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Abbreviations

Aβ: amyloid beta peptide; **AD:** Alzheimer's disease; **ALS:** amyotrophic lateral sclerosis; **APP:** Amyloid precursor protein; **CNS:** central nervous system; **EAAT:** excitatory amino acid transporter; **GFAP:** glial fibrillary acidic protein; **GABA:** γ-aminobutyric acid; **GAT:** γ-aminobutyric acid transporter; **GLUT:** glucose transporter; **GlyT:** glycine transporter; **5-HT:** 5-hydroxytryptamine, serotonin; **MCT:** monocarboxylic acid transporters; **NMDA:** N-methyl-D-aspartate; **SERT:** serotonin transporter; **SGLT:** sodium-glucose symporter; **S100B:** S100 calcium-binding protein B.

Abstract

Astrocytes play a fundamental role in maintaining the health and function of the central nervous system. Increasing evidence indicates that astrocytes undergo both cellular and molecular changes at an early stage in neurological diseases, including Alzheimer's disease. These changes may reflect a change from a neuroprotective to a neurotoxic phenotype. Given the lack of current disease modifying therapies for Alzheimer's disease, astrocytes have become an interesting and viable target for therapeutic intervention. The astrocyte transport system covers a diverse array of proteins involved in metabolic support, neurotransmission and synaptic architecture. Therefore, specific targeting of individual transporter families has the potential to suppress neurodegeneration, a characteristic hallmark of Alzheimer's disease. A small number of the four hundred transporter superfamilies' are expressed in astrocytes, with evidence highlighting a fraction of these are implicated in Alzheimer's disease. Here we review the current evidence for six astrocytic transporter subfamilies involved in Alzheimer's disease, as reported in both animal and human studies. This review confirms that astrocytes are indeed a viable target, highlights the complexities of studying astrocytes and provides future directives to exploit the potential of astrocytes in tackling Alzheimer's disease.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterised by deposition of amyloid plaques, neurofibrillary tangle formation, synaptic loss and neuronal cell death (1). Whilst understanding of AD pathology has been predominantly 'neurocentric', recent advances in techniques and experimental models provide some valuable insights into astrocytes in AD pathogenesis (2,3). Astrocytes are present in the nervous system in approximately equal numbers to neurons (4), and have intimate physical connections with synapses and blood vessels. An appreciation of the diverse range of roles they play has gathered pace in the last 20 years (5,6). Far from being passive bystanders as once thought, strong evidence highlights astrocytes as dynamic components of CNS physiology and pathophysiology. For example, through cellular connections with brain endothelial cells, astrocytes regulate neurovascular coupling which ensures activity-dependent metabolite supply to neurons, and as cellular partners of neurons at synapses, astrocytes regulate potassium ions, hydrogen ions and take up neurotransmitters to influence synaptic signalling and plasticity (7–9). Alteration in astrocyte functions could contribute to AD processes; namely, synaptic loss, neurodegeneration, amyloid deposition and tangle formation. Indeed, it is found that regional specificity, initially considered to be a neuronal phenomenon, also applies to astrocytes(10,11).

While there is no or little astrocyte loss, both human post-mortem and animal studies show there is a distinct change in astrocyte morphology and physiology associated with pathology (12–14). Research to date indicates that the number of astrocytes remains consistent in post-mortem brains as well as in animal models of AD (overexpression of Aβ) as determined by immunostaining with available astrocytic markers (GFAP, S100B and glutamine synthetase) (15–17). This change in phenotype is most often reported as an increase in astrocyte reactivity, as visualised by increased protein levels of the intermediate filament, glial fibrillary acid protein (GFAP). Increases in GFAP coincide with astrocyte hypertrophy and changes in morphology (18). While the number of reactive astrocytes correlates well with disease state in

animal models (18,19), it is important to note that human studies have revealed increased astrocyte reactivity is not only associated with disease, but also reflects ageing (12). In several transgenic mouse models of AD; astrocyte reactivity occurs before detectable amyloid plaque deposition (20,21) and therefore may be regarded an early marker of pathology (Figure 1). Currently there is debate on whether reactive astrocytes contribute to neurodegeneration, or are indeed neuroprotective (22). Although it is usually GFAP which is measured, astrocyte reactivity is associated with a wealth of molecular changes which are incompletely characterised (18), and characterisation of these molecular changes can provide insight into disease process and potential therapeutic targets.

Astrocyte functional capacity is a consequence of a complex transcriptome (23,24), and astrocytes express numerous receptors, transporters and signalling molecules which allow them to regulate CNS homeostasis, provide neuroprotection, and contribute to neurodegeneration. Amongst the proteins pivotal in the maintenance of cellular and system homeostasis are transporter proteins, with over four hundred superfamilies' (25). Astrocytes express a variety of transporters, with subfamilies and splice variants adding to their diversity (26–28). With a lack of AD-modifying treatments and an urgent need for new molecular targets to enter the discovery pipeline, astrocytic transporters provide an avenue for further research and development. Here we review evidence for the modulation of astrocyte transporters in AD and the implications for future treatment opportunities. Transporters include a diverse group of protein families with numerous members and a suitably diverse nomenclature. The rich diversity is evident in the topology of the transporters, with highly variable regions connecting transmembrane domains (Figure 2). For the purposes of this review, we have used the IUPHAR transporter nomenclature (29). A survey of the literature[1] revealed several families

[1] The authors restricted their search to PubMed using search terms: 'astrocyte and Alzheimer's disease', 'astrocyte transporters and Alzheimer's disease'. Phrases combining Alzheimer's disease and individual transporters, for example, 'EAAT2 and Alzheimer's disease' were also included in the search.

that have been implicated in AD pathology (Table 1) and some of the major families are discussed below.

1. SLC1 Amino acid transporters

The SLC1 amino acid transporter family comprises five high affinity glutamate (SLC1A1-3 and 6-7) and two neutral amino acid transporters (SLC1A4,5) with regional and cell type specific expression throughout the CNS (30). Glutamate transporters are predicted to contain eight transmembrane domains with two membrane re-entrant loops, and exist as homotrimers (see Figure 2A; 31).

The glutamate transporters regulate glutamate homeostasis at both synaptic and extrasynaptic sites through active uptake of glutamate. Cellular uptake of glutamate is coupled with three Na^+ and one H^+ and counter transports one K^+ (32). This process is secondary-active transport, as glutamate uptake does not directly involve ATP catabolism but is instead dependent on ionic gradients generated by ATP-dependent pumps, in particular Na^+/K^+ ATPase (*see section 5*). It is well established that an important function of these transporters is to prevent glutamate-induced excitotoxicity at the synapse (Figure 3), believed to be causative in neurodegeneration (33). Given this pivotal role, these proteins have been the focus of numerous studies of neurodegeneration (e.g. 32–34) and there is a wealth of data available with respect to their modulation in AD pathology.

EAAT2 (SLC1A2; rat GLT-1) is a transmembrane protein predominantly expressed in astrocytes and responsible for approximately 95% of all L-glutamate uptake in the CNS (30,37,38). Along with the uptake of glutamate from the synaptic cleft, EAAT2 is an important component of the glutamate:glutamine cycle (39,40) involved in the detoxification of glutamatergic signalling. EAAT1 (SLC1A3; rat GLAST) is also expressed at the membrane of astrocytes and mediates around 5% of the total glutamate transport in the adult CNS (41). In relation to AD, these astrocytic transporters are most important given their contribution to total glutamate uptake and their predominant expression in brain regions susceptible to AD

pathology (42). Arguably the most important of these transporters is EAAT2; indeed, the small contribution of EAAT1 to total glutamate uptake has left its role in neurodegenerative diseases, particularly AD, largely unknown.

