovative Medicine	Aug 22 2019
4th authors	Robert	Zorec	I agree to the proposed new authorship shown in section 4 /**and the addition/removal*of my name to the authorship list.**		University of Ljubljana	Aug 22 2019
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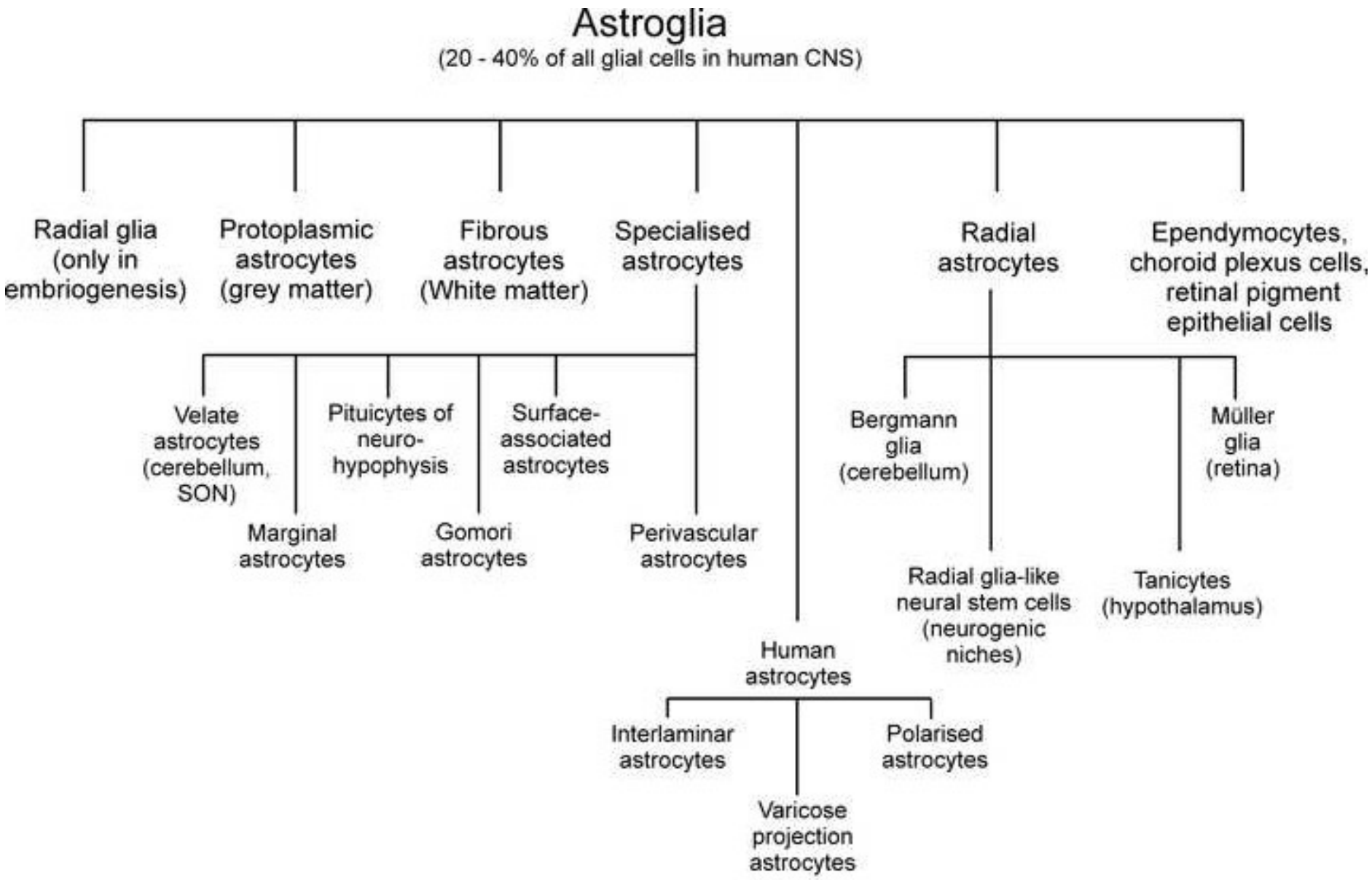

