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Astrocyte and glutamate involvement in the pathogenesis of epilepsy in Alzheimer's Disease

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Key point box

- Astrogliosis in Alzheimer's disease can lead to the changes in the expression levels of proteins which are important for the glutamate-glutamine cycle.
- Aβ oligomers can decrease the expression levels of GLT-1 and GS proteins in the brain leading to an increase in seizure susceptibility.
- Neuroinflammation in Alzheimer's disease can cause a loss of function in astrocytes, resulting in an increase in seizure susceptibility.

Summary

Alzheimer's disease (AD) can increase the risk of epilepsy by up to 10-fold compared to healthy age-matched controls. However, the pathological mechanisms which underlie this increased risk are poorly understood. Since disruption in brain glutamate homeostasis has been implicated in both AD and epilepsy, this might play a mechanistic role in the pathogenesis of epilepsy in AD. Prior to the formation of amyloid-β (Aβ) plaques, the brain can undergo pathological changes as a result of increased production of Amyloid Precursor Protein (APP) and Aβ oligomers. Impairments in the glutamate uptake ability of astrocytes due to astrogliosis is hypothesised to be an early event occurring before Aβ plaque formation. Astrogliosis may increase the susceptibility to epileptogenesis of the brain via accumulation of extracellular glutamate and resulting excitotoxicity. Here we hypothesise that Aβ oligomers and proinflammatory cytokines can cause astrogliosis and accumulation of extracellular glutamate, which then contribute to the pathogenesis of epilepsy in AD. In this review article, we consider the evidence supporting a potential role of dysfunction of the glutamate-glutamine cycle and the astrocyte in the pathogenesis of epilepsy in AD.

Keywords: Epileptogenesis, seizures, Alzheimer's disease, glutamate, astrogliosis, neuroinflammation, amyloid-β

Introduction

The bidirectional relationship between Alzheimer's disease and epilepsy

The prevalence of Alzheimer's disease continues to rise globally as the proportion of the older population in many countries continues to grow. Currently, 50 million people in the world are living with dementia, which is placing a global financial burden of approximately 1 trillion US dollars annually[1]. It is predicted that by the year 2050, there will be 150 million people living with dementia and the financial burden will double by 2030[1]. Alzheimer's disease (AD) is the most prevalent type of dementia, contributing ~60-70% of cases[1].

In addition to deteriorating cognitive function, many AD patients also experience spontaneous seizures and epilepsy. It has been reported that AD patients have up to 10-fold higher risk of developing epilepsy compared to healthy age-matched controls[2-5], although it should be noted that, in some of these reports, a diagnosis of AD was made from clinical observations, and so the enrolled patients would presumably also include cases of non-AD dementia. In addition, the relationship between AD/dementia and epilepsy is bidirectional: many patients with epilepsy also experience cognitive disturbances similar to dementia patients[6, 7], and epilepsy patients (also including a heterogeneous collection of epilepsy subtypes) have a 2-fold greater risk of suffering dementia[8] compared with controls. Despite such evidence supporting elevated risk of epilepsy in AD patients, the mechanisms involved in the pathogenesis of epilepsy in this population have not been elucidated. Moreover, there is still no cure for AD or epilepsy and the current treatments are symptomatic with no disease-modifying effect. Understanding the mechanisms of epileptogenesis in AD would potentially aid the development of targeted preventive or disease-modifying therapies.

Toward this end, animal models of AD can be powerful tools to investigate the links between AD and epilepsy. Although rodent models do not display the full AD pathology spectrum, they allow us to isolate and investigate the impact of individual pathologies associated with AD on brain physiology, which is largely not practical in humans. It has been reported in multiple studies that mouse models of AD that overexpress mutant human amyloid precursor protein (APP) have a higher mortality rate than the non-transgenic littermate[9, 10] and this may be caused by unprovoked seizures[11, 12]. Indeed, spontaneous seizures[13-15] and seizure-like events, such as interictal spikes[16], has been observed in several mouse models of AD. It was also reported that mouse models of AD with mutant APP overexpression exhibit neuronal hyperactivity, especially in the area near the amyloid β (Aβ) plaque[17]. Recently, different studies have confirmed that rodent models of AD have higher susceptibility to kainic acid[18], pentylenetetrazole[19] and kindling-induced seizures[20] compared

to the wild-type (WT) littermates. These findings support the use of rodent models of AD to investigate the pathogenesis of epilepsy in AD.