Mouse models have been a rich source of information with regards AD pathology, but provide conflicting data regarding the role and regulation of glutamate transporters. In terms of mouse AD models (which overexpress Aβ but do not recapitulate all the pathological features in human AD), there is a mixed picture of glutamate transporter alterations. The human amyloid precursor protein (APP; hAPP695 (V642I)/APP/Ld) model (43) showed no significant changes in EAAT2 or EAAT3 mRNA levels between genotypes; however transgenic animals displayed reduced B_{max} and K_D for [^{3}H] D-aspartate uptake along with significantly decreased EAAT2 and EAAT1 protein levels as analysed by immunohistochemistry (44). In the APP/PS1 model (45), both EAAT2 and synaptophysin protein levels do not change over the lifetime of these mice. To further understand a role for EAAT2 in regulating the progression of AD pathology, APP/PS1 mice heterozygous for EAAT2 ($EAAT2_{het}$/APP/PS1) were generated. While long term deficits (9 months) in cognition and Aβ load where comparable to wild type mice, there was evidence of early deficits in memory tasks and Aβ load at 6 months for the heterozygous mouse (46). In another APP transgenic mouse (APP23 mice) EAAT1 and EAAT2 protein levels in both the cortex and hippocampus show an age dependent decrease which preceded amyloid plaque deposition and gliosis (47). These studies suggest that loss of the EAAT protein renders the brain more vulnerable to neurological insults, including Aβ production.

The triple transgenic (3xTg-AD) mouse (48) shows no changes in cortical EAAT2 expression or distribution (49). This contrasts with the report by Zumkehr et al. (2015) which highlighted significant decreases in hippocampal EAAT2 protein and mRNA in a disease dependent manner (50). The contrast in observations may highlight regional deficits in EAAT2 (cortical vs hippocampal) or approaches to qualitative analysis. From a therapeutic stand point, increasing levels of EAAT2 through chronic administration of ceftriaxone restored cognitive deficits without impact on the amyloid load (50).

Given that AD is a human disease, human tissue remains the most insightful as to monitoring pathological changes. The findings from mouse models are in contrast to human data (see below) and highlights the complexity of sporadic human AD and also questions the relevance of mouse models of AD (51). Radiolabelling and immunohistochemical techniques provided initial insight into changes in EAAT protein levels and activity in post-mortem human tissue. An early study looked at EAAT2 substrate uptake and reported an AD-related decrease of around 30% in aspartate uptake (52). This study highlighted that a functional deficit in EAAT2 may underlie synaptotoxic elements of AD pathology. Subsequent studies have looked at biochemical changes in EAAT2 and reported a decrease in EAAT2 protein levels within AD brains (12,53–56). Studies have suggested a link between EAAT2 reduction and APP processing (56), Braak stage progression in AD (12), amyloid plaque deposition (55) and cognitive impairment (57). However there are conflicting reports as to changes in EAAT2 mRNA levels observed in AD brains; studies have highlighted no change (56) or a decrease (54,55) in EAAT2 mRNA in AD tissue compared to controls. Therefore, evidence exists for both pre and post transcriptional regulation of EAAT with reference to AD pathogenesis. While the above studies point to a reduction in EAAT2 in human AD, this is contested in other studies (58). As technology advances and the ability to monitor EAAT function through positron emission tomography becomes available (59), the regulation of EAAT2 in AD may become clearer.

The development of sophisticated gene profiling techniques (Gene Chip arrays, RNA sequencing) has allowed large-scale quantitative gene analysis of AD human tissue and age matched controls. These techniques have revealed decreased EAAT2 mRNA levels in hippocampal tissue taken from stage III-VI patients. Furthermore, the same study highlighted a localisation that correlated with neurofibrillary tangles and Aβ positive plaques (55). The idea that changes in EAAT2 expression reflect disease progression is further supported by work using human hippocampal membrane preparations, detailing that the progression from mild cognitive impairment (MCI) to AD involves significant decreases in EAAT2 protein levels (60).

Analysis of prefrontal cortex samples from Harvard Brain Tissue Resource Centre showed that AD tissues show greatly reduced protein and mRNA expression of EAAT2 (54).

Fewer reports have looked at EAAT1 expression in human AD post-mortem tissue and present contrasting findings. Protein and mRNA levels of EAAT1 in the frontal cortex are reportedly unchanged between AD groups and control (56). EAAT1 mRNA and protein has been shown to be reduced in prefrontal cortex from AD samples (54). Hippocampal regions also show pronounced loss of EAAT1 mRNA, particularly in later stage AD (55). Likewise, gene chip arrays and RT-PCR showed EAAT1 to be significantly reduced in the hippocampus and middle frontal gyrus (12% and 18% respectively) of AD brains. Interestingly, EAAT1, normally expressed exclusively in astrocytes, has been shown to co-localize with tau in cortical pyramidal neurons of AD human tissues, suggesting an intriguing potential link between aberrant EAAT1 expression and AD pathology (61).

Relevance to Alzheimer's disease

Glutamate is the major excitatory neurotransmitter in the central nervous system, and glutamatergic neurons are located in areas that are targeted by the AD pathological cascade (62,63). Disruption in glutamatergic signalling has been reported in both animal models of AD and people with dementia (62,64). Indeed a breakdown in glutamate homeostasis forms the basis of the 'glutamatergic' hypothesis in AD (65–67). It is also noteworthy that current symptomatic relief for AD is provided by memantine, a metabotropic glutamate receptor antagonist. Therefore the astrocyte-specific EAATs offer an intriguing therapeutic target (68–70) for manipulating excessive glutamate levels as described in AD.

Preclinical animal studies had highlighted the potential of the β-lactam antibiotic, ceftriaxone, to increase glutamate transport (71–74). However, this was not shown further in clinical trials investigating the efficacy of ceftriaxone for the neurodegenerative condition amyotrophic lateral sclerosis (ALS) (75). This has stimulated researchers to adopt new approaches in order to develop compounds that can activate EAAT protein expression at a translational level (76).

High throughput screening has revealed a number of compounds that act as EAAT2 activators in expression systems (71,76,77), thus acting to maintain extracellular glutamate concentration at physiological levels (in the low micromolar range). Most recently, a small molecule activator has been developed and characterised in animal models of neurodegeneration (78,79). In an ALS rodent model, the novel EAAT2 activator delayed motor function deficits and prolonged lifespan (78). How this will translate to treating humans remains to be seen, but any impact on disease onset would offer real hope to those at risk of developing AD or those in the early stages of the disease. Providing neuroprotection through modulation of EAAT function is therefore possible in animal models of disease; however the challenge will be translating these findings into a clinical application (80). Therefore, it remains to be seen which methods to target astrocytic glutamate transport will provide a safe and long term therapeutic benefit to all AD patients.

2. SLC2A Glucose Transporters

There are three families of glucose transporter proteins in mammals, each with multiple isoforms. These are the facilitative hexose transporters (GLUTs) of the SLC2 family, the sodium-glucose symporters (SGLTs) of the SLC5 family and the more recently characterised glucose transporters of the SLC50 family (SWEETs). The SLC2 family members; GLUT1 (SLC2A1), GLUT2 (SLC2A2), GLUT3 (SLC2A3), GLUT4 (SLC2A4), GLUT6 (SLC2A6), GLUT8 (SLC2A8), GLUT9 (SLC2A9), GLUT10 (SLC2A10), GLUT11 (SLC2A11) and GLUT12 (SLC2A12) have twelve transmembrane domains and transport glucose (see Figure 2B; 80). The SLC5 family mostly have fourteen transmembrane domains whose family members SGLT1 (SLC5A1), SGLT2 (SLC5A2), SGLT4 (SLC5A9), SGLT5 (SLC5A10) and SMIT1 (SLC5A3) mediate active transport of glucose against its concentration gradient (82). Currently SWEET1 (SLC50A1) is the only reported SLC50 family member to be expressed in mammalian cells and is predicted to have seven transmembrane domains (83). Also relevant to the uptake of metabolic fuels for CNS metabolism are monocarboxylic acid transporters

(MCTs) of the SLC16 family allowing facilitative transport of lactate and pyruvate, namely MCT1 (SLC16A1), MCT2 (SLC16A7), MCT3 (SCL16A8) and MCT4 (SLC16A3) (84).

