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Astrocytes in Alzheimer's disease and other age-associated dementias; a supporting player with a central role

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Abstract

Astrocytes have essential roles in the central nervous system and are also implicated in the pathogenesis of neurodegenerative disease. Forming non-overlapping domains, astrocytes are highly complex cells. Immunohistochemistry to a variety of proteins can be used to study astrocytes in tissue, labelling different cellular components and subpopulations, including GFAP, ALDH1L1, CD44, NDRG2 and amino acid transporters, but none of these label the entire astrocyte population. Increasing heterogeneity is recognised in the astrocyte population, a complexity that is relevant both to their normal function and pathogenic roles. They are involved in neuronal support, as active components of the tripartite synapse and in cell interactions within the neurovascular unit, where they are essential for blood brain barrier maintenance and neurovascular coupling. Astrocytes change with age, and their responses may modulate the cellular effects of neurodegenerative pathologies, which alone do not explain all of the variance in statistical models of neurodegenerative dementias. Astrocytes respond to both the neurofibrillary tangles and plaques of Alzheimer's disease, to hyperphosphorylated tau and Aβ, eliciting an effect which may be neuroprotective or deleterious. Astrocyte hypertrophy, in the form of gliosis, occurs, but also astrocyte injury and atrophy. Loss of normal astrocyte functions may contribute to reduced support for neurons and dysfunction of the neurovascular unit. Understanding how astrocytes contribute to dementia requires an understanding of the underlying heterogeneity of astrocyte populations, and the complexity of their responses to pathology. Enhancing the supportive and neuroprotective components of the astrocyte response has potential translational applications in therapeutic approaches to dementia.

Abbreviations: Aβ Beta-amyloid; AD Alzheimer's disease; ALDH1L1 aldehyde dehydrogenase 1 family member L1; ARTAG aging related tau astrogliopathy; BBB blood brain barrier; CAA cerebral amyloid angiopathy; CBF cerebral blood flow; CFAS Cognitive Function and Ageing Study; CNS central nervous system; CLU clusterin; CR1 complement receptor 1; EAAT excitatory amino acid transporter; EGFP – enhanced green fluorescent protein; ET-1 Endothelin-1; FTD frontotemporal dementia; GFAP glial fibrillary acidic protein; GLAST glutamate/aspartate transporter; GLT-1 glutamate transporter-1; GWAS genome-wide association studies; MND Motor Neurone Disease; NDRG-2 N-myc downregulated gene 2; NFT neurofibrillary tangle; NVU Neurovascular unit; SASP senescence associated secretory phenotype; SOD-1 superoxide dismutase-1; TREM2 triggering receptor expressed on myeloid cells 2

Astrocytes in the Context of Dementia Research

The neuron is the target for many of the cellular pathologies of dementia, such as neurofibrillary tangles (NFTs) and Lewy bodies, whilst extracellular beta-amyloid (Aβ) may also target neurons and their processes. These classical pathologies remain central to theories of dementia pathogenesis and to neuropathological diagnosis, and much of the focus in dementia research has been on the neuron. However, although important correlates of dementia, other factors may also be important. Population based studies such as the UK Medical Research Council Cognitive Function and Ageing Study (CFAS), and other large autopsy series, have shown that classical neuropathological lesions alone do not account for all of dementia in statistical models, particularly at the oldest ages (1-8). This is also recognised in clinical trials with the identification of individuals with Alzheimer's Disease (AD)-like clinical presentations but with little pathology on amyloid imaging or on neuropathology, so-called AD with insufficient neuropathology (9). Different molecular forms of amyloid, tau and other protein pathologies are important on-going areas of therapeutic targets, but other factors may contribute to age-related cognitive decline either independently or by modulating the effect of the classical cellular and molecular pathologies on neuronal fate and function. This includes other cell types and the interactions between them.

Neurons function within the context of other cell types, and recent theories have emphasised the importance of dysfunction of the neurovascular unit (NVU) in ageing and in development of neurodegeneration and dementia (10, 11). The NVU includes the neuron, the microvasculature composed of endothelial cells and pericytes, astrocytes and the blood brain barrier (BBB). Oligodendrocytes and microglia are additional key components. A neurovascular hypothesis of dementia would then seek to integrate changes in the neuron with alterations of other cell types and their interaction within the NVU.

Long thought of as "just" a supporting cell, or brain glue, the astrocyte has important physiological roles in relation to the neuron, to synaptic function, and to blood brain barrier and neurovascular coupling. Astrocyte dysfunction may therefore be an important contributor to dysfunction of the NVU, with effects on neurons and microvasculature. This review is focused on the role of the astrocyte in the brain and how perturbation of its function may contribute to dementia pathogenesis, particularly focusing on AD.

Astrocyte Biology

Classification

Astrocytes were first described by Virchow in 1846 and were originally thought to be a homogenous population of cells which function to support neurons. The term 'astrocyte' was first used in 1893 by Michael von Lenhossek and refers to the stellate morphology of the cells (12). There has been much debate around what constitutes an astrocyte given the diversity of this cell type and the commonalities with other cell types of the central nervous system (CNS). A review by Kimelberg et al., sets out the following criteria for defining this cell type : 1) Nonexcitability; 2) A very negative membrane potential which is determined by the K^+ gradient; 3) Glutamate and GABA uptake by astrocyte-specific transporters; 4) the presence of intermediate filament bundles; 5) the presence of glycogen granules; 6) cell processes surrounding blood vessels and synapses; 7) the presence of gap junctions formed from connexins (13).

Astrocytes have been classified into two major morphological groups on the basis on their morphology and anatomical location; the fibrous astrocytes of the white matter and the protoplasmic astrocytes of the grey matter. They have distinct morphologies, initially identified through the use of Golgi staining, with protoplasmic astrocytes having a more complex structure, with many finely branching processes (12). Although both Golgi staining and immunolabelling of protoplasmic astrocytes have provided insights into the structure of these cells it is estimated that they reveal only a small proportion of total astrocyte volume. Immunohistochemistry for glial fibrillary acidic protein (GFAP), a structural protein found only in the main branches of the astrocyte, has been estimated to identify around 15% of the cell. Die injection studies enable a larger proportion of the total astrocyte volume to be revealed, and have identified that protoplasmic astrocytes organise themselves into distinct domains with no overlap apparent (14). The significance of these domains is not well understood but they are functional territories with autonomous features that are thought to be central to the astrocytes ability to coordinate synaptic activity and blood flow (12, 15). Fibrous astrocytes send out long processes which are less elaborate than their grey matter counterparts. These processes contact axons at nodes of Ranvier as well as establishing perivascular end-feet. Fibrous astrocytes are organised along white matter tracts and unlike protoplasmic astrocytes do not organise themselves into domains.

This binary classification, however, is an oversimplification. Several morphological types can be recognised in human cortex and are more complex than those found in other animals, such as rodents, that are often used for disease modelling. For example, the interlaminar astrocyte, which is found in superficial cortex with long processes extending into cortex, is unique to higher primates (16, 17).

Much of the complexity of human brain is thought to arise from this phylogenetic evolution of astrocytes. Functional complexity is also being recognised but, as yet, has not been accounted for in neurodegeneration research.

Developmental origin

There are three distinct pools of glial progenitors in the cerebral cortex, radial cells of the ventricular zone, postnatal glial progenitor cells of the subventricular zone (SVZ) and glial restricted precursors. The protoplasmic astrocytes of the grey matter are mostly generated by embryonic radial glia whereas the fibrous astrocytes of the white matter are derived from neonatal SVZ progenitors (18). It is generally accepted that astrocytes develop after neurons in the developing central nervous system although in spite of this they are thought to play a role in guiding axon migration and synaptic pruning (19) Further, astrocyte-like cells residing in the adult subventricular zone can function as multi-potent stem cells in vivo and are the major source of adult neurogenesis in the olfactory bulb and hippocampus (20, 21). However, it is important to consider that the developmental origins of astrocytes are fundamentally different across species, suggesting that the contributions of astrocytes to the functioning CNS also differ across species (22).

Glial Fibrillary Acidic Protein

Since their initial classification numerous techniques have been used to further investigate astrocyte morphology and phenotype including immunohistochemical techniques. GFAP is the prototypical marker for identifying astrocytes immunohistochemically. GFAP is a class III intermediate filament protein, encoded by a gene on chromosome 17q21. There are several isoforms, including GFAP-α, which is the main isoform in the CNS, and GFAP-δ, which is particularly expressed by neurogenic subventricular astrocytes. A single nucleotide frameshift produces GFAP+1 variants that are expressed in a subset of astrocytes (23). Mutation in GFAP produces Alexander disease, a primary astrogliopathy characterised by Rosenthal fibre accumulation (24).

GFAP expression increases with age in animal models, in which oxidative stress may play a role (25). It is also upregulated non-specifically under pathological conditions in the hypertrophic response of (astro)gliosis. This is a complex phenomenon, reflecting a spectrum of changes, from up-regulation of GFAP, increasing GFAP with proliferation and loss of domain structure through to compact glial scar formation with disruption of tissue architecture (26). The term isomorphic gliosis has been used where gliosis does not disrupt the underlying cytoarchicture of the brain, so that remodelling of neuronal networks is permitted, whereas the term anisomorphic gliosis has been used where a permanent glial scar disrupts tissue architecture, preventing remodelling (27).

GFAP up-regulation, detectable by immunohistochemistry, is a reliable marker for identifying reactive astrocytes, but astrogliosis also involves up-regulation of other intermediate filament proteins, namely vimentin, nestin and synemin (28, 29). Although astrogliosis appears to be a stereotypic reaction histologically, transcriptomic analysis shows that the pattern of gene expression within reactive astrocytes varies according to the stimulus, which is relevant to the functional role that astrocytes are likely to play in different pathological states (30).

Astrocytes in the neurovascular unit

The main areas of astrocyte function are summarised in Figure 2. Protoplasmic astrocytes form extensive contacts with blood vessels and the microvasculature and play a central role in regulating blood flow in the brain. They release prostaglandins, nitric oxide and arachidonic acid in order to cause vasoconstriction or vasodilation of blood vessels, often in response to changes in synaptic activity. They have a role in neurovascular coupling, by which local cerebral blood flow is matched to neuronal activity and energy requirements (31). In addition to effects on vasculature from neurons themselves, astrocytes appear to be able to mediate both vasoconstriction and vasodilation. Changes in astrocyte intracellular calcium with activity lead to formation of arachidonic acid and release of vasoactive substances. Although incompletely understood, astrocyte responses may contribute to changes in functional MRI signals and these responses may be altered in ageing and CNS disease states.