The Glutamate-Glutamine Cycle

Glutamate is the most abundant excitatory neurotransmitter in the central nervous system (CNS) and is responsible for nearly all excitatory synaptic activities in the brain. The movement and metabolism of glutamate is tightly controlled by the glutamate-glutamine cycle. This cycle involves uptake of extracellular glutamate into nearby astrocytes (driven by Glutamate Transporter 1 (GLT-1)), astrocytic conversion of glutamate to glutamine (via the Glutamine Synthetase (GS) enzyme), transfer of glutamine to the extracellular milieu via the SNAT3 transporter, and then into the pre-synaptic neuron via the SNAT1 transporter[21], and finally conversion of glutamine into glutamate by GLS, an enzyme highly enriched in the mitochondria of the pre-synaptic neurons[22]. The rapid uptake and conversion of glutamate by astrocytes prevents prolonged post-synaptic glutamatergic receptor activation, which if left unchecked, could trigger excitotoxicity and seizures, thus extracellular glutamate must be removed promptly after release[22-24]. Changes in the function or expression of any of these key glutamate-glutamine proteins would be anticipated to cause a shift in the equilibrium of extracellular glutamate[25] (Figure 1A). In this review, we propose that the Glutamate-Glutamine cycle plays a role in the pathogenesis of epilepsy in Alzheimer's disease. While other mechanisms may be very relevant[26, 27], the following section will discuss the evidence from the literature on the role of alterations to the Glutamate-Glutamine cycle in the pathogenesis of AD and epilepsy.

The Glutamate-Glutamine Cycle: a link between Alzheimer's disease and epilepsy?

It is known that dysregulation of glutamate in the brain can contribute to the pathogenesis of both AD[28, 29] and of epilepsy[24]. However, the mechanism that links AD pathologies, such as Aβ, APP and tau, to the dysregulation of glutamate and epileptic seizure susceptibility in patients is not elucidated. Since AD is not associated with just one pathology but rather a combination of many, such as Aβ, tau phosphorylation and neuroinflammation[30], it is important to investigate the physiological target that these pathologies may have in common. We propose here that AD pathologies can increase the risk of seizure by disrupting the functional role of astrocytes in the Glutamate-Glutamine cycle, which leads abnormal glutamate homeostasis.

The main physiological function of the astrocyte is to support and protect neurons by providing nutrients and regulating neurotransmitter levels. Astrocytes are heavily involved in maintaining glutamate homeostasis in the CNS via the tripartite synapse structure, which consists of a pre-synapse, a post-synapse and an astrocytic process[31] (Figure 1A). Changes in the expression levels of astrocyte-specific protein such as GLT-1 and GS may therefore lead dysregulated glutamate homeostasis. Astrogliosis is a process triggered by CNS damage and/or disease involving activation and proliferation of astrocytes. In such conditions, the cells undergo striking morphological, metabolic and functional transformations as part of the hosts endogenous defence mechanism to minimise damage and restore homeostasis. Astrogliosis and glutamate homeostasis dysfunction have been associated with the pathogenesis of AD[29, 32] and of epilepsy[33, 34]. Astrogliosis may occur in response to AD pathologies such as Aβ[29], which can then disrupt normal astrocytic glutamate uptake[35]. Since astrocytes are the most important regulator of extracellular glutamate[36], disruption in glutamate uptake via astrogliosis may result in neurotransmitter accumulation in the synaptic cleft. Below, we discuss mechanisms that AD pathologies – in particular Aβ species – interact with astrocytes and the Glutamate-Glutamine cycle, and how this may lead to increased seizure susceptibility (Figure 1B).

Interaction between amyloid-β and the glutamate-glutamine cycle increases risk of seizure

Aβ is one of the major hallmarks of AD and it has been reported that this pathology is associated with astrogliosis[37-39]. Rodent models of AD which overexpress Aβ demonstrate an astrogliosis phenotype, and this typically precedes formation of Aβ plaques[40]. This might mean that Aβ can affect the glutamate-glutamine cycle before plaque formation, with evidence from animal studies also suggesting that cognitive and neuronal network impairments are observed prior to formation of Aβ plaques[41-43]. In addition, astrocytes (and microglia) are involved in the clearance and degradation of Aβ[44]. In AD, this clearance is decreased due to the overproduction of Aβ and/or impairments in the phagocytic capability of glial cells[45, 46]. This accelerates the accumulation of Aβ protein in the brain, which can further enhance reactive astrogliosis. This section will discuss the interaction between Aβ and astrocytes and how associated mechanisms can affect the glutamate-glutamine cycle. Below, we will further elaborate on alternate mechanisms relating to reactive astrocyte release of proinflammatory cytokines which also modulate excitability.