The CNS, without its own significant fuel stores, is critically dependent on uptake of glucose from the bloodstream. The brain accounts for at least 20% of whole body glucose utilisation (85). A summary of the cellular expression of the main glucose and MCTs in human and rodent CNS is provided by Nijland et al. (2014). The glucose transporter GLUT1 (SLC2A1) accounts for the majority of glucose entry into CNS tissue through brain vascular endothelial cells, although there is evidence that SGLTs can also contribute to this process (87). The main astrocytic glucose transporter is GLUT1, while neurons, by contrast, express mainly GLUT3 which has a higher transport activity than GLUT1 (88). In addition, both astrocytes and neurons also express the insulin-regulated glucose transporter GLUT4 (88). In relation to the MCTs, there is a cell specific pattern with astrocytes expressing MCT1 and MCT4 (89,90), while neurons express MCT2 which displays a higher substrate affinity than MCT1 (84).

Neuronal function is absolutely dependent on supply of metabolic fuel, and to this end neuronal activation is efficiently coupled to energy utilisation and increased blood flow (85). Astrocytes represent an essential cellular link required for this neurovascular coupling and are therefore considered vital for the effective supply of blood and oxygen to meet the metabolic needs of neurons. Following glucose uptake, astrocytes can synthesise and store glycogen (91). Breakdown of glycogen in astrocytes is not able to protect the CNS from hypoxia, but can provide short term support to neurons when blood glucose availability does not meet demand (91). There are several models and some controversy about the metabolic substrate supplied by astrocytes. Magistretti and colleagues have proposed the glucose-lactate shuttle, whereby glucose taken up by astrocytes via GLUT1 is metabolised to lactate by glycolysis. Lactate then exits astrocytes via MCT1 and MCT4 and is taken up by neurons through MCT2, where it can be oxidised in the TCA pathway (Figure 3; 92,93). Other investigators suggest that glucose which is transported into the CNS through capillary endothelial cells avoids astrocytes entirely, and is in fact taken up directly into neurons via GLUT3 (94). Other models

show glucose uptake via astrocytes at the capillary surface and released directly at synapses to be taken up by neurons (95).

While debate surrounds astrocytic metabolic supply and demand, it is clear that brain glucose metabolism is decreased in preclinical patients (96), animal models of dementia (97) and people with a diagnosis of AD (98). The molecular mechanisms behind this are poorly understood, although human AD studies highlight that astrocyte/endothelial GLUT1 (as well as neuronal GLUT3) levels are reduced in post-mortem AD brains (99,100). Loss of astrocyte glucose transporters (GLUT1) is also found in the arcAβ (expressing both the Swedish and Arctic APP mutations) transgenic animal model, with no detectable changes in neuronal glucose transporters (97).

Relevance to Alzheimer's disease

Perturbed glucose metabolism is a contributing factor to the pathogenesis of AD (97,101,102). This disruption is reported to be one of the earliest features of AD, in that a reduction in brain glucose metabolism can be detected (103) and is used as a diagnostic tool (104). However a much more dynamic picture of glucose metabolism is emerging (105,106), with reference to timeline and impact of ageing.

Recent work suggests that contribution of astrocytic glucose transporters is less significant than the importance of the vascular endothelial transporters (107). Further work also suggests that glial glucose transporters might not provide an appropriate target for preventing AD: as in *drosophila* AD models overexpression of GLUT1 in glia had no effect against Aβ induced neurotoxicity (108). However, this lack of effect could have been due to the genetic approaches used in this specific study and provides a cautionary tale in terms of interpreting genetic studies. In contrast, studies have highlighted a role for GLUT1 in memory formation and that GLUT1 induction is not mirrored by the neuronal GLUT3 protein (109). This indicates a level of selectivity for the astrocytic GLUT1 protein which seems to be task dependent. This remains to be therapeutically exploited. Therapeutic interventions seeking to enhance

astrocytic lactate transport may provide a more fruitful endeavour. MCT1 knockout mice show deficits in long term memory (109) and there is clear evidence that MCT1 and MCT4 are critical for memory formation in the hippocampus (110). The cancer field has developed some non-specific MCT inhibitors (111) which are currently in clinical trials (AZD3965; http://www.clinicaltrials.gov/show/NCT01791595). There remains the potential for these inhibitors developed further in a neuroscience context.

Metformin, the antidiabetic drug, has received a lot of attention with regards to its potential act as a novel therapeutic strategy in managing AD (112–114). This links into the emerging hypothesis that AD should be classed as a third type of diabetes (115,116). However, reports are conflicting as to its effectiveness as a therapeutic intervention and the risk of developing AD (117,118). This may underlie the fact that the extent of metformin's molecular pharmacology is still under investigation (31,119,120). In relation to transporter activity, reports have highlighted that GLUT4 translocation could potentially underlie its regulation of glucose metabolism (121). Modulation of the GLUT4 transporter to elevate glucose levels to maintain physiological levels may provide a potential route for pharmacological intervention. However, given the dynamic regulation of this transporter via endogenous means (e.g. increasing energy demands; see (122)) using intrinsic mechanisms rather than exogenous applications may prove to be a better therapeutic strategy to target GLUT4 in the fight against AD (123). Longer term studies are needed to reveal the role of metformin administration in human subjects and the elevated risk, if any, of developing AD. Here there is a particular interest in the APOE-ε4 genotype given its known associated risk with AD (124).

Astrocytes have a greater glycolytic capacity than their neuronal counterparts (125,126); indeed, this may contribute to the resilience against Aβ accumulation in contrast to the catastrophic neuronal loss (127). It is interesting that most transgenic rodent models fail to reproduce neuronal loss on the same scale as the human disease. This may indicate a species-specific expression of key metabolic enzymes, such as PDK1. This could lead to drug discovery programmes targeting these enzymes. However, this may be a short term protective

mechanism prior to symptom onset. The long-term potential of aerobic glycolysis to match the continual decline in glucose availability remains to be determined, however transporter activity can feed into this enhancement of astrocytic metabolism. For example, BDNF, which has received a lot of interest as an Alzheimer's therapeutic target (128), has been shown to modulate the expression levels of GLUT3 with astrocyte transporter levels not determined (129). Furthermore, lactate can stimulate BDNF production in both human astrocytes and SHSY5Y cells in a time-dependent manner (130). Such studies highlight the importance of a therapeutic timeline in which to stimulate astrocytes to respond to the growing deficits in neuronal energy supply.

There are some indirect interventions which may lead to improved glucose metabolism in AD. For example, a recent study has reported the use of a cannabinoid receptor type 2 agonist to enhance CNS glucose uptake (131). Interventions targeting β-adrenoceptors, which induce glucose uptake and storage and breakdown of glycogen in astrocytes, also have promise to improve the supply of glucose and lactate to neurons and modify disease progression (132,133).

Multiple mechanisms, some restricted to astrocytes, can therefore influence brain metabolism. The challenge is to generate specific compounds that target these proteins; which may be through direct interaction with the astrocyte transporters or indirectly through modulation of cellular processes that impact of energy metabolism. What is clear is that astrocytes are key to the regulation of brain glucose metabolism and should be considered as part of a solution in redressing the energy deficit in neurodegeneration.