Close apposition of astrocytic end-feet with the vasculature is important in the induction and maintenance of the BBB (32). In vitro studies have demonstrated that astrocytes can induce barrier properties in cerebral endothelial cells and it has been shown in vivo that loss of GFAP-positive astrocytes from the inferior colliculus of rat brain is accompanied by widespread loss of the tight junctional proteins claudin-5, occludin and ZO-1 (33, 34). Perivascular astrocytes are also vital for ionic and water homeostasis. Their end-feet are enriched in potassium channels, important for the spatial buffering of K^+ in the extracellular space, whilst water movement through aquaporin-4 channels is essential to osmotic balance (35).

The tripartite synapse and astrocyte signalling

Astrocytes have been shown to regulate the stability, dynamics, and maturation of dendritic spines (36, 37) and take part in the regulation of synaptic plasticity and synaptic transmission (38). The tripartite synapse was a phrase coined 16 years ago (39) to define the close proximity of astrocyte processes to pre- and post-synaptic neuronal elements (40) (Figure 2). This strategic positioning of astrocytes enables an interactive dialogue to occur between these two cell types which is central to the local and widespread communication of information across the brain.

The exchange begins with the release of neurotransmitters from the pre-synaptic cleft, which triggers activation of metabotropic or ionotropic receptors on the astrocyte cell surface. Neuronally released transmitters such as glutamate, noradrenaline, histamine and acetylcholine are all capable of binding to receptors on the surface of astrocytes, which leads to a transient increase in calcium levels within the cell, the extent of which is dependent on the intensity of synaptic activity. In rat brain a single hippocampal astrocyte is estimated to be associated with approximately 140000 hippocampal synapses (41) meaning that the processes of one astrocyte are in close proximity to a number of stimuli of differing intensities. Astrocytes integrate external synaptic activity and generate a calcium wave of corresponding magnitude that can exist in the small microdomain of a process or propagate throughout the cell. More recently it has been shown that calcium signalling in astrocytic processes can occur independently of signalling in the cell body, and this can compartmentalise neuronal activity to a specific region of the astrocyte (42). Calcium waves are not restricted to one astrocyte and can propagate via gap junctions to neighbouring astrocytes. Importantly, calcium oscillations in astrocytes are not just a chemical conversion of synaptic activity but can occur spontaneously and excite neighbouring neurons (43).

In response to neuronal activity astrocytes produce gliotransmitters to facilitate communication between neurons. Gliotransmitter release occurs via a number of different mechanisms; calcium dependent exocytosis (44), non-exocytotic release from cytosolic pools; and calcium-independent channel mediated release. The molecular mechanisms that regulate the release of these neuroactive molecules (e.g glutamate, adenosine, GABA, ATP and D-serine) is not fully understood but is thought to be predominantly controlled by calcium (45). Astrocytic release of gliotransmitters offers multiple ways of controlling synaptic activity as astrocytes are able to selectively release a range of different gliotransmitters to individual synaptic inputs, each one having different mechanisms of action at the pre or post-synaptic neuronal elements (46). In addition gliotransmitters interact with other cell types in the vicinity such as microglia and vascular cells.

Astrocytes can further regulate signalling in the brain via the efficient removal of synaptically released inhibitory and excitatory neurotransmitters respectively known as ϒ-aminobutyrate (GABA) and glutamate, which are taken up through transporters on perisynaptic astrocytic processes (47). Astrocytes can directly convert glutamate to glutamine (48) which upon release from the astrocyte is used by neurons to replenish intracellular stores of glutamate and GABA. Uptake of glutamate is also important for astrocytic amino acid metabolism. The speed astrocytes clear neurotransmitters from the synaptic cleft affects the level of post-synaptic activation (49). Thus activity at the synapse can be regulated by both the release and uptake of neuroactive molecules, and activity at a single synapse can quickly lead to ripples of bidirectional chemical crosstalk between a network of astrocytes and neurons.

Finally, astrocytes are also capable of simultaneously integrating information beyond the synapse, such as from vascular cells and microglia, which allows them to finely tune the complex neuronal circuitry within a dynamic microenvironment. Astrocytes form networks, connected via gap junctions, which include connexins, which add a further layer to the integration of astrocytic and neuronal function (50).

Astrocyte metabolism and neuronal support

Astrocyte support in the form of neurotransmitter recycling, provision of energy substrates and cholesterol are important mechanisms by which astrocytes support the viability and function of neurons (Figure 2). Such astrocyte metabolic activities are important in astrocyte involvement in memory and learning (51) and the positioning of these cells means they are able to provide glucose and other molecules for neuronal metabolism, and to sense changes in neural activity in order to facilitate this. Astrocytes are known to avidly take up glucose and typically present a high glycolytic rate (52) and the metabolic needs of active neurons are at least partly met by non-oxidative glucose metabolism. The astrocyte-neuron lactate shuttle hypothesis proposes that astrocytes metabolise glucose to lactate which is then taken up by neurons and used as a fuel source (53). In addition to this proposed function, astrocytes also store glucose in the form of glycogen which can act as a short-term energy buffer during periods of high neuronal activity, for example during learning and memory when astrocytic glycogen can be used to liberate lactate as an energy substrate for neurons (54, 55). Astrocyte cholesterol metabolism is a further important area. Cholesterol is essential for normal brain function, including synaptic and receptor function. Astrocytes are the main source for neuronal cholesterol, via the APOE lipoproteins (56, 57).

Cytopathology of Astrocytes in Ageing and Neurodegeneration

Identifying astrocytes in tissue-based studies

Many neuropathological studies of astrocytes have relied on the use of GFAP to detect these cells. However, many healthy astrocytes do not express detectable levels of GFAP, and expression of GFAP can depend upon the anatomical location of astrocytes as well as the species in which GFAP expression is being examined (26). This can skew studies into the role of astrocytes in disease as only the population with up-regulated GFAP, reflecting the more reactive cells, is detected. This limitation of current detection methods should be kept in mind when interpreting the results of pathological studies. Thus, although GFAP might still be considered the 'gold standard' astrocyte marker there is a need for additional tissue markers to better capture the full spectrum of astrocytic

cells. A number of other potential markers are available and can be used on human post mortem tissue (Figure 1).

1. S100β marks many astrocytes, but is non-specific, labelling many other neuroglial cells (58-60).

2. The excitatory amino acid transporters (EAATs), involved in glutamate uptake from the synaptic cleft. There are 5 types of EAAT in human brain with EAAT1 and EAAT-2 primarily expressed by astrocytes (61). The rodent analogues of these are designated GLAST (glutamate/aspartate transporter) and GLT-1 (glutamate transporter-1) respectively. Double labelling shows that EAAT-2 and GFAP do not entirely overlap, so that there are EAAT2+/GFAP+, EAAT2+/GFAP- and EAAT2-/GFAP+ populations (62).

3. Glutamine synthetase is expressed in cell bodies and processes of astrocytes throughout the cortex (63).

4. CD44 is a cell surface glycoprotein that is a receptor for hyaluronic acid and may act as an adhesion molecule for astrocytes. This marker appears to be distributed more to processes in astrocytes and to identify particular subsets (64).

5. ALDH1L1 (aldehyde dehydrogenase 1 family, member L1, an enzyme involved in folate metabolism) is a marker identified from transcriptomic analysis of astrocytes. ALDH1L1 immunohistochemistry labels astrocyte cell bodies and processes and appears to label more astrocytes than GFAP (65). In our hands, ALDH1L1 immunohistochemistry also labels oligodendrocytes. In mice, it is expressed in cortical and spinal astrocytes and studies in the G93A mutant SOD1 (superoxide dismutase 1) motor neuron disease model suggest that it is upregulated in chronic neurodegeneration. These authors also showed that the gene expression profiles of ALDH1L1+ and GLT-1+ cells are similar, suggesting that they share astroglial identity (66).

6. NDRG2 (N-myc downregulated gene 2) is expressed in the cell bodies and processes of astrocytes in humans and rodents. It appears to be involved in responses to stress but is downregulated in reactive astrocytes near a cortical glial scar. It has been suggested that NDRG2 may be a marker for mature non-reactive astrocytes (67, 68).

In summary, there are a number of astrocytic markers in addition to GFAP, which show varying labelling patterns. To date, ALDHL1 and NDRG2 hold out the best promise for general markers that pick up the largest proportion of the astrocyte population. However, some of these markers require further validation in human tissue and whilst they provide options for the analysis of astrocytes, cannot be considered a universal marker; considerations of astrocyte heterogeneity and subpopulations will still be required.

Astrocytes in ageing

Ageing is the leading risk factor for the common dementias, including AD, dementia with Lewy bodies and vascular dementia.. Whilst age may operate partly via development of the classical

neurodegenerative and vascular pathologies, age-related mechanisms may impair cells of the brain via a number of mechanisms (69) which could damage NVU function to act independently or in concert with classical neuropathologies. Astrocytes are affected by ageing, potentially impairing their complex functions or biasing their response to pathology to a less advantageous pattern.

GFAP mRNA and protein levels appears to increase with ageing in rodents and humans, accompanied (in some studies) by S100β (25, 70-72). Astrocytes in the ageing brain show features of senescence and expression of a senescence associated secretory phenotype (SASP). Transcriptomic studies also suggest a shift to a more proinflammatory phenotype in astrocytes with ageing (73-75) . These age-related astrocytic changes might contribute to declining brain function via loss of function and neuroinflammation, and contribute to the background in which neurodegenerative disease develops.

Astrocytic inclusions

AD is generally not associated with astroglial inclusions. However, tau positive thorny astrocytes are a feature of brain ageing (76, 77) . These are found particularly in subpial, subependymal and perivascular locations, particularly in mesial temporal structures and around brainstem. The inclusions may be Gallyas positive, suggesting that some of the tau is fibrillar, and immunohistochemical studies suggest that the inclusions are of 4-repeat tau. The significance and origin of these is currently uncertain, but they do not appear to be associated with dementia and studies have found either no, or a weak association with AD neuropathology. Those in a subpial location, however, are associated with subpial tau positive neuritic processes (78). Whether the astrocytes derive the tau from neuronally produce phospho-tau is currently unknown. Recently a consensus approach has been developed for assessment of the age related form of tau, which also includes "astrocytes with finely granular (tau) immunoreactivity in processes", under an umbrella term of Aging Related Tau Astrogliopathy (ARTAG) (79).