Fig. 1

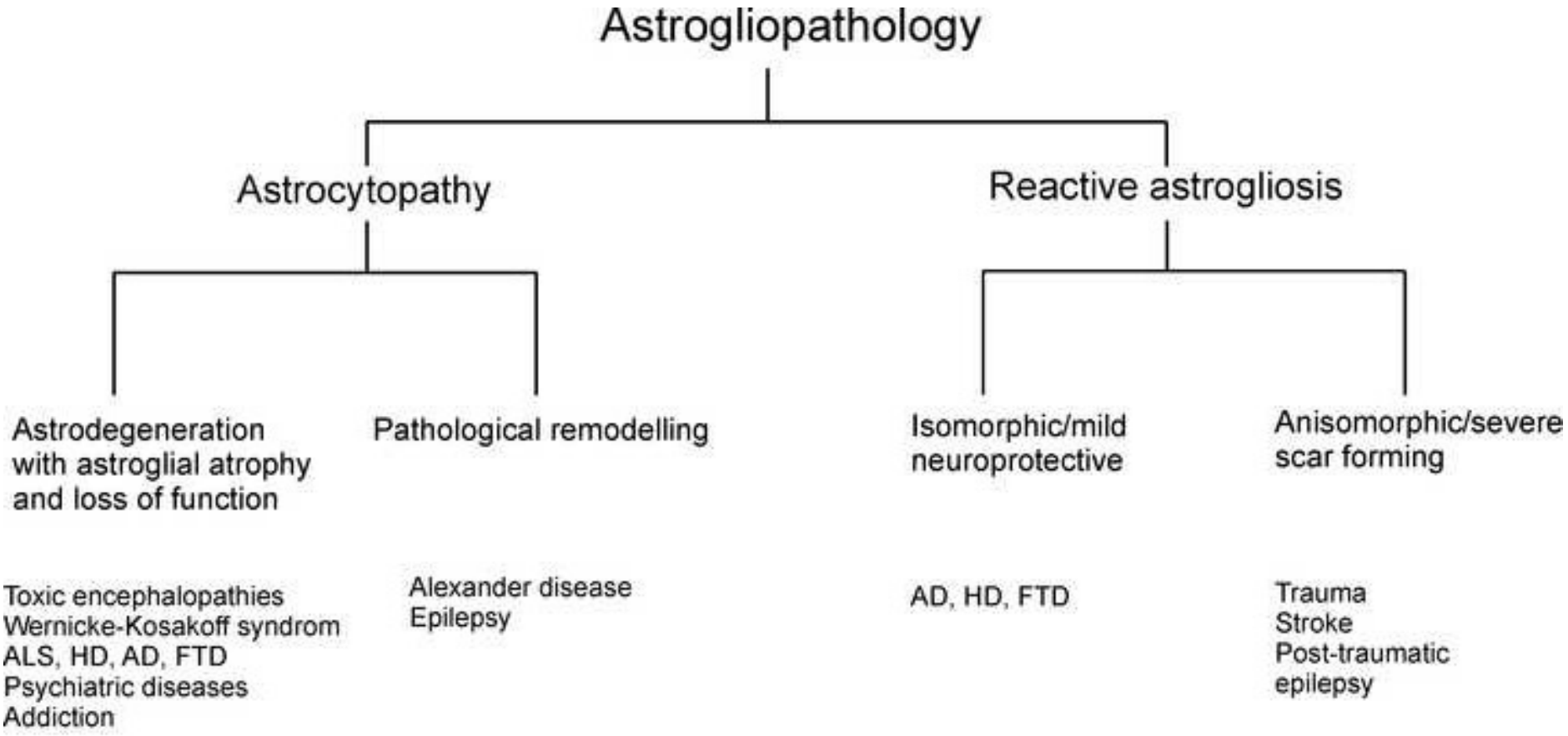

Fig. 2

Figure 3

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