3. SLC6 Neurotransmitter Transporters

The neurotransmitter family (SL6) incorporates GABA, glycine, monoamine and neutral amino acid transporters. Neurotransmitter transport is Na^+ and Cl^- dependent, with the stoichiometry of ionic coupling being subfamily specific (134). The predicted topology of twelve transmembrane domains (see Figure 2B; 108) has been confirmed from crystal structure

studies using a prokaryote homologue, the leucine transporter (LeuT; (136)). These transporters are expressed throughout the CNS and are critical in maintaining physiological levels of neurotransmitters making them an interesting target for therapeutic intervention.

GABA and glycine are vital inhibitory neurotransmitters influencing information processing, network synchrony and neuronal plasticity. An imbalance in excitatory and inhibitory neuronal activity has been implicated in AD, with a focus on modulating excitation to reduce neurodegeneration associated with excess excitatory tone (33). The ability of astrocytic GABA and glycine transporters to suppress excitation has previously been demonstrated (137,138). To date there has been limited research into these transporters and their involvement in AD.

Astrocytes show a predominant expression of the GAT3 (SLC6A11) protein, with GAT2 (SLC6A13) and BGT-1 (SLC6A12) also expressed but not exclusively in astrocytes (139). Disruption of GABAergic systems has been implicated in the pathogenesis of AD (140). Loss of GABAergic terminals has been reported in human AD brain (141), network imbalance in the hAPPJ20 transgenic model of AD has been linked to reduced GABAergic activity (142) and transplantation of GABAergic interneurons restores AD related cognitive decline in mice (143). The strongest evidence for a role of astrocytes has come from research by Wu et al., (2014), looking at both transgenic models of disease and human tissue. Using the 5xFAD model of disease (144) they reported both neuronal and astrocytic GABA modulation, high GABA content in astrocytes and upregulation of the α5 subunit of the $GABA_A$ receptor. Astrocyte enriched GAT3/4 (SLC6A11; human GAT3/mouse GAT4) was implicated in releasing excess GABA leading to elevated tonic inhibition of signalling in the dentate gyrus (Figure 3). GABA transporters can operate in either direction, either taking up or releasing GABA, in both physiological and pathological conditions (145).

Wu et al., (2014) highlighted that modulation of GAT3/4 could be a novel AD therapy, although current pharmacological tools would need to be developed for this purpose. Furthermore, they suggest that increased astrocytic GABA content may provide a biomarker for AD; increases

in GABA occurred in line with development of Aβ deposition. Highlighting the need to replicate AD animal model studies in humans, Wu et al., (2014) also observed increased GABA content in human AD samples, giving further weight to the role of astrocytic GABA in AD. There are interesting links to the loss of GABAergic signalling with ageing and APoE4 expression (143,146), two known risk factors for AD. Whether this is an astrocyte-dependent process merits further investigation. It remains to be determined if GABAergic dysfunction is found in all forms of AD, which will impact on the drug development targeting the astrocytic GABA transporters.

In comparison, glycine transport has so far received little attention in the field of AD. Astrocytes express the GlyT-1 (SLC6A9) transporter; although expression is not restricted to astrocytes. Furthermore there is conflicting evidence as regards the expression of GlyT-2 (SLC6A5) with early studies suggesting a neuronal specific isoform and later studies proposing a neuronal and astrocyte localisation (28,137,147); differences in cellular preparations may underlie these discrepancies. Importantly glycine is a co-agonist of the NMDA receptor which has been linked to AD pathology (148). A study by Timmer et al., (2014) indicated a decrease in the levels of glycine in an animal model of AD which they proposed may influence the glutamatergic system via NMDA receptors (149). Therefore, mechanisms to increase synaptic glycine levels could be exploited to restore NMDA signalling which is lost in AD. To this end, a GlyT-1 inhibitor (ASP2535) improved cognition in the scopolamine-induced model of AD, signalling the potential for targeting glycine transport (150). However, the failure of this model to replicate the progressive nature of the AD limits our interpretation (151).

The serotonin transporter (SERT, SLC6A4), which facilitates transport of 5-hydroxytryptamine (5-HT, serotonin), has largely been perceived as neuronal specific (152). However studies have shown the expression of functional SERT in astrocytes (27,153,154). These studies have been carried out in a range of *in vitro* preparations, so the significance of SERT astrocytic expression *in vivo* remains unresolved. Increasing evidence highlights a role for 5-HT in AD pathogenesis; human studies indicate a loss of 5-HT (155) and changes in 5-HT receptor

levels (5-HT_{1A}, 5-HT_{2A}, 5-HT_4 and 5-HT_6) in AD brains (156–160). A number of 5-HT_6 antagonists are currently in clinical trials as so-called cognitive enhancers (161,162). Their ability to improve cognition is linked to modulation of the cholinergic and glutamatergic signalling rather than alterations in 5-HT levels (163,164). Furthermore there is recent evidence that may suggest a role for SERT in AD pathology (165,166). Human data presents an interesting debate surrounding polymorphisms within the *SLC6A4* gene, with studies reporting an association with the etiopathogenesis of AD (167–169) and others the contrary (170–172).

Relevance to Alzheimer's disease

Disrupted neurotransmitter signalling has long been associated with AD pathology (65,173). While much of the research focuses on the cholinergic and glutamatergic pathways, recently scientists have proposed changes in a host of other neurotransmitters (174,175).

Based on preclinical work, AD therapy would look to inhibit astrocytic GATs which would reduce tonic GABAergic inhibition and improve cognition. To date, GAT1 inhibitors have been developed for the treatment of epilepsy (e.g. tiagabine; (176)). In addition, a GAT2 inhibitor, EF1502, has been used in preclinical studies and showed additional anticonvulsant effects when administered with tiagabine (177,178). While we await clinical development of astrocyte specific GATs inhibitors, examining astrocyte GABAergic transport may provide both insight into potential biomarker validation (astrocyte GABA content) and the efficacy of targeting astrocytic GABA transporters in combatting AD pathology.

While the contribution of astrocyte-mediated uptake of serotonin is not completely established, it is possible that they are targets for AD therapeutics. Given that both antagonists and agonists of 5-HT_6 receptors are reported to improve cognition, it is unclear if activation or inhibition of astrocytic SERT activity would benefit AD patients. To this end, the ability to pharmacologically target astrocyte rather than neuronal SERT will be a challenge, based on previous reports (179). Overall, modulation of 5-HT transport has the potential to modulate

non-cognitive deficits in AD, such as depression; however, the use of selective 5-HT reuptake inhibitors in AD shows limited efficacy (180). Where alterations in 5-HT transport may provide benefits in frontotemporal dementia, where the serotoninergic system is disrupted, although studies to date have showed that effects are limited to the non-cognitive domains (181).

Increasing NMDA mediated synaptic transmission via GlyT1 modulation may only serve to modulate disease progression at certain points in the pathogenesis of AD (i.e. prior to excitotoxic events; Figure 3). While this would not prevent the disease from developing, it may provide a disease-modifying treatment which would be a significant advance on the current symptomatic treatments available. However, it would not provide a long-term strategy to manage the disease as it progresses. This may highlight the need for a progressive strategy to tackle AD and finding the therapeutic window for different neurotransmitter targeted treatments and how to exploit them will be key to developing future therapeutic strategies.

4. SLC3/SLC7 Heteromeric Amino Acid Transporters

The cystine/Glu antiporter system xc– consists of two membrane spanning proteins: the light chain, XCT (SLC7A11) with twelve or fourteen transmembrane spanning domains that associates with the heavy chain protein 4F2hc (SLC3A2), also known as FRP (fusion regulatory protein) and CD98hc. 4F2hc has a single transmembrane domain (see Figure 2C; 152). Together these proteins mediate the heteroexchange of cystine and glutamate with a stoichiometry of cystine:glutamate of 1:1 at the plasma membrane. The complex can operate in either direction, but is generally considered to import cystine and export glutamate from the cell (183,184).