Astrocytes in Alzheimer's disease

The initial observation that astrocytes appeared 'activated' in AD brain was thought to be a secondary and non-specific response to the disease process (80), and a neurocentric view has predominated in AD and neurodegeneration research generally. However, astrocytes are central to pathogenic mechanisms in neurodegeneration. This may include toxic gains-of-function, such as production of cytokines and chemokines, or loss of their complex physiological functions, such as neuronal support (e.g. neurotransmitter recycling, energy substrate provision) and spatial buffering (e.g. K^+, H^+) (26). Essential to synaptic maintenance, disruption of the normal glio-neuronal interaction can lead to synaptic dysfunction and contribute to cognitive impairment (81). Studies in Motor Neurone Disease (MND) have shown that astrocytes affect disease progression (82-84), giving rise to a non-cell-

autonomous theory of MND, and establishing the principle that astrocytes have a deterministic role in pathogenic progression of a neurodegenerative disease.

The astrocytic response to beta-amyloid

The first clear evidence that astrocytes play an active role in the AD process was provided by Wyss-Corey et al., (2003) who showed that astrocytes were able to uptake and degrade Aβ using an in vitro system of cultured mouse astrocytes (85). However, this was not the first evidence for the uptake of Aβ by astrocytes since earlier studies had shown the presence of Aβ-containing astrocytes (86, 87). Astrocytes respond to plaques, along with microglia and their activation is important in pathogenesis. Since they can take up Aβ they are involved in plaque progression, with death of Aβ loaded astrocytes giving rise to secondary plaques (88).

The mechanisms governing the receptor-mediated uptake of Aβ are not fully understood, particularly whether the uptake of Aβ induces a change in astrocyte phenotype which alters their usual neuro-supportive function. One receptor identified as being involved in uptake and clearance of Aβ is the Low density lipoprotein receptor-related protein 1 (LRP1) (89, 90). LRP1 is also a receptor for the uptake of apoE and complexes of apoE-Aβ and highlights the importance of this receptor, as well as apoE, in astrocytic clearance of Aβ (91). However, it is possible that a number of as yet unidentified receptors are also involved in Aβ uptake.

Whether the response of astrocytes to Aβ is a protective mechanism or results in further damage is not clear with conflicting evidence which likely reflects differences in experimental approach, although it is worth considering that there may be an impact of age on the ability of astrocytes to uptake and degrade Aβ effectively. Evidence from cell culture models of AD suggest that the astrocyte response to Aβ actively contributes to the disease process; Garwood et al. (2011) have shown that the toxic properties of Aβ are enhanced in cultures of primary neurons with small contaminating numbers of astrocytes and that changes in tau phosphorylation and cleavage are only observed in the presence of astrocytes (92). This toxicity is associated with a distinct astrocytic inflammatory profile which might, amongst other things, increase the amyloidogenic processing of APP in astrocytes (93). This same paper has evidence showing $Aβ_{1-42}$ itself can further increase astrocytic BACE1, APP and β-secretase processing resulting in further increases in oligomeric and fibrillary Aβ, providing evidence astrocytes contribute to the production, as well as the degradation, of Aβ.

Astrocytes and neuroinflammation in AD

Neuroinflammation is a prominent and early feature of AD which plays a key role in modulating the progression of disease via a range of inflammatory mediators and neurotoxic compounds. Genome-wide association studies (GWAS) have identified several immune-associated genes that are associated with an increased risk of developing AD, including CLU (clusterin), CR1 (complement receptor 1) and TREM2 (triggering receptor expressed on myeloid cells 2) (94-97). While the role of microglia in the neuroinflammatory response in AD is well established [reviewed in (98)], several studies indicate that astrocyte-mediated inflammatory processes also contribute to neurodegeneration in AD through increased astrocytic expression of pro-inflammatory cytokines and chemokines, activation of the complement cascade, as well as reactive oxygen and nitrogen species [reviewed in ((99-101)]. Transcriptional analysis of GFAP$^+$ astrocytes isolated from cortex of Alzheimer's mice (APPswe/PS1dE9) reveals a proinflammatory phenotype compared to their wildtype littermates, with the number of genes induced and the fold-change in expression more pronounced in astrocytes than in microglia, suggesting that astrocytes contribute to cytokine production and play a central role in the disease process. These findings were further supported by comparisons with a human AD astrocyte transcriptomic data set, which showed a similar alteration in inflammatory changes (102). Astrocytes can also suppress innate immunity through αβ-crystallin, suggesting that they have a modulatory effect on neuroinflammation. Loss of this function (through knockout of the dopamine D2 receptor) enhances immune responses (103). Anti-inflammatory therapies targeting astrocytes in animal models of AD suggest that the astrocyte contribution to neuroinflammation is a potential and relevant therapeutic target (104).

Astrocyte hypertrophy and atrophy in Alzheimer's disease

Astrocyte hypertrophy has previously been documented in Alzheimer's disease brain (105, 106). The hypertrophic response, as described earlier, is characterised typically by increased GFAP expression and loss of domain structure. The CFAS neuropathology cohort is a population-based ageing brain (>65yrs) cohort that spans the spectrum of ageing brain pathologies. CFAS, and other population cohorts, allows assessment of relationships between pathology, risk factors and dementia without the biases inherent in pre-selection into clinical diagnostic groups [reviewed in (3, 107, 108)], providing complementary information to the usual case-control design. Studies in CFAS showed that the astrocyte hypertrophic response, characterised by GFAP expression, increases early in relation to the development of AD neuropathology as defined by Braak NFT stage. GFAP showed a closer relationship to compact than diffuse plaques. The trend for GFAP expression was the opposite of that for EAAT2 expression, suggesting loss of astrocyte function with AD progression. (62).

Although there is some differential expression of GFAP isoforms in terms of astrocyte sub-populations, transcript levels of most isoforms appear to increase in concert in AD and mouse AD-models, without evidence for differential AD-related isoform changes (109, 110). However, isoform

GFAP-ε (also designated GFAP-δ) appears able to bind presenilin-1 (111), so it remains to be determined whether isoforms play selective functional roles in the disorder.

Astrogliosis, may have functional pathological effects. Gliosis contributes to neuronal hyperexcitability through alterations to glutamate, GABA and chloride channels (112). Neuronal hyperexcitability occurs in AD, whilst in APPmt-transgenic AD models hyperexcitability is worsened by Aβ immunization (113). Whether astrocytes contribute to this as a further mechanism by which astrocytes contribute to neuronal dysfunction is unclear at present.

Whilst much of the work in human tissue has focused on hypertrophy, which is easily visualised using the currently available markers, there is also evidence for astrocyte atrophy. Careful morphological studies in transgenic models have suggested that, in addition to gliosis, astrocytes undergo atrophy particularly at early disease stages, supporting the importance of reduced function and atrophy in addition to hypertrophic and gain of function responses (114, 115). The fragmentation of calpain-positive glial processes (116) and the loss of EAAT2 (62) in human tissue may be reflections of this process. Loss of astrocyte processes are also seen in age related white matter lesions associated with serum plasma protein uptake and BBB dysfunction, producing clasmatodendritic astrocytes (117, 118). Astrocyte atrophy may reflect loss of function, so confining study to hypertrophic astrocytes identified by GFAP up-regulation may miss important changes.

Oxidative stress and the DNA damage response in AD

In addition to the hypertrophic response, there is evidence of cellular damage to astrocytes. Oxidative DNA damage can be found in astrocytes in the ageing brain, and is early in relation to AD stage, and the DNA damage response in astrocytes appears to parallel that in neurons and endothelium (119, 120). Unrepaired DNA damage may induce senescence or apoptosis. Glia express apoptosis-related markers in AD, such as p53 and CD95 (121), and β-galactosidase expression, a marker for senescence, can be found in astrocytes, although the quantitative relationship of this to AD is unclear (120).

Astrocyte injury may contribute to loss of their function or to a proinflammatory state via the SASP, and a number of factors may drive this injury. Oxidative stress is likely to be important and an early feature of AD (122-124). Oxidative stress is also a feature of ageing in the brain (125), and indeed the presence of oxidative DNA damage at the earliest AD stages suggests that oxidative DNA damage in astrocytes may be partly related to ageing mechanisms, a further point of interaction between ageing and neurodegeneration. AD-related molecular pathology may also contribute to astrocyte injury; aggregated Aβ can induce DNA damage and apoptosis (126, 127) and astrocytes around amyloid

deposits in both cored and non-cored plaques express apoptosis related molecules caspase 3 and CD95 (128).

Whilst it is clear that multiple factors may drive astrocyte pathology, related to both ageing and neurodegeneration, the relative partitioning of injury in vivo is uncertain. Further, different pathological drivers may produce different pathological molecular responses in astrocytes. So, for example, DNA damage but not AD pathology correlates with nuclear retention of the signalling molecule FOXO3a, suggesting differential effects of stressors on signalling in astrocytes (63).

Alterations in astrocytic signalling pathways.

Astrocytes host a complex network of signaling pathways which are altered with the development of disease pathology. Indeed signalling pathways involved in orchestrating glial activation which include STAT3, NFκB, and MAP kinases, have previously been investigated as potential therapeutic targets (129).

Gene expression analysis is one approach to dissecting out molecular responses in tissue and, combined with laser capture microdissection, can be applied to specific cell types (130). It should be noted that the laser capture method will produce a sample that, whist highly enriched for the cell-type targeted, is not a pure sample. Our group have previously used microarray-based gene expression analysis to define changes in astrocyte gene expression associated with AD progression. Analysis showed down-regulation of key cellular pathways, particularly in relation to signalling, including insulin, IGF-1, calcium and MAP-kinase signalling (131). The protein components of the insulin and IGF-1 pathways are present and functional in human cultured astrocytes and can be modulated by insulin-fructose treatment and IGF-1R monoclonal antibody treatment respectively (132). Insulin regulates metabolic processes such as glycogen synthesis and proliferation in astrocytes (133). IGF-1 signalling is important for astrocyte protection of neurons from oxidative stress (134) and interaction of IGF-1 and calcineurin in astrocytes has been implicated in pathogenesis in AD models (135). Insulin resistance is described in neurons in AD, and is a potential therapeutic target (136-139). Derangement of metabolic pathways regulated by insulin and IGF-1 in astrocytes may therefore also contribute to impairment of astrocyte function and their support for neurons as AD progresses.