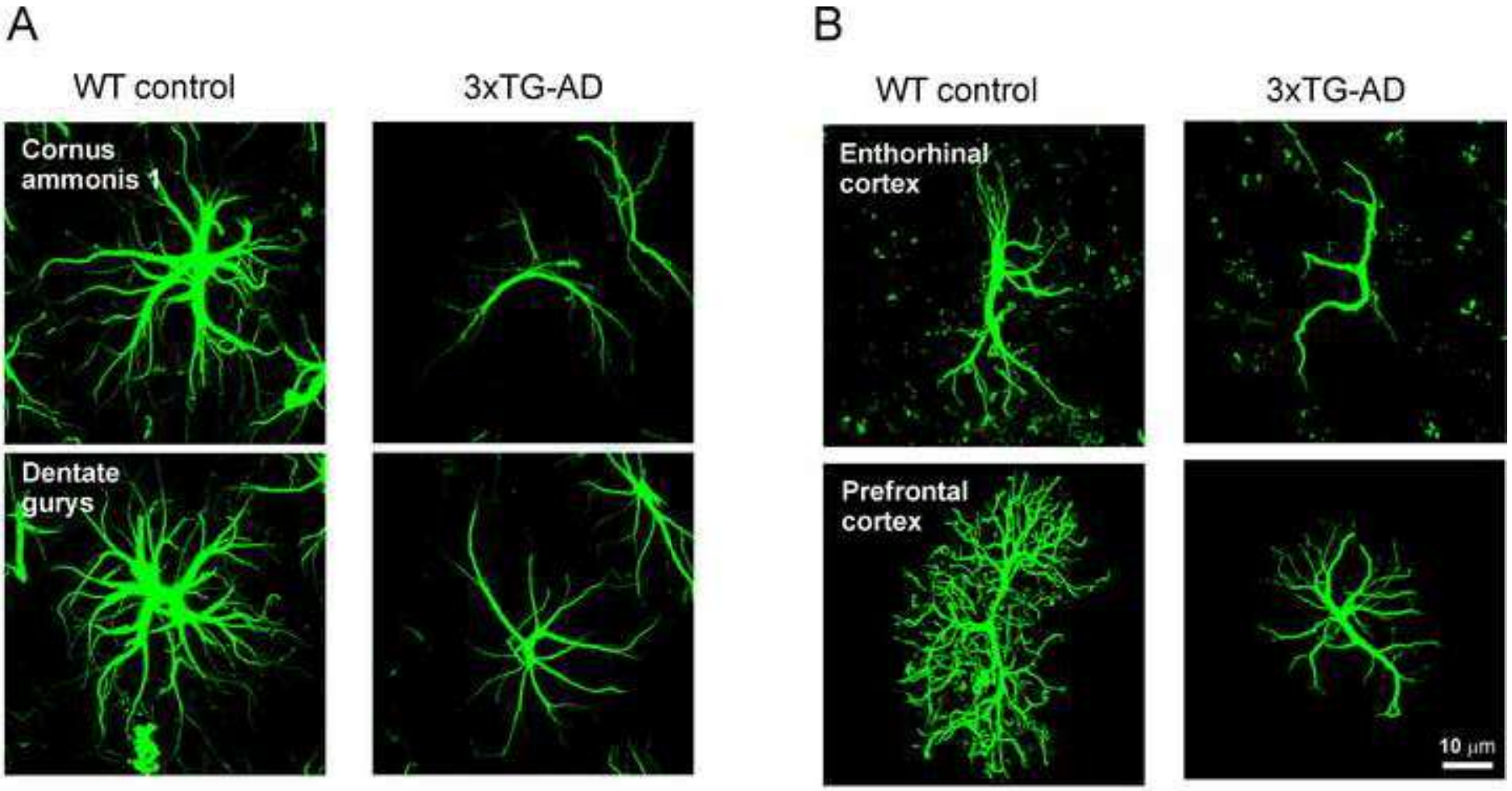

Fig. 3

Figure 4

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