In the CNS, system xc- is predominantly expressed on glial cells, particularly astrocytes (185). Astrocytes are rich in glutathione compared to neurons, and release glutathione which can then be taken up by neurons (186). This mechanism is considered to be one of the most important ways that astrocytes confer neuroprotective effects, through reducing neuronal susceptibility to oxidative stress, and it is system xc- which controls glutathione levels:

imported cystine is reduced intracellularly to cysteine, which is used in the synthesis of glutathione (GSH) (187). System xc- also mediates glutamate release from astrocytes (Figure 3). The non-vesicular (calcium-independent) glutamate release from astrocytes via system xc- is sufficient to activate neuronal glutamate receptors in the CNS and therefore system xc- directly contributes to neuronal excitability and synaptic plasticity (184,188,189).

Relevance to Alzheimer's disease

In relation to system xc- as a therapeutic target, most published work to date has focused on developing methods to inhibit system xc- as it has been identified as a key system in several cancers. For AD, upregulation of system xc- activity should be disease-modifying by increasing glutathione; however, conversely, increased system xc- activity may contribute to increased extracellular glutamate levels, potentially leading to excitotoxic neuronal death (see Section 1). To date, however, there are no studies characterising changes in CT, 4F2hc levels or xc- activity in AD or animal models of AD disease. Nonetheless, neuroprotective properties of ceftriaxone in an animal model of motor neuron disease have been attributed to its ability to induce XCT (190) but, paradoxically, deletion of XCT reduces symptoms in a transgenic mouse model of motor neuron disease (191). Therefore, it remains to be demonstrated if system xc- is a worthwhile target in the search for novel AD therapeutics.

5. Na^+.K^+-ATPases

The ubiquitous plasma membrane protein sodium-potassium ATPase (Na^+.K^+-ATPase), commonly known as the Na^+ pump is classified as an enzyme (EC 3.6.3.9). Na^+.K^+-ATPase is a primary active transporter transporting Na^+ out of cells and K^+ into cells against their concentration gradients with a stoichiometry of three Na^+ exported for two K^+ imported. Na^+.K^+-ATPase is a heterodimer of α and β subunits. α subunits have ten transmembrane domains (see Figure 2D) and possess all the essential domains for ATP hydrolysis and ion transport with four family members identified in man: α1 (*ATPA1*), α2 (*ATPA2*), α3 (*ATPA3*), α4 (*ATPA4*). Beta subunits are glycoproteins with a single transmembrane domain, of which there

are four family members in man: β1 (*ATPB1*), β2 (*ATPB2*), β3 (*ATPB3*), β4 (*ATPB4*) (192). There are also γ-subunits of the FXYD protein family which alter $Na^+.K^+$-ATPase activity through modulating affinity for Na^+ and K^+(193).

Most focus on the Na^+ pump in the nervous system has been on its role in maintaining Na^+ and K^+ gradients in neurons, with estimates that 60-70% of energy utilisation in the CNS is thought to provide ATP to power $Na^+.K^+$-ATPase. While neurons express the α3 isoform, astrocytes predominantly express the α2 and α1 isoforms together with β2 (194–196). The α2 isoform has a higher K_d than the α1 isoform, suggesting that it is most important in astrocytes at times of high Na^+ load, for example following neuronal activity (Figure 3). In those circumstances, there is a need for astrocytes to take up large quantities of glutamate via EAATs (see section 1) with a concomitant uptake of Na^+. Supporting a close association between EAAT and $Na^+.K^+$-ATPase function, there is a physical interaction between the $Na^+.K^+$-ATPase α2 subunit and the major astrocyte SLC1A/EAATs: GLT-1 and GLAST (197) and stimulation of glutamate uptake results in an up-regulation of $Na^+.K^+$-ATPase activity (198). Disruption of astrocyte $Na^+.K^+$-ATPase activity, as found in human *ATP1A2* gene mutations, leads to both epileptic seizures and familial hemiplegic migraine: these two examples of neuronal hyperexcitability syndromes are likely a consequence of reduced ability of astrocytes to take up K^+ (199). Further supporting a role of astrocyte $Na^+.K^+$-ATPase in supporting neuronal function, heterozygous mice with reduced Na+.K+-ATPase α2 subunit (astrocytes) or α3 subunit (neurons) display impaired spatial learning (200). In addition, protein levels of the α2 subunit have been shown to increase following inhibitory avoidance learning (109).

In post-mortem AD brain tissue, there is a marked decrease in $Na^+.K^+$-ATPase α subunits as determined by binding of the $Na^+.K^+$-ATPase inhibitor, ouabain (201). Ouabain is a pan Na+.K+-ATPase inhibitor displaying differing affinities for the three alpha isoforms and therefore does not discriminate between neuronal and glial α isoforms (202). Other studies have shown decreased $Na^+.K^+$-ATPase activity and α subunits with a concomitant decrease

in α3 mRNA, but an increase in α1 mRNA, attributed to up-regulation of α1 mRNA rather than mRNA degradation in reactive astrocytes (203,204). Likewise, α3 subunit protein levels and $Na^+.K^+$-ATPase activity are reduced in a double transgenic (APP and PS-1) mouse model of AD (205). With reference to the glial isoforms, exogenous application of $A\beta_{25-35}$ resulted in a decrease in α1 subunit protein levels (206). Most recently, the α3 neuronal specific $Na^+.K^+$-ATPase isoform is a target for Aβ assemblies, presenting a potential novel therapeutic strategy (207).

Relevance to Alzheimer's disease

Whether corresponding therapeutic opportunities exist to stimulate astrocyte $Na^+.K^+$-ATPase and confer neuroprotection is an open question. Alterations in Na^+ and K^+ concentrations have been reported in AD tissue (208), with some reports highlighting a two-fold increase in intracellular Na^+ and a 15% increase in intracellular K^+ (206). These concomitant increases in intracellular levels of both Na^+ and K^+ highlights that while changes in $Na^+.K^+$-ATPase activity may occur, they cannot contribute solely to the global changes reported in AD. It is clear, however, that individual elements of AD pathology including amyloid beta peptide and oxidative stress can modulate learning and memory through modulation of $Na^+.K^+$-ATPase activity (209–211). This has led to an increasing number of $Na^+.K^+$-ATPase modulators being proposed as AD therapeutics (211). Many of these are preclinical observations tested in expression systems that remain to be robustly tested, of note however is the observation that currently approved AD therapeutics rivastigmine and memantine have been reported to increase $Na^+.K^+$-ATPase activity (212,213). At a molecular level, phosphorylation of the neuronal isoform is dynamically regulated by PKC and calcineurin (214,215). It is likely that a similar phosphorylation switch occurs in astrocytes, however given differences in Ca^{2+} sensitivity between the isozymes (α1β1 shows insensitivity to Ca^{2+}; (216,217)) Na^+ may play a more prominent role here. This reinforces the suggestion that regulation of Na^+ homeostasis is of greater importance than Ca^{2+} in astrocytes (218,219).

6. ATP-binding cassette transporter family

ATP binding cassette (ABC) transporters are membrane proteins which facilitate ATP dependent movement of a diverse array of substrates. Individual subunits are typically comprised of two six transmembrane-spanning domains with two nucleotide binding domains (see Figure 2E). This architecture permits two modes of transport; namely import and export. Five subfamilies (ABCA, ABCB, ABCC, ABCD and ABCG) allow for the transport of a diverse range of substrates, including many therapeutic drugs. Furthermore, various isoforms within the subfamilies enhance both the cellular and substrate specificity.