Astrocyte subtypes in relation to AD pathology

In addition to physiological subtypes, there may also be pathological heterogeneity of astrocytes related to their spatial relationships to specific pathological lesions. Increased gliosis with AD progression correlates with NFTs, but the relationship to plaques is more complex in that gliosis diverges from plaque burden, but glia increase in the vicinity of plaques (140). The association of

peri-plaque astrocytes with better cognitive preservation may suggest that the response of astrocytes to this lesion is neuroprotective (141). Calpain upregulation with AD progression is observed in the long processes of interlaminar astrocytes (116), and there are alterations in astrocytes in white matter in AD (142, 143). Astrocytes cannot be considered as a homogeneous population and the differential effects of astrocytes in these various pathological compartments is a significant question.

The influence of APOE on astrocyte responses.

The mechanisms by which possession of the ε4 allele contributes to a higher risk of late-onset AD remain to be fully defined, but influence on astrocyte responses may be a contributory factor. Astrocytes respond to Aβ in an APOE-dependent manner (144) and possession of the ε4 allele is associated with a lower peri-plaque astrocyte response (141). Possession of ε4 is associated with higher degree of GFAP expression in demented individuals (145). There may also be interaction with therapy. Use of non-steroidal anti-inflammatory drugs in AD is associated with lower astrocytic counts that is more marked in association with ε4 (146). In a gene expression analysis of astrocytes, transcriptomic changes associated with AD progression were seen at earlier Braak NFT stage in association with ε4 allele possession, although this was a subsidiary analysis of the study based on few cases (131). Overall, however, these studies suggest a picture whereby the astrocyte response is more detrimental in individuals with the ε4 polymorphism.

Evidence for the role of astrocytes from other dementias

The involvement of astrocytes in dementia is not restricted to Alzheimer's disease. Vascular dementia is associated with dysregulation of cerebral blood flow, blood-brain barrier breakdown and increased inflammation and oxidative stress (147). There is considerable overlap between vascular dementia and AD, with evidence of cerebral amyloid angiopathy (CAA) frequently found in AD brains. CAA is the vascular accumulation of Aβ. Interactions of astrocytic endfeet with the vasculature, a critical component of the NVU, is thought to be disrupted by Aβ, with impairment of vascular responses. Hence the presence of CAA is likely to disrupt the NVU and could contribute to reduce blood flow to the brain in AD (148). In the ArcAβ mouse model, which exhibits severe vascular pathology, there are changes in astrocytes early in the disease process. These changes include retraction and swelling of astrocyte endfeet surrounding vascular Aβ deposits and reduced expression of the glucose transporter GLUT1 and monocarboxylate transporter 1 (MCT1) and were accompanied by evidence of neurovascular uncoupling and disruption of the BBB (149).

There is also evidence that changes in astrocytic endothelin-1 are involved in dementia associated with stroke. Endothelin-1 (ET-1), a potent constrictor, is synthesized by both astrocytes and endothelial cells in stroke and in AD brain where it plays an important role in neurovascular reactions.

In a study investigating the role of astrocytic ET-1 in post-stroke cognitive deficit it was found that astrocytic ET-1 overexpression contributed to neurodegeneration (in the GET-1 mouse model) (150).

In frontotemporal dementia (FTD), there is evidence that the severity of the disease is directly related to the degeneration of astrocytes. This is evidenced by an increase in astrocytes with beaded processes as well as apoptotic features which correlated with the degree of neuronal loss and the stage of the disease (151) and there is a suggestion that as the disease progresses unreactive, quiescent astrocytes become involved, further exacerbating the situation. An earlier study investigating astrocytic degeneration in sporadic FTD found that degenerating astrocytes correlated with decreased cerebral blood flow (CBF) and that areas of significant astrogliosis corresponded to areas of hypoperfusion, suggesting a potential causal role for astrocytes in disturbed CBF in FTD (152). It should be noted that these studies preceded current molecular subclassification of the frontotemporal lobar degenerations (the pathological pattern that underlies FTD).

Finally, astrocytic star-like inclusions, which resemble tufted astrocytic fibrillary tangles, are present in the brains of those with Dementia with Lewy Bodies and may contain α-synuclein, suggesting that in DLB a primary degenerative process takes place in both glial cells and neurons (153, 154). Astrocytes may be directly affected by inclusion formation, particularly with the deposition of phosphorylated tau, in specific neurodegenerative disorders. These include tufted astrocytes in progressive supranuclear palsy, astrocytic plaques in corticobasal degeneration and globular astroglial inclusions in globular glial tauopathy (155).

CONCLUSION

So what is the involvement of astrocytes in dementia? In MND there is evidence for a central role of astrocytes in the disease process; the "non-cell autonomous" theory (156, 157). Whether this applies to AD and other dementias is unknown, but the evidence for their cellular pathology and perturbation of function certainly suggests an important role. Whilst the potential dichotomy between loss of normal function and gain of a toxic function is well recognised, the complexity of this cell, including intrinsic heterogeneity and different responses in relation to different molecular and cell pathologies suggest that a more detailed and nuanced understanding of the variations in astrocyte responses is required. Given the central role of this cell in microvascular regulation and neuronal and synaptic support, enhancing the potential neurosupportive and neuroprotective functions of astrocytes, whilst inhibiting their proinflammatory injurious effects offers an additional therapeutic avenue to support brain function in dementia.

AUTHOR CONTRIBUTIONS
CJG and SBW wrote the paper with input from LER, JES, PRH and PGI.

There are no conflicts of interest to disclose.

FIGURE LEGENDS

Figure 1: Immunohistochemistry illustrating variation in immunostaining patterns for astrocyte markers in human cerebral cortex and white matter which showed gliosis. Sections from an (anonymised) case (Braak NFT stage I) from the CFAS ageing brain cohort. The left column shows low power views of cortex, the centre column high power view of cortex, right column high power view of white matter. Variation in staining patterns with antibodies to: GFAP (A, B, C); EAAT2 (D, E, F); glutamine synthetase (G, H, I); ALDH1L1 (J,K,L). Note variation in relative staining of cortical vs white matter astrocytes.

Figure 2: **The many roles of the astrocyte**: (A) Astrocytes provide energy substrates for neurons, primarily in the form of lactate which is converted from glucose as well as providing cholesterol to support neuronal function. (B) The intimate association of astrocytic processes with neuronal synapses enables the finely-tuned regulation of synaptic transmission. Neurotransmitters are released from the pre-synaptic terminal such as glutamate, noradrenaline, histamine or ACh that are capable of binding to post-synaptic receptors or to receptors present on the surface of astrocytic processes. This binding can trigger a calcium response in the astrocyte that results in the release of gliotransmitters such as GABA, ATP or D-serine that will regulate synaptic activity. Glutamate can also be recycled back to the precursor glutamine, which is released into the extracellular space and taken up by neurons to be resynthesised into glutamate. (C) Astrocytes couple together synaptic activity with local blood flow to ensure neurons have a sufficient energy supply. Prostaglandin (PG), nitric oxide (NO) and arachidonic acid (AA) are all released in response to changes in intracellular calcium levels, which causes vasoconstriction or dilation. Aquaporin 4 and K^+ channels are also present to maintain osmotic balance and for spatial K+ buffering, respectively. (D). Gap junctions such as connexin 43 enable dialogue between neighbouring astrocytes, which is mediated by intracellular changes in calcium levels.

References

1.	Matthews FE, Brayne C, Lowe J, McKeith I, Wharton SB, Ince P. Epidemiological pathology of dementia: attributable-risks at death in the Medical Research Council Cognitive Function and Ageing Study. PLoS Med. 2009 Nov;6(11):e1000180. PubMed PMID: 19901977. Pubmed Central PMCID: 2765638. Epub 2009/11/11. eng.

2.   Savva GM, Wharton SB, Ince PG, Forster G, Matthews FE, Brayne C. Age, neuropathology, and dementia. N Engl J Med. 2009 May 28;360(22):2302-9. PubMed PMID: 19474427. Epub 2009/05/29. eng.

3.   Wharton SB, Brayne C, Savva GM, Matthews FE, Forster G, Simpson J, Lace G, Ince PG. Epidemiological neuropathology: the MRC Cognitive Function and Aging Study experience. J Alzheimers Dis. 2011;25(2):359-72. PubMed PMID: 21422529. Epub 2011/03/23. eng.

4.   Fotuhi M, Hachinski V, Whitehouse PJ. Changing perspectives regarding late-life dementia. Nat Rev Neurol. 2009 Dec;5(12):649-58. PubMed PMID: 19918254. Epub 2009/11/18. eng.

5.   Boyle P, Wilson R, Yu L, Barr A, Honer W, Schneider J, Bennett D. Much of late life cognitive decline is not due to common neurodegenerative pathologies. Ann Neurol. 2013;74:478-9.

6.   Bennett D, Schneider J, Arvanitakis Z, Kelly J, Aggarwal N, Shah R, Wilson R. Neuropathology of older persons without cognitive impairment from two community-based studies. Neurology. 2006;66:1837-44.

7.   Haroutunian V, Schnaider-Beeri M, Schmeidler J, Wysocki M, Purohit D, Perl D, Libow L, Lesser G, Maroukian M, HT G. Role of the neuropathology of Alzheimer disease in dementia in the oldest old. Arch Neurol. 2008;65:1211-7.

8.   Prohovnic I, Perl D, Davis K, Libow L, Lesser G, Haroutunian V. Dissociation of neuropathology from severity of dementia in late-onset Alzheimer's disease. Neurology. 2006;66:49-55.

9.   Serrano-Pozo A, Qian J, Monsell S, Blacker D, Gomez-Isla T, Betensky R, Growdon J, Johnson K, Frosch M, Sperling R, Hyman B. Mild to moderate Alzheimer dementia with insufficient neuropathological changes. . Ann Neurol. 2014;75:597-601.

10.   Sagare A, Bell R, Zhao Z, Ma Q, Winkler E, Ramanathan A, Zlokovic B. Pericyte loss influences Alzheimer-like neurodegeneration in mice. . Nat Commun. 2013;2:2932.

11.   Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. Nat Rev Neurosci. 2011 Dec;12(12):723-38. PubMed PMID: 22048062. Epub 2011/11/04. eng.

12.   Oberheim NA, Goldman SA, Nedergaard M. Heterogeneity of astrocytic form and function. Methods in molecular biology (Clifton, NJ). 2012;814:23-45. PubMed PMID: 22144298. Pubmed Central PMCID: PMC3506190. Epub 2011/12/07. eng.