Changes in GLT-1 and GS expression levels are associated with higher seizure susceptibility

There is evidence from human studies[47-50] and rodent models[51, 52] suggesting that GLT-1 gene and protein expression is reduced by AD pathologies. In human temporal lobe epilepsy (TLE), one study reported a significant increase in GLT-1 protein and mRNA in hippocampus samples collected from TLE patients [55]. This increase in expression might be caused by a compensatory mechanism that results in the upregulation of GLT-1 expression to counteract elevated glutamate levels associated with seizures [55]. However, others studies failed to identify differences GLT-1 protein expression in neocortex and temporal cortex of patients, compared to the healthy controls [53, 54]. Despite these findings, data from human study is currently lacking and the effect of epilepsy on GLT-1 expression in humans remains to be clarified.

On the other hand, there is extensive evidence from rodent models to support the hypothesis that loss of GLT-1 could mediate increase in seizure susceptibility in AD. This pathway involves the presence of oligomeric Aβ, which can reduce expression of GLT-1 in rat astrocyte cultures via the transcriptional regulator nuclear factor of activated t-cells (NFAT)[56]. In addition, evidence from *ex vivo* studies provided insights into the functional interactions between AD pathologies and GLT-1 function. One study demonstrated that Aβ oligomers decrease astrocytic glutamate uptake via the promotion of GLT-1 internalisation from the surface of the astrocyte[57]. Also, Aβ oligomers could significantly inhibit glutamate uptake in rat astrocytic cultures via an increase in oxidative stress and ubiquitination of GLT-1[57, 58]. The consequences of reduced GLT-1 appears to be detrimental: cross-breeding APP/PS1 mice (a prominent AD mouse model) with heterozygous GLT-1 mice resulted in significantly accelerated cognitive decline, compared to APP/PS1 with wildtype GLT-1 levels[59]. Furthermore, enhancing brain GLT-1 expression with β-lactam antibiotic such as ceftriaxone improved cognitive function in mouse models of AD[52, 60]. Together, these reports suggest that AD pathologies, such as Aβ and mutant human APP overexpression, may decrease expression of GLT-1 in the brain and this loss of GLT-1 expression correlates with cognitive decline. Furthermore, astrocytic dysfunction and consequential changes in glutamate homeostasis may take place in the presymptomatic stages of AD, without relying on the presence of Aβ plaques[37].

Since astrocytic GLT-1 is important for synaptic glutamate uptake, sustained and progressive loss of GLT-1 expression due to AD pathology could increase the risk of seizure. There is well established evidence from rodent models to support the association between the loss of GLT-1 expression and epileptic seizures. A landmark study by Tanaka and colleagues

demonstrated that GLT-1 deficient mice display a lethal spontaneous seizure phenotype[61], and a subsequent study also confirmed that GLT-1 knock-out mice develop spontaneous seizures after 2 weeks with only 50% of the knock-out mice surviving for 8 weeks[62]. Additionally, inhibiting GLT-1 function with dihydrokainic acid increases extracellular glutamate and epileptiform activity in rats[63]. Further, selectively knocking out astrocytic GLT-1 in mice significantly reduced glutamate uptake from the extracellular space, and this was accompanied by lower survival rate of knock-out mice due to seizures[64]. On the contrary, selectively knocking out neuronal GLT-1 does not produce any abnormal neuronal discharge or seizure[64]. This evidence supports the theory that astrocytic, rather than neuronal, GLT-1 plays an essential role in the prevention of synaptic overexcitation and seizures. On the other hand, transgenic mice with enhanced astrocytic GLT-1 expression display lower acute mortality and less frequent seizures caused by pilocarpine induced-status epilepticus (SE) compared to WT mice[65]. One time-course study investigated the effect of kainic acid induced-SE on the expression of GLT-1 in mice. This study found that there was a transient increase in the GLT-1 expression 1 day after SE, followed by a significant decrease in the expression 4 to 7 days after SE[66]. Despite that, GLT-1 expression returned to the baseline level in the majority of hippocampal regions 30 days after SE[66]. These results suggest that expression of GLT-1 is modified as a consequence of SE, perhaps as compensation for the immediate increase in glutamate release caused by SE. From the current evidence, it appears that loss of GLT-1 expression can be associated with AD pathologies, such as Aβ and mutant APP overexpression, and this may increase the seizure susceptibility due to dysregulation of glutamate homeostasis.

In addition to changes in GLT-1 levels, evidence also suggests that GS, the enzyme responsible for metabolising glutamate to glutamine, is involved in the pathology of both AD and epilepsy. In human AD patients, the density of Aβ plaques is anti-correlated with GS protein levels in the brain[67], and there is also significant loss of enzymatic activity of GS compared to age-matched controls[68]. Changes in GS protein have also been associated with epilepsy (reviewed in [69]). In human mesial TLE patients, decreases in GS protein expression in hippocampus have been found compared to healthy controls, and this was accompanied by significantly lower GS enzymatic activity[70 71]. Furthermore, genetic mutations that lead to GS enzyme deficiency are associated with seizures in new-born humans[72].