ABCA transporters (ABCA1-13) have been shown to regulate lipid transport and gene expression data highlights that human astrocytes express ABCA1-3 at the mRNA level (220). There is however contrasting evidence at the protein level (221,222). More recently, reports have detected expression of ABCA5 in astrocytes although to a lesser extent than neuronal expression (223). This is of interest given the observation that the ABCA5 transporter has been linked to APP processing *in vitro*. This modulation of APP processing resulted in a reduction of the $A\beta_{1-40}$ and $A\beta_{1-42}$ variants (223). While the role of the astrocytic ABCA5 in Aβ generation remains to be determined, it is clear that transporter function can be regulated by key elements of AD pathology. Further evidence is needed as to the nature of the astrocyte ABCA transporters with reference to their expression, at both the mRNA and protein level and to their functional activity. For example, ABCA7 has been strongly implicated in APP processing, Aβ generation and the onset of dementia (224,225). However, the individual cellular role(s) of ABCA7 remains unresolved, and this is key given its widespread distribution (220,225). This shortfall in our understanding currently limits a critical appraisal of the role these astrocytic transporters may play in revealing a novel strategy to better manage AD pathology.

P-glycoprotein 1 (PGP,MDR1, ABCB1) is highly expressed in the blood brain barrier, with protein detected in both the endothelial component and astrocytes (226). Therefore these

transporters have a key role in the elimination of Aβ from the brain, which is believed to be dysfunctional in late onset AD (227). Interestingly, when overexpressed in cancer cells, P-glycoprotein 1 confers multidrug resistance through its export of cytotoxic messengers (228). *In vitro* evidence suggests that Aβ can act as a substrate for the transporters, and blockade of P-glycoprotein transport elevates extracellular levels of Aβ in a time dependent manner (229,230). Animal models of AD (3xTg and Tg2576) have revealed a link between both P-glycoprotein 1 expression and activity in relation to disease progression (231,232). This evidence suggests that accumulation of the toxic form of Aβ can lead to downregulation of the P-glycoprotein 1 transporter. This correlates with human studies that reported elevated levels of Aβ and decreases in P-glycoprotein 1 levels (233,234). The relative contributions to Aβ clearance from capillaries and astrocytes remains to be fully understood.

Multidrug resistance proteins (Mrps1-9) are members of the ABCC subfamily of the ABC transporters. Mrps are export pumps that use ATP to facilitate the efflux of organic cations (235). Regarding astrocyte expression, Mrp1 protein has been reported in rodent and human astrocytes (226,236); Mrp2 at the mRNA and protein level is lacking in astrocytes (237); Mrp3-5 have been reported at both the mRNA level and protein levels in various astrocyte preparations (237,238). Evidence in the literature supports a role of Mrp1 in AD; however, this is not conclusive. For example, oxidative modifications of Mrp1 have been shown in AD brain, with lipid peroxidation a contributing factor (239). It remains to be see if this is a consequence of the disease process or a contributing factor.

Relevance to Alzheimer's disease

There is a wealth of evidence that links lipid metabolism and AD (240,241) and this highlights the potential to target ABCA transporters given their role in cholesterol metabolism (222). This is pertinent for AD, given the genetic links to APOE-ε4 which is predominantly synthesised within astrocytes (242). While activation of the ABCA transporters can be achieved via agonists of the retinoid X receptor system (e.g. bexarotene), numerous other cellular proteins

are targeted by this system and therefore this limits the selectivity of agonists (243). This led to the discovery of an ABCA1 agonist that increased transporter activity, with a subsequent rise in cholesterol efflux (244). The use of a small molecule ABCA1 agonist (CS-6253) has produced some interesting results both *in vitro* and *in vivo*. *In vivo* studies highlight that injection of the CS-6253 peptide can restore cognitive deficits in APOE transgenic mice (245), and the peptide can modulate both central and peripheral lipid metabolism (246). This gives a clear indication that ABCA1 transporters can reverse cognitive decline, through modulation of ApoE4. While work remains to be done to look at the specific signalling cascades involved, the preliminary results are promising. It would be of interest to see whether the activation of astrocytic ABCA1 contributed to these effects. This could be achieved using astrocyte-directed genetic strategies to selectively knock out ABCA1 in astrocytes.

Mrps alongside P-glycoprotein-1 are of interest from an AD perspective given their expression in the cellular constituents of the blood brain barrier (BBB; (247)). Thus, they may provide a route by which to enhance toxic Aβ clearance from the brain or indeed to facilitate transfer of a therapeutic agent into the brain. Aβ clearance is a subject of growing interest, albeit secondary to Aβ formation (248,249). Enhancing transporter mediator clearance of Aβ with a view to redressing the Aβ balance in the brain would be a therapeutic strategy worth pursuing. In support of this *in vivo* evidence highlights the upregulation of the P-glycoprotein transporters via pharmacological activation of the nuclear receptor pregnane X receptor (232). The diverse range of substrates for these transporters should facilitate drug discovery programmes to generate preclinical compounds of interest to the AD research community. Conversely, this may prevent the development of selective tools and another approach may be needed. One such strategy could explore transporter protein modification to alter the kinetics of Aβ efflux via the P-glycoprotein transporter. This type of strategy will be aided by the use of positron emission tomography (PET) studies, which can reveal P-glycoprotein transport kinetics in vivo (250,251).

Future perspectives

Our knowledge of the roles astrocytes play in both physiology and pathology has increased greatly in the last decade and they are now seen as pivotal parts of the complex cellular network that maintain CNS function. As such, they are becoming of interest in neurodegenerative research (252). Neurological disorders such as AD are difficult to combat, although recent clinical trials with the anti-amyloid antibodies, Solanezumab (Eli Lilly) and Aducanamab (Biogen), may provide some hope (253,254). Given the lack of success for novel therapies targeting neuronal entities, it is perhaps surprising that to date few astrocyte targets have been identified. Advances in technology have increased our understanding of astrocytes and their interactions (255), which is still limited by poor astrocyte-specific markers which lack the robust nature of neuronal markers (5). The publication of the astrocytic transcriptome (24) revealed potential new cellular biomarkers that provide tools for generating specific therapeutic approaches to target astrocytes for the purpose of reducing neurodegeneration in AD.

One challenge that still remains is understanding the heterogeneity of the astrocyte population (256) and being able to replicate this in experimental models. This may in part be overcome by the development of induced pluripotent stem cell (iPSC) technology. Again here, neuronal research has led the way and there is now an extensive research literature using this technology to further our understanding of neuronal cell death in AD (257–260). The ability to develop patient-derived cell lines gives researchers the opportunity to study disease pathology directly. In recent years investigators have developed methods to derive and maintain astrocytes from patient iPSCs (261–263). The question as to whether these cells truly represent functioning astrocytes in aged human brains remains to be answered (264). However initial studies have provided interesting insights into neurodegenerative diseases. Studies in AD-derived astrocytes are undoubtedly underway and we await the findings with interest.

Using these tools will unravel the complex role of astrocyte transporters at both a cellular and network level. The interplay between individual astrocyte transporters is evident (e.g. $Na^+.K^+$-ATPase and SLC1A2/SLC1A3) and dysregulation of one set of transporters will impact on the functionality of other associated transporters (Figure 3). The challenge will be to dissect the timeline of this dysregulation to pinpoint molecular targets and their role in AD pathology. Many astrocyte proteins are intimately involved in the pathology of a disease which leads to neuronal cell death. Understanding if this dysregulation in transporter function occurs pre-symptomatically, and the preliminary research indicates that this is the case, will be key to determining a therapeutic strategy. This has been helped by a growing awareness of astrocytes and their biology in the last decade. The purpose of this review was to highlight changes in select astrocytic transporter families and in doing so highlight a host of potential targets for AD. These targets can be classified accordingly to biological research themes which cover key areas not only for AD research but neuroscience research in general (Figure 4). For these targets to become a realisation in terms of clinical products will require further research efforts in translatable neuroscience with direct clinical applications, allowing us to cross the 'valley of death' in drug discovery to provide much needed therapies for tackling AD.