13.   Kimelberg HK. Functions of mature mammalian astrocytes: a current view. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry. 2010 Feb;16(1):79-106. PubMed PMID: 20236950. Epub 2010/03/20. eng.

14.   Bushong E, Martone M, Jones Y, Ellisman M. Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. . J Neurosci. 2002;22:183-92.

15.   Parpura V, Heneka MT, Montana V, Oliet SH, Schousboe A, Haydon PG, Stout RF, Jr., Spray DC, Reichenbach A, Pannicke T, Pekny M, Pekna M, Zorec R, Verkhratsky A. Glial cells in (patho)physiology. J Neurochem. 2012 Apr;121(1):4-27. PubMed PMID: 22251135. Pubmed Central PMCID: PMC3304021. Epub 2012/01/19. eng.

16.   Oberheim NA, Takano T, Han X, He W, Lin JH, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M. Uniquely hominid features of adult human astrocytes. J Neurosci. 2009 Mar 11;29(10):3276-87. PubMed PMID: 19279265. Pubmed Central PMCID: PMC2819812. Epub 2009/03/13. eng.

17.   Oberheim NA, Wang X, Goldman S, Nedergaard M. Astrocytic complexity distinguishes the human brain. Trends Neurosci. 2006 Oct;29(10):547-53. PubMed PMID: 16938356. Epub 2006/08/30. eng.

18.   Sovrea AS, Bosca AB. Astrocytes reassessment - an evolving concept part one: embryology, biology, morphology and reactivity. Journal of molecular psychiatry. 2013;1:18. PubMed PMID: 26019866. Pubmed Central PMCID: PMC4445578. Epub 2013/01/01. eng.

19.   Kessaris N, Pringle N, Richardson WD. Specification of CNS glia from neural stem cells in the embryonic neuroepithelium. Philosophical transactions of the Royal Society of London Series B,

Biological sciences. 2008 Jan 12;363(1489):71-85. PubMed PMID: 17282992. Pubmed Central PMCID: PMC2605487. Epub 2007/02/07. eng.

20.   Garcia AD, Doan NB, Imura T, Bush TG, Sofroniew MV. GFAP-expressing progenitors are the principal source of constitutive neurogenesis in adult mouse forebrain. Nature neuroscience. 2004 Nov;7(11):1233-41. PubMed PMID: 15494728. Epub 2004/10/21. eng.

21.   Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell. 1999 Jun 11;97(6):703-16. PubMed PMID: 10380923. Epub 1999/06/25. eng.

22.   Chaboub LS, Deneen B. Developmental origins of astrocyte heterogeneity: the final frontier of CNS development. Developmental neuroscience. 2012;34(5):379-88. PubMed PMID: 23147551. Pubmed Central PMCID: PMC3576470. Epub 2012/11/14. eng.

23.   Yang Z, Wang K. Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. Trends Neurosci. 2015;38:364-74.

24.   Messing A, Brenner B, Feany M, Nedergaard M, Goldman J. Alexander Disease. J Neurosci. 2012;32:5017-23.

25.   Morgan T, Rozovsky I, Goldsmith S, Stone D, Yoshida T, Finch C. Increased transcription of the astrocyte gene GFAP during middle-age is attenuated by food restriction: implications for the role of oxidative stress. . Free Radic Biol Med. 1997;23:524-8.

26.   Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. Acta Neuropathol. 2010 Jan;119(1):7-35. PubMed PMID: 20012068. Pubmed Central PMCID: 2799634. Epub 2009/12/17. eng.

27.   Verkhratsky A, Butt A. General pathophysiology of glia. . Glial Neurobiology A textbook Chichester, England: John Wiley and Sons; 2007. p. 155-66.

28.   Pekny M, Nilsson M. Astrocyte activation and reactive gliosis. Glia. 2005 Jun;50(4):427-34. PubMed PMID: 15846805. Epub 2005/04/23. eng.

29.   Jing R, Wilhelmsson U, Goodwill W, Li L, Pan Y, Pekny M, Skalli O. Synemin is expressed in reactive astrocytes in neurotrauma and interacts differentially with vimentin and GFAP intermediate filament networks. . J Cell Sci. 2007;120:1267-77.

30.   Zamanian J, Xu L, Foo L, Nouri N, Zhou L, Giffard R, Barres B. Genomic analysis of reactive astrogliosis. J Neurosci. 2012;32:6391-410.

31.   Howarth C. The contribution of astrocytes to the regulation of cerebral blood flow. . Front Neurosci. 2014;8:Article 103.

32.   Lecuyer M-A, Kebir H, Prat A. Glial influences on BBB functoins and molecular players in immune cell trafficking. . Biochim Biophys Acta. 2015;In Press. http://dx.doi.org/10.1016/j.bbadis.2015.10.004.

33.   Willis CL, Leach L, Clarke GJ, Nolan CC, Ray DE. Reversible disruption of tight junction complexes in the rat blood-brain barrier, following transitory focal astrocyte loss. Glia. 2004 Oct;48(1):1-13. PubMed PMID: 15326610. Epub 2004/08/25. eng.

34.   Willis CL, Nolan CC, Reith SN, Lister T, Prior MJ, Guerin CJ, Mavroudis G, Ray DE. Focal astrocyte loss is followed by microvascular damage, with subsequent repair of the blood-brain barrier in the apparent absence of direct astrocytic contact. Glia. 2004 Mar;45(4):325-37. PubMed PMID: 14966864. Epub 2004/02/18. eng.

35.   Abbott N, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci. 2006;7:41-53.

36.   Haber M, Zhou L, Murai KK. Cooperative astrocyte and dendritic spine dynamics at hippocampal excitatory synapses. J Neurosci. 2006 Aug 30;26(35):8881-91. PubMed PMID: 16943543. Epub 2006/09/01. eng.

37.   Nishida H, Okabe S. Direct astrocytic contacts regulate local maturation of dendritic spines. J Neurosci. 2007 Jan 10;27(2):331-40. PubMed PMID: 17215394. Epub 2007/01/12. eng.

38.   Ullian EM, Sapperstein SK, Christopherson KS, Barres BA. Control of synapse number by glia. Science. 2001 Jan 26;291(5504):657-61. PubMed PMID: 11158678. Epub 2001/02/07. eng.

39.   Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci. 1999 May;22(5):208-15. PubMed PMID: 10322493. Epub 1999/05/14. eng.

40.   Auld DS, Robitaille R. Glial cells and neurotransmission: an inclusive view of synaptic function. Neuron. 2003 Oct 9;40(2):389-400. PubMed PMID: 14556716. Epub 2003/10/15. eng.

41.   Bushong EA, Martone ME, Jones YZ, Ellisman MH. Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. J Neurosci. 2002 Jan 1;22(1):183-92. PubMed PMID: 11756501. Epub 2002/01/05. eng.

42.   Di Castro MA, Chuquet J, Liaudet N, Bhaukaurally K, Santello M, Bouvier D, Tiret P, Volterra A. Local Ca2+ detection and modulation of synaptic release by astrocytes. Nature neuroscience. 2011 Oct;14(10):1276-84. PubMed PMID: 21909085. Epub 2011/09/13. eng.

43.   Nett WJ, Oloff SH, McCarthy KD. Hippocampal astrocytes in situ exhibit calcium oscillations that occur independent of neuronal activity. Journal of neurophysiology. 2002 Jan;87(1):528-37. PubMed PMID: 11784768. Epub 2002/01/11. eng.

44.   Bezzi P, Volterra A. Imaging exocytosis and recycling of synaptic-like microvesicles in astrocytes. Cold Spring Harb Protoc. 2014 May;2014(5). PubMed PMID: 24786509. Epub 2014/05/03. eng.

45.   Parpura V, Verkhratsky A. Homeostatic function of astrocytes: Ca(2+) and Na(+) signalling. Translational neuroscience. 2012 Dec;3(4):334-44. PubMed PMID: 23243501. Pubmed Central PMCID: PMC3520132. Epub 2012/12/18. Eng.

46.   Perea G, Navarrete M, Araque A. Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci. 2009 Aug;32(8):421-31. PubMed PMID: 19615761. Epub 2009/07/21. eng.

47.   Schousboe A, Sarup A, Bak LK, Waagepetersen HS, Larsson OM. Role of astrocytic transport processes in glutamatergic and GABAergic neurotransmission. Neurochem Int. 2004 Sep;45(4):521-7. PubMed PMID: 15186918. Epub 2004/06/10. eng.

48.   Norenberg MD, Martinez-Hernandez A. Fine structural localization of glutamine synthetase in astrocytes of rat brain. Brain Res. 1979 Feb 2;161(2):303-10. PubMed PMID: 31966. Epub 1979/02/02. eng.

49.   Arnth-Jensen N, Jabaudon D, Scanziani M. Cooperation between independent hippocampal synapses is controlled by glutamate uptake. Nature neuroscience. 2002 Apr;5(4):325-31. PubMed PMID: 11896395. Epub 2002/03/16. eng.

50.   Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N. Astroglial networks: a step further in neuroglial and gliovascular interactions. . Nat Rev Neurosci. 2010;11:87-99.

51.   Gibbs M, Hutchinson D, Hertz L. Astrocytic involvement in learning and memory consolidation. Neurosci Biobehav Rev. 2008;32:927-44.

52.   Belanger M, Allaman I, Magistretti PJ. Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. Cell metabolism. 2011 Dec 7;14(6):724-38. PubMed PMID: 22152301. Epub 2011/12/14. eng.

53.   Newington JT, Harris RA, Cumming RC. Reevaluating Metabolism in Alzheimer's Disease from the Perspective of the Astrocyte-Neuron Lactate Shuttle Model. Journal of neurodegenerative diseases. 2013;2013:234572. PubMed PMID: 26316984. Epub 2013/01/01. eng.

54.   Muller MS. Functional impact of glycogen degradation on astrocytic signalling. Biochemical Society transactions. 2014 Oct;42(5):1311-5. PubMed PMID: 25233408. Pubmed Central PMCID: PMC4179473. Epub 2014/09/19. eng.

55.   Brown AM, Ransom BR. Astrocyte glycogen and brain energy metabolism. Glia. 2007 Sep;55(12):1263-71. PubMed PMID: 17659525. Epub 2007/07/31. eng.

56.   Allaman I, Belanger M, Magistretti P. Astrocyte-neuron metabolic relationships: for better and for worse. Trends Neurosci. 2011;34:76-87.