Evidence from rodent models also support the involvement of GS in AD and epilepsy: in the 3xTg mouse model of AD, expression levels of GS were significantly decreased in the medial prefrontal cortex during the early and intermediate stages of pathology[73] and in the

hippocampus during later stages[74]. Furthermore, astrocytes in close proximity to Aβ plaques have lower GS expression levels than astrocytes distal from the plaque[74]. Since GS plays an important role in the conversion of glutamate to glutamine in the astrocyte, the reduction in GS protein or decreased enzymatic activity would be expected to result in loss of astrocytic neuroprotective properties against glutamate-induced excitotoxicity[75]. With regards to epilepsy, one important study demonstrated that selectively knocking out GS from the cortex led to spontaneous seizures in mice[76]. This study also found that GS deficient mice had 30% decrease in survival rate and a significant increase in astrogliosis in the brain. Interestingly, knocking out the GS enzyme also resulted in a significant reduction in expression of cerebral GLT-1[76], suggesting a compensatory mechanism that involves the alternation of GLT-1 expression to match the change in GS expression level in order to maintain normal glutamate homeostasis.

The current evidence suggests that loss of GS expression may increase seizure susceptibility, but that seizures *per se* may not reduce GS expression. For example, there is evidence showing a transient increase in GS expression following kainic acid[77] and kindling-induced seizures[78]. On the other hand, pilocarpine-induced SE in rats results in a gradual decrease in GS levels[79]. Although these results are not consistent, they do indicate that changes in the level of GS enzyme in the brain can be associated with epilepsy. Currently, there is not sufficient evidence to determine whether inhibiting or enhancing GS levels in the brain may be beneficial for the treatment of epilepsy.

In summary, the current evidence suggests that the expression and function of both GLT-1 and GS can be altered by AD pathologies, and the loss of these proteins would be expected to alter glutamate homeostasis and consequently increase seizure susceptibility.

Influence of proinflammatory cytokines on seizure susceptibility in AD

Since proinflammatory cytokines have been implicated in the pathogenesis of both AD[80] and epilepsy[81], and these are associated with gliosis, it is also important to consider how cytokines themselves contribute to the increase in seizure susceptibility in AD. In addition to affecting the expression of proteins that are involved in the glutamate/glutamine cycle, soluble Aβ can also act as a damage-associated molecular pattern (DAMP)[82]. As such, increasing soluble Aβ can exacerbate release of proinflammatory cytokines such as interleukin 1 beta (IL-1β) from glial cells, which in turn may contribute to the increase in seizure susceptibility[82, 83].

Proinflammatory cytokines released by reactive astrocytes as a consequence of astrogliosis can act through both autocrine and paracrine manners to enhance the expression of other proinflammatory cytokines[39]. For example, IL-1β and TNF-α can induce astrocytic release of IL-6[84, 85]. In addition to perpetuating and accentuating the neuroinflammatory cycle, cytokines released by these cells can trigger mechanisms that also disrupt glutamate homeostasis and consequently increase in seizure susceptibility. We propose two primary mechanisms that might be relevant to this: alterations in gliotransmission, and facilitation of excitatory neurotransmission.

Gliotransmission describes the release of neurotransmitter such as glutamate from glial cells for the purpose of cell to cell communication. There is evidence to suggest that proinflammatory cytokines, including IL-6, can trigger the release of gliotransmitters such as glutamate from reactive astrocytes[86-88]. This release of glutamate is Ca^{2+}-dependent[89] and it has been shown that IL-6[90] can increase the influx of Ca^{2+} into astrocytes, thus increasing the release of glutamate into the synaptic cleft. The amount of glutamate released from the reactive astrocyte is sufficient to activate high affinity glutamate receptors such as the N-methyl-D-aspartate (NMDA) receptor[91] and therefore this increase in glutamatergic activation can contribute to brain excitability. Whether this distinct mechanism contributes to epilepsy in AD warrants exploration.