Encouragingly some drugs target some transporters highlighted in this review (e.g. EF1502; Section 3), but with a lack of selectivity for astrocytic isoforms it will hopefully be the next generation of compounds that target astrocyte specific transporters. Preclinical strategies here could use chemogenetic delivery systems as described for selective targeting of astrocyte adenosine receptors (265). This allows for the modulation of target activity through delivery of a synthetic ligand and has distinct advantages over the invasive optogenetic approaches (266). While this chemogenetic approach works well for G-protein coupled receptors (GPCRs), it is unclear if this approach could yield positive results for transporter proteins which are not exclusively coupled in a similar fashion. Research into tailor-made delivery systems is on-going and with pharmaceutic companies waking up to the idea of using the so called 'support cells' to provide patient benefit (e.g. Astrocyte Pharmaceuticals), it will hopefully not

be long before astrocyte-targeting drugs are being considered as viable therapeutic strategies to combat complex neurological conditions, including AD.

Figures/Table:

Figure 1. A timeline of astrocyte involvement in AD pathology. A schematic representation of how astrocytes are believed to be involved in the pathogenesis of AD. (A) GFAP expression is routinely reported to increase in animal models of AD, with available human data indicating this may relate to physiological ageing other than AD specifically. However, the phenotypic change presented is consistent and highlights that this switch occurs before both amyloid deposition and neurodegeneration. This therefore highlights the importance of astrocytes in the early stages of disease and with it the potential to target them for patient benefit. (B) Schematic representation of the phenotypic changes reported in astrocytes from models of AD. (Left) A cartoon of a pre-symptomatic astrocyte showing elaborated processes and regulated volume. (Right) A cartoon of an astrocyte from an AD brain. Astrocyte atrophy is noted in several animal models of AD and can be defined by a variety of parameters including surface area.

Table 1. An overview of the astrocyte transporters with proposed relevance to AD pathology. A review of the literature revealed several astrocytic transporters that have been implicated in AD pathology. Here we present an overview of the main protagonists, their reported involvement and models used to present lines of evidence, as discussed in the text. Where multiple substrates exist the predominant one is mention here. (TgAD= transgenic animal model). *it is unclear as to the contribution of astrocyte SERT to brain physiology; therefore, these observations may reflect neuronal SERT. #it is unclear the role of astrocyte ABCA7, expression levels are observed within all cells of the brain with microglia showing higher levels in comparison to neurones and astrocytes.

Figure 2. Topology of the astrocytic transporters. A schematic representation of the topology of astrocyte transporters. The transporters illustrated here highlight the diversity amongst the transporter families. (A) SLC1 transporter is shown regarding Section 1; (B) SLC2, 6 and 16 are used to illustrate Sections 2 and 3; (C) A SLC7/SLC3 heterodimeric complex is shown to illustrate Section 4; (D) EC 3.6.3.9 is shown to highlight the interplay between α, β and γ subunits to make a functional transporter. This is highlighted in Section 5; (E) MDR1 is shown as an illustration of the ATP-binding cassette transporter family. This family is discussed in Section 6.

Figure 3. Astrocyte transporters coupled to AD signalling cascades. 1) EAAT2 transports glutamate from the synapse into the cytosol using ionic currents generated from primary active transporters which breakdown ATP. Therapeutically increasing the activity of this transporter may reduce glutamate-induced excitotoxicity evident in Alzheimer's disease (AD). 2) Increasing GLUT1 levels or transporter activity could increase astrocyte glycolysis, improving the supply of lactate to neurons. Lactate can be taken up by neurons through MCT1 transporters. 3) GAT3 inhibition could limit GABA release from reactive astrocytes, preventing long term deficits in neuronal activity. Targeting reactive astrocytes this way could increase neuronal activity in AD. GlyT-1 transporter inhibition may potentiate NMDA signalling by increasing the bioavailability of glycine at the synapse. Glycine is a co-agonist of the NMDA receptor and could act to modulate glutamatergic neurotransmission. 4) Glutathione (GSH) is the predominant antioxidant in astrocytes and is depleted in AD brains. Increasing cystine transport, via modulation of System xc-, could boost GSH antioxidant capacity. 5) Targeting Na^+/K^+ ATPase could have mixed effects. Inhibition could decrease sodium efflux from astrocytes but dampen neuronal activity. Conversely, activation would stimulate neuronal activity through increasing synaptic Na^+ concentration but delay repolarisation via increased K^+ influx. Furthermore, due to the interaction between Na^+/K^+ ATPase and EAAT2, targeting this pump may impact glutamate uptake dynamics (numbers refer to the relevant sections within the text).

Figure 4. Schematic of therapeutic opportunities for targeting astrocytes in AD. As outlined within the review various aspects of astrocyte physiology/pathology contribute to neurological disorders. Three specific areas are highlighted as indicated above with the molecular targets also listed. More detail about the specific transporters can be found in the relevant sections of the review.

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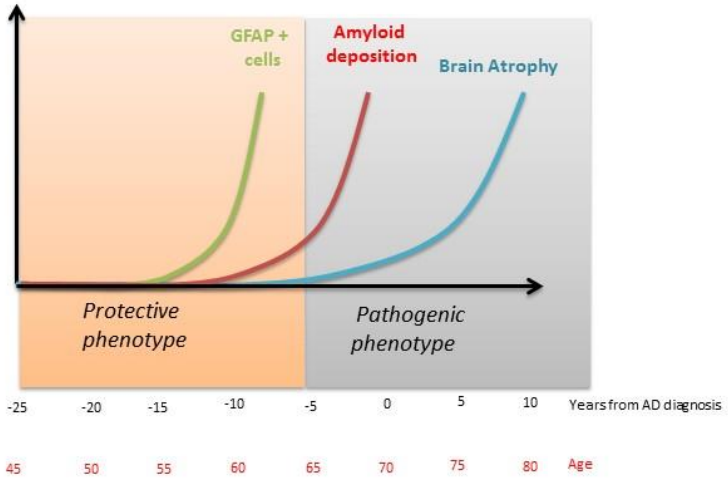
Figure 1.

A.


GFAP + cells
Amyloid deposition
Brain Atrophy
Protective phenotype
Pathogenic phenotype
-25
-20
-15
-10
-5
0
5
10
Years from AD diagnosis
45
50
55
60
65
70
75
80
Age

B.

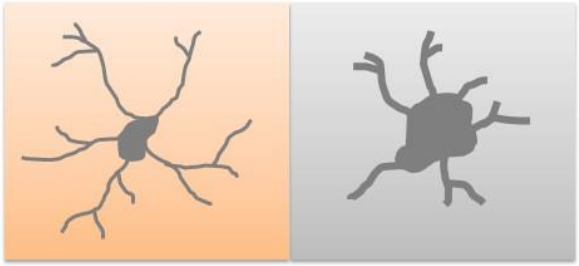


Figure 2.