57.   Pfrieger F, Ungerer N. Cholesterol metabolism in neurons and astrocytes. Prog Lipid Res. 2011;50:357-71.

58.   Vives V, Alonso G, Solal AC, Joubert D, Legraverend C. Visualization of S100B-positive neurons and glia in the central nervous system of EGFP transgenic mice. The Journal of comparative neurology. 2003 Mar 17;457(4):404-19. PubMed PMID: 12561079. Epub 2003/02/01. eng.

59.   Hachem S, Aguirre A, Vives V, Marks A, Gallo V, Legraverend C. Spatial and temporal expression of S100B in cells of oligodendrocyte lineage. Glia. 2005 Aug 1;51(2):81-97. PubMed PMID: 15782413. Epub 2005/03/23. eng.

60.   Steiner J, Bernstein HG, Bielau H, Berndt A, Brisch R, Mawrin C, Keilhoff G, Bogerts B. Evidence for a wide extra-astrocytic distribution of S100B in human brain. BMC neuroscience. 2007;8:2. PubMed PMID: 17199889. Pubmed Central PMCID: PMC1769505. Epub 2007/01/04. eng.

61.   Lee A, Pow D. Astrocytes: glutamate transport and alternate splicing of transporters. . Int J Biochem Cell Biol. 2010;42:1901-6.

62.   Simpson JE, Ince PG, Lace G, Forster G, Shaw PJ, Matthews F, Savva G, Brayne C, Wharton SB. Astrocyte phenotype in relation to Alzheimer-type pathology in the ageing brain. Neurobiol Aging. 2010 Apr;31(4):578-90. PubMed PMID: 18586353. Epub 2008/07/01. eng.

63.   Fluteau A, Ince P, Minett T, Matthews F, Brayne C, Garwood C, Ratcliffe L, Morgan S, Heath P, Shaw P, Wharton S, Simpson J. The nuclear retention of transcription factor FOXO3a correlates with a DNA damage response and increased glutamine synthetase expression by astrocytes suggesting a neuroprotective role in the ageing brain. . Neurosci Lett. 2015;609:11-7.

64.   Akiyama H, Tooyama I, Kawamata T, Ikeda K, McGeer P. Morphological diversities of CD44 positive astrocytes in the cerebral cortex of normal subjects and patients with Alzheimer's disease. . Brain Res. 1993;632:249-59.

65.   Cahoy J, Emery B, Kaushal A, Foo L, Zamanian J, Christopherson K, Xing Y, Lubisher J, Krieg P, Krupenko S, Thompson W, Barres B. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. . J Neurosci. 2008;28:264-78.

66.   Yang Y, Vidensky S, Jin L, Jie C, Lorenzini I, Frankl M, Rothstein J. Molecular comparison of GLT1$^+$ and ALDH1L1$^+$ astrocytes in vivo in astroglial reporter mice. . Glia. 2011;59:200-7.

67.   Flugge G, Araya-Callis C, Garea-Rodriguez E, Stadelmann-Nessler C, Fuchs E. NDRG2 as a marker protein for brain astrocytes. Cell and tissue research. 2014 Jul;357(1):31-41. PubMed PMID: 24816982. Pubmed Central PMCID: PMC4077251. Epub 2014/05/13. eng.

68.   Lin K, Yin A, Yao L, Li Y. N-myc downstream-regulated gene 2 in the nervous system: from expression pattern to function. . Acta Biochim Biophys Sin. 2015;47:761-6.

69.   Lopez-Otin C, Blasco M, Partridge L, Serrano M, Kroemer G. The hallmarks of ageing. Cell. 2013;153:1194-217.

70.   Sheng J, Mrak R, Rovnaghi C, Kozlowska E, Van Eldik L, Griffin W. Human brain S100β and S100β mRNA expression increases with age: pathogenic implications for Alzheimer's disease. Neurobiol Aging. 1996;17:359-63.

71.   Wu Y, A-Q Z, Yew D. Age related changes of various markers of astrocytes in senescence-accelerated mice hippocampus. Neurochem Int. 2005;46:565-74.

72.   Nichols N, Day J, Laping N, Johnson S, Finch C. GFAP mRNA increases with age in rat and human brain. Neurobiol Aging. 1993;14:421-9.

73.   Orre M, Kamphuis W, Osborn L, Melief J, Kooijman L, Huitinga I, Klooster J, Bossers K, Hol E. Acute isolation and transcriptome characterization of cortical astrocytes and microglia from young and aged mice. . Neurobiol Aging. 2014;35:1-14.

74.   Salminen A, Ojala J, Kaarniranta K, Haapasalo A, Hiltunen M, Soininen H. Astrocytes in the aging brain express characteristics of senescence-associated secretory phenotype. Eur J Neurosci. 2011 Jul;34(1):3-11. PubMed PMID: 21649759. Epub 2011/06/09. eng.

75.   Bhat R, Crowe EP, Bitto A, Moh M, Katsetos CD, Garcia FU, Johnson FB, Trojanowski JQ, Sell C, Torres C. Astrocyte senescence as a component of Alzheimer's disease. PLoS One. 2012;7(9):e45069. PubMed PMID: 22984612. Pubmed Central PMCID: PMC3440417. Epub 2012/09/18. eng.

76.	Schultz C, Ghebremedhin E, del Tredici K, Rub U, Braak H. High prevalence of thorn-shaped astrocytes in the aged human medial temporal lobe. Neurobiol Aging. 2004;25:397-405.

77.	Lace G, Ince P, Brayne C, Savva G, Matthews F, de Silva R, Simpson J, Wharton S. Mesial temporal astrocyte tau pathology in the MRC-CFAS ageing brain cohort. Dement Geriatr Cogn Disord. 2012;34:15-24.

78.	Wharton S, Minett T, Drew D, Forster G, Matthews F, Brayne C, Ince P. Epidemiological pathology of tau in the ageing brain: application of staging for neuropil threads (BrainNet Europe Protocol) to the MRC Cognitive Function and Ageing Brain Study. . Acta Neuropathol Commun. In Press.

79.	Kovacs G, Ferrer I, Grinberg L, Alafuzoff I, Attems J, Budka H, et al. Aging-related tau astrogliopathy (ARTAG): harmonized evaluation strategy. . Acta Neuropathol. 2016;131:87-102.

80.	Nicoll JA, Weller RO. A new role for astrocytes: beta-amyloid homeostasis and degradation. Trends Mol Med. 2003 Jul;9(7):281-2. PubMed PMID: 12900213. Epub 2003/08/06. eng.

81.	Chung WS, Welsh CA, Barres BA, Stevens B. Do glia drive synaptic and cognitive impairment in disease? Nature neuroscience. 2015 Nov;18(11):1539-45. PubMed PMID: 26505565. Pubmed Central PMCID: PMC4739631. Epub 2015/10/28. eng.

82.	Wang L, Gutmann D, Roos R. Astrocyte loss of mutant SOD1 delays ALS disease onset and progression in G85R transgenic mice. Hum Mol Genet. 2011;20:286-93.

83.	Yamanaka K, Chun S, Boillee S, Fujimori-Tonou N, Yamashita H, Gutmann D, Takahashi R, Misawa H, Cleveland D. Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. . Nature Neurosci. 2008;11:251-3.

84.	Ince PG, Highley JR, Kirby J, Wharton SB, Takahashi H, Strong MJ, Shaw PJ. Molecular pathology and genetic advances in amyotrophic lateral sclerosis: an emerging molecular pathway and the significance of glial pathology. Acta Neuropathol. 2011 Dec;122(6):657-71. PubMed PMID: 22105541. Epub 2011/11/23. eng.

85.	Wyss-Coray T, Loike JD, Brionne TC, Lu E, Anankov R, Yan F, Silverstein SC, Husemann J. Adult mouse astrocytes degrade amyloid-beta in vitro and in situ. Nature medicine. 2003 Apr;9(4):453-7. PubMed PMID: 12612547. Epub 2003/03/04. eng.

86.	Thal DR, Schultz C, Dehghani F, Yamaguchi H, Braak H, Braak E. Amyloid beta-protein (Abeta)-containing astrocytes are located preferentially near N-terminal-truncated Abeta deposits in the human entorhinal cortex. Acta Neuropathol. 2000 Dec;100(6):608-17. PubMed PMID: 11078212. Epub 2000/11/15. eng.

87.	Funato H, Yoshimura M, Yamazaki T, Saido TC, Ito Y, Yokofujita J, Okeda R, Ihara Y. Astrocytes containing amyloid beta-protein (Abeta)-positive granules are associated with Abeta40-positive diffuse plaques in the aged human brain. Am J Pathol. 1998 Apr;152(4):983-92. PubMed PMID: 9546359. Pubmed Central PMCID: PMC1858251. Epub 1998/04/18. eng.

88.	Nagele RG, Wegiel J, Venkataraman V, Imaki H, Wang KC, Wegiel J. Contribution of glial cells to the development of amyloid plaques in Alzheimer's disease. Neurobiol Aging. 2004 May-Jun;25(5):663-74. PubMed PMID: 15172746. Epub 2004/06/03. eng.

89.	Basak JM, Verghese PB, Yoon H, Kim J, Holtzman DM. Low-density lipoprotein receptor represents an apolipoprotein E-independent pathway of Abeta uptake and degradation by astrocytes. J Biol Chem. 2012 Apr 20;287(17):13959-71. PubMed PMID: 22383525. Pubmed Central PMCID: PMC3340151. Epub 2012/03/03. eng.

90.	Kim J, Castellano JM, Jiang H, Basak JM, Parsadanian M, Pham V, Mason SM, Paul SM, Holtzman DM. Overexpression of low-density lipoprotein receptor in the brain markedly inhibits amyloid deposition and increases extracellular A beta clearance. Neuron. 2009 Dec 10;64(5):632-44. PubMed PMID: 20005821. Pubmed Central PMCID: PMC2787195. Epub 2009/12/17. eng.

91.	Thal DR. The role of astrocytes in amyloid beta-protein toxicity and clearance. Experimental neurology. 2012 Jul;236(1):1-5. PubMed PMID: 22575598. Epub 2012/05/12. eng.

92.	Garwood CJ, Pooler AM, Atherton J, Hanger DP, Noble W. Astrocytes are important mediators of Abeta-induced neurotoxicity and tau phosphorylation in primary culture. Cell Death

Dis. 2011;2:e167. PubMed PMID: 21633390. Pubmed Central PMCID: 3168992. Epub 2011/06/03. eng.

93.   Zhao J, O'Connor T, Vassar R. The contribution of activated astrocytes to Abeta production: implications for Alzheimer's disease pathogenesis. Journal of neuroinflammation. 2011;8:150. PubMed PMID: 22047170. Pubmed Central PMCID: PMC3216000. Epub 2011/11/04. eng.

94.   Bettens K, Sleegers K, Van Broeckhoven C. Genetic insights in Alzheimer's disease. Lancet Neurol. 2013;12:92-104.

95.   Jiang Q, Jin S, Jiang Y, Liao M, Feng R, Zhang L, Liu G, Hao J. Alzheimer's disease variants with genome-wide significance are significantly enriched in immune pathways and active in immune cells. . Mol Neurobiol. 2016;Epub ahead of print.

96.   Lambert J, Grenier-Boley B, Chouraki V, Heath S, Zelenika D, Fievet N, Hannequin D, Pasquier F, Hanon O, Brice A, Epelbaum J, Berr C, Dartigues J, Tzourio C, Campion D, Lathrop M, Amouyel P. Implication of the immune system in Alzheimer's disease: evidence from genome-wide pathway analysis. . J Alzheimers Dis. 2010;20:1107-18.

97.   Jones L, Holmans P, Hamshere M, Harold D, Moskvina V, Ivanov D, et al. Genetic evidence implicates the immune system and cholesterol metabolism in the aetiology of Alzheimer's disease. . PLoS One. 2010;5:e13950.

98.   Heppner R, Ransohoff R, Becher B. Immune attack: the role of inflammation in Alzheimer disease. . Nat Rev Neurosci. 2015;16:358-72.

99.   Rubio-Perez JM, Morillas-Ruiz JM. A review: inflammatory process in Alzheimer's disease, role of cytokines. TheScientificWorldJournal. 2012;2012:756357. PubMed PMID: 22566778. Pubmed Central PMCID: PMC3330269. Epub 2012/05/09. eng.

100.   Heneka MT, O'Banion MK, Terwel D, Kummer MP. Neuroinflammatory processes in Alzheimer's disease. Journal of neural transmission (Vienna, Austria : 1996). 2010 Aug;117(8):919-47. PubMed PMID: 20632195. Epub 2010/07/16. eng.

101.   Phillips EC, Croft CL, Kurbatskaya K, O'Neill MJ, Hutton ML, Hanger DP, Garwood CJ, Noble W. Astrocytes and neuroinflammation in Alzheimer's disease. Biochemical Society transactions. 2014 Oct;42(5):1321-5. PubMed PMID: 25233410. Epub 2014/09/19. eng.

102.   Orre M, Kamphuis W, Osborn LM, Jansen AH, Kooijman L, Bossers K, Hol EM. Isolation of glia from Alzheimer's mice reveals inflammation and dysfunction. Neurobiol Aging. 2014 Dec;35(12):2746-60. PubMed PMID: 25002035. Epub 2014/07/09. eng.

103.   Shao W, Zhang S-Z, Tang M, Zhang X, Zhou Z, Yin Y, Zhou Q, Huang Y, Liu Y, Wawrousek E, Chen T, Li S-B, Xu M, Zhou J, Hu G, Zhou J. Suppression of neuroinflammation by astrocytic dopamine D2 receptors via αB-crystallin. Nature. 2013;494:90-4.

104.   Furman J, Sama D, Gant J, Beckett T, Murphy M, Bachstetter A, Van Eldick L, Norris C. Targeting astrocytes ameliorates neurologic changes in a mouse model of Alzheimer's disease. . J Neurosci. 2012;32:16129-40.

105.   Vijayan VK, Geddes JW, Anderson KJ, Chang-Chui H, Ellis WG, Cotman CW. Astrocyte hypertrophy in the Alzheimer's disease hippocampal formation. Experimental neurology. 1991 Apr;112(1):72-8. PubMed PMID: 2013308. Epub 1991/04/01. eng.

106.   Rodriguez JJ, Olabarria M, Chvatal A, Verkhratsky A. Astroglia in dementia and Alzheimer's disease. Cell Death Differ. 2009 Mar;16(3):378-85. PubMed PMID: 19057621. Epub 2008/12/06. eng.

107.   Stephan B.C.M. WSB, Simpson J., Matthews F.E., Ince P., Brayne C. The epidemiological neuropathology of dementia and the implications for drug development. Neurodegenerative Disease Management. 2012;2(5):471-82.

108.   Brayne C, Barker R, Grupe A, Harold D, Ince P, Savva G, Williams J, Williams-Gray C, Wharton S. From molecule to clinic and community for neurodegeneration: research to bridge translational gaps. J Alzheimers Dis. 2012;33 Suppl 1:S385-96.

109.   Kamphuis W, Mamber C, Moeton M, Kooijman L, Sluijs J, Jansen A, Verveer M, de Groot L, Smith V, Rangarajan S, Rodriquez J, Orre M, Hol E. GFAP isoforms in adult mouse brain with a focus

on neurogenic astrocytes and reactive astrogliosis in mouse models of Alzheimer disease. . PLOS ONE. 2012;7:e42823.

110.	Kamphuis W, Middeldorp J, Kooijman L, Sluijs J, Kooi E-J, Moeton M, Freriks M, Mizee M, Hol E. Glial fibrillary acidic protein isoform expression in plaque related astrogliosis in Alzheimer's disease. . Neurobiol Aging. 2014;35:492-510.

111.	Nielsen A, Holm I, Johansen M, Bonven B, Jorgensen P, Jorgensen A. A new splice variant of glial fibrillary acidic protein, GFAPε, interacts with the presenilin proteins. . J Biol Chem. 2002;277:29983-91.

112.	Robel S, Sontheimer H. Glia as drivers of abnormal neuronal activity. Nat Neurosci. 2016;19:28-33.

113.	Busche M, Grienberger C, Keskin A, Song B, Neumann U, Staufenbiel M, Forstl H, Konnerth A. Decreased amyloid-β and increased neuronal hyperactivity by immunotherapy in Alzheimer's models. . Nat Neurosci. 2015;18:1725-8.

114.	Olabarria M, Noristani HN, Verkhratsky A, Rodriguez JJ. Concomitant astroglial atrophy and astrogliosis in a triple transgenic animal model of Alzheimer's disease. Glia. 2010 May;58(7):831-8. PubMed PMID: 20140958. Epub 2010/02/09. eng.

115.	Verkhratsky A, Olabarria M, Noristani HN, Yeh CY, Rodriguez JJ. Astrocytes in Alzheimer's disease. Neurotherapeutics. 2010 Oct;7(4):399-412. PubMed PMID: 20880504. Epub 2010/10/01. eng.

116.	Garwood C, Faizullabhoy A, Wharton SB, Ince PG, Heath P, Shaw PJ, Baxter L, Gelsthorpe C, Forster G, Matthews FE, Brayne C, Simpson JE. Calcium dysregulation in relation to Alzheimer-type pathology in the ageing brain. Neuropathol Appl Neurobiol. 2013 Feb 19. PubMed PMID: 23421725. Epub 2013/02/21. Eng.

117.	Tomimoto H, Akiguchi I, Suenaga T, Nishimura M, Wakita H, Nakamura S, Kimura J. Alterations of the blood-brain barrier and glial cells in white-matter lesions in cerebrovascular and Alzheimer's disease patients. Stroke. 1996;27:2069-74.

118.	Simpson JE, Fernando M, Clark L, Ince P, Matthews F, Forster G, O'Brien J, Barber R, Kalaria R, Brayne C, Shaw P, Lewis C, Wharton S. White matter lesions in an unselected cohort of the elderly: astrocytic, microglial and oligodendrocyte precursor cell responses. . Neuropathol Appl Neurobiol. 2007;33:410-9.

119.	Garwood CJ, Simpson JE, Al Mashhadi S, Axe C, Wilson S, Heath PR, Shaw PJ, Matthews FE, Brayne C, Ince PG, Wharton SB. DNA damage response and senescence in endothelial cells of human cerebral cortex and relation to Alzheimer's neuropathology progression: a population-based study in the Medical Research Council Cognitive Function and Ageing Study (MRC-CFAS) cohort. Neuropathol Appl Neurobiol. 2014 Dec;40(7):802-14. PubMed PMID: 24861546. Epub 2014/05/28. eng.

120.	Simpson JE, Ince PG, Haynes LJ, Theaker R, Gelsthorpe C, Baxter L, Forster G, Lace GL, Shaw PJ, Matthews FE, Savva GM, Brayne C, Wharton SB. Population variation in oxidative stress and astrocyte DNA damage in relation to Alzheimer-type pathology in the ageing brain. Neuropathol Appl Neurobiol. 2010 Feb;36(1):25-40. PubMed PMID: 19422529. Epub 2009/05/09. eng.

121.	De La Monte S, Sohn Y, Ganju N, Wands J. p53 and CD95 associated apoptosis in neurodegenerative disease. Lab Invest. 1998;78:401-11.

122.	Nunomura A, Tamaoki T, Motohashi N, Nakamura M, McKeel D, Tabaton M, Lee H-G, Smith M, Perry G, Zhu X. The earliest stage of cognitive impairment in transition from normal aging to Alzheimer disease is marked by prominent RNA oxidation in vulnerable neurons. . J Neuropathol Exp Neurol. 2012;71:233-41.

123.	Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj E, Jones P, Ghanbari H, Wataya T, Shimohama S, Chiba S, Atwood C, Petersen R, Smith M. Oxidative damage is the earliest event in Alzheimer Disease. J Neuropathol Exp Neurol. 2001;60:759-67.

124.	Lovell M, Soman S, Bradley M. Oxidatively modified nucleic acids in preclinical Alzheimer's disease (PCAD) brain. . Mech Ageing Dev. 2011;132:443-8.

125.	Venkateshappa C, Harish G, Mahadevan A, Srinivas Bharath M, Shankar S. Elevated oxidative stress and decreased antioxidant function in the human hippocampus and frontal cortex with increasing age: implications for neurodegeneration in Alzheimer's disease. . Neurochem Res. 2012;37:1601-14.

126.	Blasko I, Stampfer-Kountchev M, Robatscher P, Veerhuis R, Eikelenboom P, Grubeck-Loebenstein B. How chronic inflammation can affect the brain and support the development of Alzheimer's disease in old age: the role of microglia and astrocytes. Ageing Cell. 2004;3:169-76.