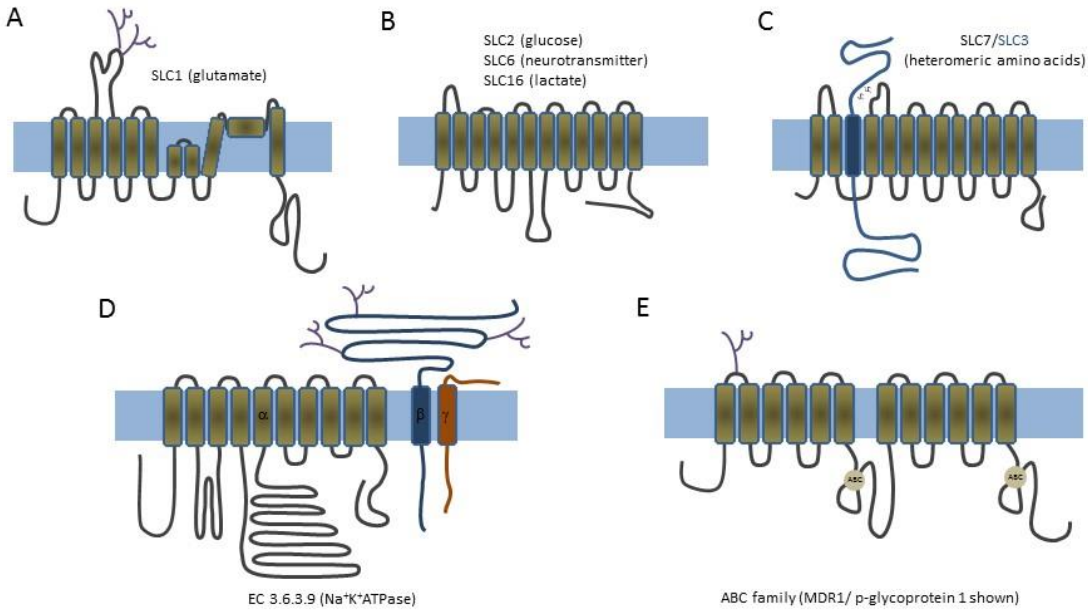

A
SLC1 (glutamate)
B
SLC2 (glucose)
SLC6 (neurotransmitter)
SLC16 (lactate)
C
SLC7/SLC3
(heteromeric amino acids)
D
α
β
γ
E
ABC
ABC
EC 3.6.3.9 (Na+K+ATPase)
ABC family (MDR1/ p-glycoprotein 1 shown)

Figure 3.

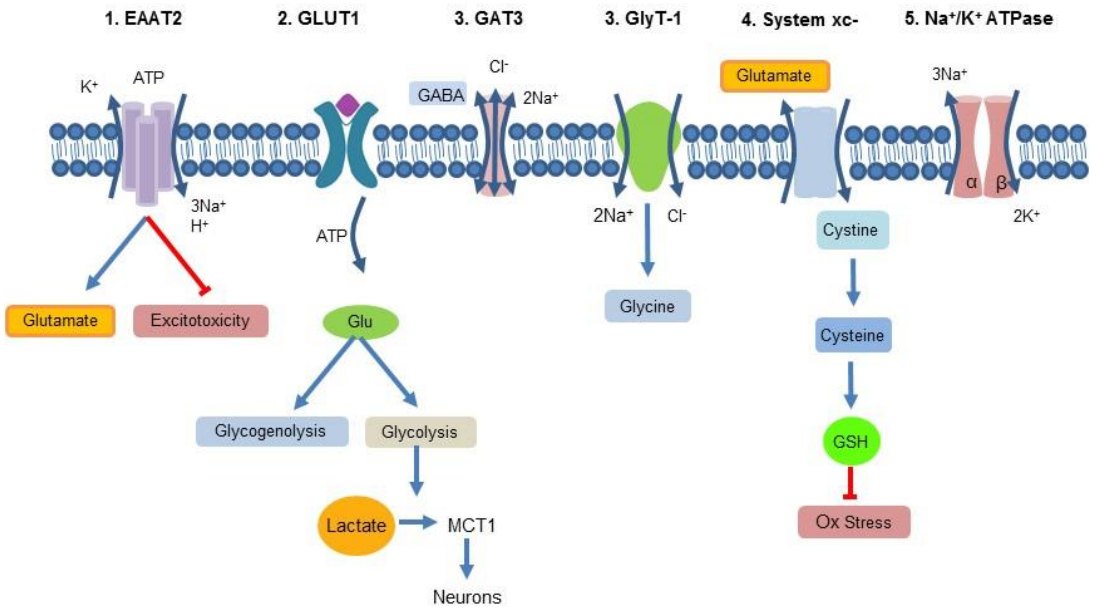
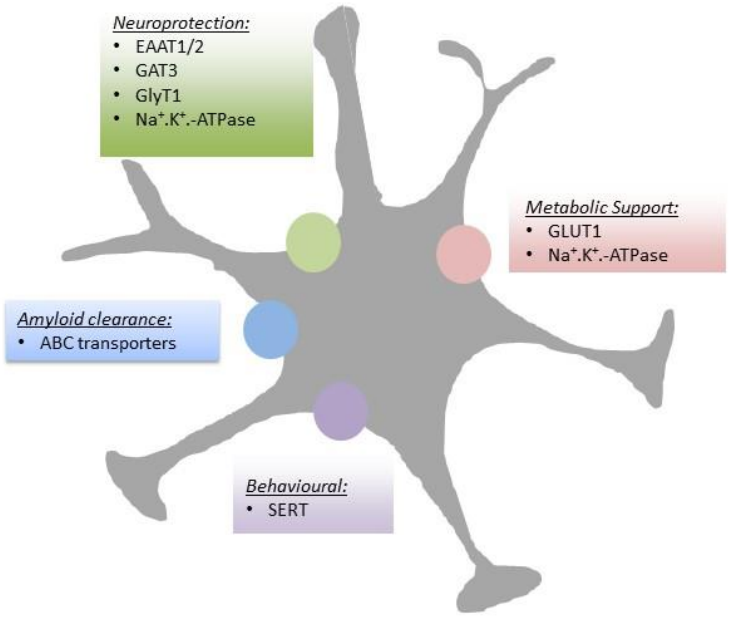

1. EAAT2
2. GLUT1
3. GAT3
3. GlyT-1
4. System xc-
5. Na+/K+ ATPase
K+
ATP
3Na+
H+
Glutamate
Excitotoxicity
ATP
Glu
Glycogenolysis
Glycolysis
Lactate
MCT1
Neurons
Cl-
GABA
2Na+
2Na+
Cl-
Glycine
Glutamate
Cystine
Cysteine
GSH
Ox Stress
3Na+
α
β
2K+

Table 1.

Transporter	Synonym(s)	Substrate	Models	Observations	Impact
EAAT1	SLC1A3/ GLAST	Glutamate	TgAD, Human	Decrease in expression/activity	Increased excitotoxicity
EAAT2	SLC1A2/GLT1	Glutamate	TgAD, Human	Decrease in expression/activity	Increased excitotoxicity
GLUT1	SLC2A1	Glucose	TgAD, Human	Decrease in expression/activity	Hypometabolism
GAT3	SLC6A11	GABA	TgAD, Human	Increase in expression/activity	Increased inhibitory tone.
GlyT1	SLC6A9	Glycine	TgAD	Increased glycine levels	Increased NMDA signalling
SERT*	SLC6A4	Serotonin	TgAD	Decrease in expression/activity	Decrease serotonin uptake
$Na^+.K^+$.ATPase α1 subunit	Atpa1a3	Na^+/K^+	Human	Increase in expression/activity	Decreased excitotoxicity
ABCA7#	ABCX	Lipids	TgAD, Human	Decrease in expression/activity	Increase in Aβ production

Figure 4.


Neuroprotection:
• EAAT1/2
• GAT3
• GlyT1
• Na+.K+.-ATPase
Metabolic Support:
• GLUT1
• Na+.K+.-ATPase
Amyloid clearance:
• ABC transporters
Behavioural:
• SERT