127.	Butterfield D, Boyd-Kimball D. Amyloid β-peptide (1-42) contributes to the oxidative stress and neurodegeneration found in Alzheimer disease brain. Brain Pathol. 2004;14:426-32.

128.	Kobayashi K, Hayashi M, Nakano H, Shimazaki M, Sugimori K, Koshino Y. Correlation between astrocyte apoptosis and Alzheimer changes in gray matter lesions in Alzheimer's disease. . J Alzheimers Dis. 2004;6:623-32.

129.	Munoz L, Ammit AJ. Targeting p38 MAPK pathway for the treatment of Alzheimer's disease. Neuropharmacology. 2010 Mar;58(3):561-8. PubMed PMID: 19951717. Epub 2009/12/03. eng.

130.	Waller R, Woodroofe MN, Francese S, Heath PR, Wharton SB, Ince PG, Sharrack B, Simpson JE. Isolation of enriched glial populations from post-mortem human CNS material by immuno-laser capture microdissection. J Neurosci Methods. 2012 Apr 26. PubMed PMID: 22609336. Epub 2012/05/23. Eng.

131.	Simpson JE, Ince PG, Shaw PJ, Heath PR, Raman R, Garwood CJ, Gelsthorpe C, Baxter L, Forster G, Matthews FE, Brayne C, Wharton SB. Microarray analysis of the astrocyte transcriptome in the aging brain: relationship to Alzheimer's pathology and APOE genotype. Neurobiol Aging. 2011 Oct;32(10):1795-807. PubMed PMID: 21705112. Epub 2011/06/28. eng.

132.	Garwood CJ, Ratcliffe LE, Morgan SV, Simpson JE, Owens H, Vazquez-Villasenor I, Heath PR, Romero IA, Ince PG, Wharton SB. Insulin and IGF1 signalling pathways in human astrocytes in vitro and in vivo; characterisation, subcellular localisation and modulation of the receptors. Molecular brain. 2015;8(1):51. PubMed PMID: 26297026. Pubmed Central PMCID: PMC4546315. Epub 2015/08/25. eng.

133.	Heni M, Hennige AM, Peter A, Siegel-Axel D, Ordelheide AM, Krebs N, Machicao F, Fritsche A, Haring HU, Staiger H. Insulin promotes glycogen storage and cell proliferation in primary human astrocytes. PLoS One. 2011;6(6):e21594. PubMed PMID: 21738722. Pubmed Central PMCID: PMC3124526. Epub 2011/07/09. eng.

134.	Genis L, Davila D, Fernandez S, Pozo-Rodrigalvarez A, Martinez-Murillo R, Torres-Aleman I. Astrocytes require insulin-like growth factor I to protect neurons against oxidative injury. F1000Research. 2014;3:28. PubMed PMID: 24715976. Pubmed Central PMCID: PMC3954172. Epub 2014/04/10. eng.

135.	Fernandez A, Jimenez S, Mecha M, Davila D, Guaza C, Vitorica J, Torres-Aleman I. Regulation of the phosphatase calcineurin by insulin-like growth factor 1 unveils a key role of astrocytes in Alzheimer's pathology. Mol Psychiat. 2012;17:705-18.

136.	Talbot K, Wang HY, Kazi H, Han LY, Bakshi KP, Stucky A, Fuino RL, Kawaguchi KR, Samoyedny AJ, Wilson RS, Arvanitakis Z, Schneider JA, Wolf BA, Bennett DA, Trojanowski JQ, Arnold SE. Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. The Journal of clinical investigation. 2012 Apr 2;122(4):1316-38. PubMed PMID: 22476197. Pubmed Central PMCID: PMC3314463. Epub 2012/04/06. eng.

137.	Bosco D, Fava A, Plastino M, Montalcini T, Pujia A. Possible implications of insulin resistance and glucose metabolism in Alzheimer's disease pathogenesis. J Cell Mol Med. 2011;15:1807-21.

138.	Picone P, Giacomazza D, Vetri V, Carrotta R, Militello V, San Biagio P, Di Carlo M. Insulin-activated Akt rescues Aβ oxidative stress-induced cell death by orchestrating molecular trafficking. . Ageing Cell. 2011;10:832-43.

139. Zhao WQ, De Felice FG, Fernandez S, Chen H, Lambert MP, Quon MJ, Krafft GA, Klein WL. Amyloid beta oligomers induce impairment of neuronal insulin receptors. FASEB J. 2008 Jan;22(1):246-60. PubMed PMID: 17720802. Epub 2007/08/28. eng.

140. Serrano-Pozo A, Mielke ML, Gomez-Isla T, Betensky RA, Growdon JH, Frosch MP, Hyman BT. Reactive glia not only associates with plaques but also parallels tangles in Alzheimer's disease. Am J Pathol. 2011 Sep;179(3):1373-84. PubMed PMID: 21777559. Pubmed Central PMCID: 3157187. Epub 2011/07/23. eng.

141. Mathur R, Ince PG, Minett T, Garwood CJ, Shaw PJ, Matthews FE, Brayne C, Simpson JE, Wharton SB. A reduced astrocyte response to beta-amyloid plaques in the ageing brain associates with cognitive impairment. PLoS One. 2015;10(2):e0118463. PubMed PMID: 25707004. Pubmed Central PMCID: PMC4338046. Epub 2015/02/24. eng.

142. Kobayashi K, Hayashi M, Nakano H, Fukutani Y, Sasaki K, Shimazaki M, Koshino Y. Apoptosis of astrocytes with enhanced lysosomal activity and oligodendrocytes in white matter lesions in Alzheimer's disease. Neuropathol Appl Neurobiol. 2002;28:238-51.

143. Sjobeck M, Englund E. Glial levels determine severity of white matter disease in Alzheimer's disease: a neuropathological study of glial changes. Neuropathol Appl Neurobiol. 2003;29:159-69.

144. Koistinaho M, Lin S, Wu X, Esterman M, Koger D, Hanson J, Higgs R, Liu F, Malkani S, Bales K, Paul S. Apolipoprotein E promotes astrocyte colocalisation and degradation of deposited amyloid-β peptides. Nature Med. 2004;10:719-26.

145. Overmyer M, Helisalmi S, Soininen H, Laakso M, Riekkinen P, Alafuzoff I. Astrogliosis and the ApoE genotype. An immunohistochemical study of postmortem human brain tissue. . Dement Geriatr Cogn Disord. 1999;10:252-7.

146. Alafuzoff I, Overmyer M, Helisalmi S, Soininen H. Lower counts of astroglia and activated microglia in patients with Alzheimer's disease with regular use of non-steroidal anti-inflammatory drugs. J Alzheimers Dis. 2000;2:37-46.

147. Enciu AM, Constantinescu SN, Popescu LM, Muresanu DF, Popescu BO. Neurobiology of vascular dementia. Journal of aging research. 2011;2011:401604. PubMed PMID: 21876809. Pubmed Central PMCID: PMC3160011. Epub 2011/08/31. eng.

148. Kimbrough I, Robel S, Roberson E, Sontheimer H. Vascular amyloidosis impairs the gliovascular unit in a mouse model of Alzheimer's disease. . Brain. 2015;138:3716-33.

149. Merlini M, Meyer EP, Ulmann-Schuler A, Nitsch RM. Vascular beta-amyloid and early astrocyte alterations impair cerebrovascular function and cerebral metabolism in transgenic arcAbeta mice. Acta Neuropathol. 2011 Sep;122(3):293-311. PubMed PMID: 21688176. Pubmed Central PMCID: PMC3168476. Epub 2011/06/21. eng.

150. Hung VK, Yeung PK, Lai AK, Ho MC, Lo AC, Chan KC, Wu EX, Chung SS, Cheung CW, Chung SK. Selective astrocytic endothelin-1 overexpression contributes to dementia associated with ischemic stroke by exaggerating astrocyte-derived amyloid secretion. J Cereb Blood Flow Metab. 2015 Jun 24. PubMed PMID: 26104290. Epub 2015/06/25. Eng.

151. Broe M, Kril J, Halliday GM. Astrocytic degeneration relates to the severity of disease in frontotemporal dementia. Brain. 2004 Oct;127(Pt 10):2214-20. PubMed PMID: 15282215. Epub 2004/07/30. eng.

152. Martin JA, Craft DK, Su JH, Kim RC, Cotman CW. Astrocytes degenerate in frontotemporal dementia: possible relation to hypoperfusion. Neurobiol Aging. 2001 Mar-Apr;22(2):195-207. PubMed PMID: 11182469. Epub 2001/02/22. eng.

153. Terada S, Ishizu H, Haraguchi T, Takehisa Y, Tanabe Y, Kawai K, Kuroda S. Tau-negative astrocytic star-like inclusions and coiled bodies in dementia with Lewy bodies. Acta Neuropathol. 2000 Nov;100(5):464-8. PubMed PMID: 11045667. Epub 2000/10/25. eng.

154. Terada S, Ishizu H, Yokota O, Tsuchiya K, Nakashima H, Ishihara T, Fujita D, Ueda K, Ikeda K, Kuroda S. Glial involvement in diffuse Lewy body disease. Acta Neuropathol. 2003;105:163-9.

155. Kovacs G. Neuropathology of tauopathies: principles and practice. . Neuropathol Appl Neurobiol. 2015;41:3-23.

156.	Meyer K, Ferraiuolo L, Miranda CJ, Likhite S, McElroy S, Renusch S, Ditsworth D, Lagier-Tourenne C, Smith RA, Ravits J, Burghes AH, Shaw PJ, Cleveland DW, Kolb SJ, Kaspar BK. Direct conversion of patient fibroblasts demonstrates non-cell autonomous toxicity of astrocytes to motor neurons in familial and sporadic ALS. Proc Natl Acad Sci U S A. 2014 Jan 14;111(2):829-32. PubMed PMID: 24379375. Pubmed Central PMCID: PMC3896192. Epub 2014/01/01. eng.

157.	Haidet-Phillips AM, Hester ME, Miranda CJ, Meyer K, Braun L, Frakes A, Song S, Likhite S, Murtha MJ, Foust KD, Rao M, Eagle A, Kammesheidt A, Christensen A, Mendell JR, Burghes AH, Kaspar BK. Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. Nature biotechnology. 2011 Sep;29(9):824-8. PubMed PMID: 21832997. Pubmed Central PMCID: PMC3170425. Epub 2011/08/13. eng.