Proinflammatory cytokines are also recognised to facilitate excitatory neurotransmission. Here, the role of IL-1β has been highlighted since it is a potent proinflammatory cytokine that is associated with the pathogenesis of epilepsy via long-term and rapid mechanisms[92]. The long-term effects of IL-1β involve genomic events, which lead to structural and functional changes in glial and neuronal networks[92]. For example, the expression of genes that are reported to be associated with epilepsy, such as HCN1, can be significantly increased[92]. These changes cause neurons to be prone to excitatory synaptic transmission by neurotransmitters such as glutamate, theoretically leading to susceptibility to seizures[93]. Alternatively, the rapid mechanism involves kinases and changes in ion channel function[81]. In this mechanism, IL-1β released by the reactive astrocyte activates neuronal kinase systems such as Src tyrosine kinase via the interleukin-1 receptor type 1, which then cause ion channels such as NMDA receptor to be phosphorylated[94]. This phosphorylation can directly increase Ca^{2+} influx and decrease seizure threshold via the glutamatergic pathway[95], leading to neuronal hyperexcitability. Therefore, the increase in the level of proinflammatory cytokines in AD may contribute to increased seizure susceptibility.

Future Research Directions

In conclusion, there is converging evidence supporting the role of astrocytes and the glutamatergic pathway in the pathogenesis of epilepsy in AD. In AD, increased production of Aβ can result in astrogliosis, alterations in glutamate homeostasis and elevated proinflammatory cytokines, which may increase susceptibility to epilepsy. Furthermore, astrogliosis can be one of the earliest events in AD pathology triggered by Aβ oligomers, and does not rely on the presence of mature plaques. Because astrocytes play many crucial roles in the maintenance of healthy brain function, it would be beneficial if astrocytic dysfunction in AD can be corrected, for instance by enhancing GLT-1 expression level pharmacologically, to restore glutamate uptake efficiency. Interactions and relationship between soluble Aβ and proinflammatory cytokines should also be the focus of future research, especially since the human data on this topic is lacking. Such experimental investigations are warranted, based on existing evidence.

Currently, there is no reliable early biomarker for epilepsy in AD, it is important to identify specific, sensitive and stable candidates[96]. The current literature suggests that astrogliosis in AD can take place prior to Aβ plaque formation[40] and therefore investigating how this pathway is affected by the early AD pathology might enhance our chance of identifying a biomarker for the presymptomatic or the mild cognitive impairment stage of AD. This may allow AD to be diagnosed earlier, which will be critical for the prevention of seizures in AD patients. Early diagnosis of AD would also allow important data on the mechanisms that link AD to epilepsy to be generated.

Future research should also aim to detect regional *in vivo* level of extracellular glutamate, rather than the total level of glutamate. In the CNS, the level of intracellular glutamate is higher than the extracellular glutamate[97] and therefore changes in the level of extracellular glutamate can be overshadowed by the intracellular glutamate level when using such techniques as magnetic resonance spectroscopy, or tissue homogenisation. Therefore, measuring astrocytic glutamate uptake from the synaptic cleft would provide more insights into a potential compensatory mechanism that can counter the disruption in the brain glutamate homeostasis caused by the AD pathology. Furthermore, there is little evidence on the effect of AD pathologies on the expression of GLS, SNAT1 and SNAT3 – other key enzymes involved in the glutamate-glutamine cycle, particularly in human subjects. Thus, more investigation is needed to understand the effect of AD pathologies on the glutamate-glutamine cycle as a whole. These insights on the involvement of astrocyte and glutamate-

glutamine cycle may reveal novel drug targets for reducing seizure susceptibility in AD or slowing the progression of AD pathologies.

We also must consider the strengths and limitations of our current 'animal models of AD'. To date, these are generated almost exclusively by manipulating the genome of mice using transgenic technologies. Gene mutations identified in patients can be simply inserted into mouse DNA, oftentimes with that gene overexpressed, allowing us to examine the impact of, say, mutant APP on epilepsy susceptibility. These methods have proven invaluable in creating a greater understanding of the pathological development of plaques, and of facilitating development of treatments targeting plaques. But they are first and foremost models of Aβ, not of AD, since the pathology of AD involves other aspects, notably neurofibrillary tangles composed of hyperphosphorylated tau, as well as substantial neuronal loss – features missing from our current models. The other primary criticism is that these are based on models of familial AD, which only accounts for a small proportion of all AD (sporadic forms are estimated to be >95% of all AD patients)[30]. However, the field is moving in the right direction. Perhaps driven by failures of multiple clinical trials which were initiated following success in animal models[98], there is a growing appreciation of the need to develop new models of disease, incorporating risk factors, such as APOε4 and environmental cues[99], as well as moving from a reliance on rodent models[100], and moving away from mutations in APP and PSEN genes. In the current context, much of the evidence supporting a role for the glutamate/glutamine cycle as a mechanism driving seizure susceptibility in AD is generated from these animal models. While this is currently compelling, future studies must also take advantage of the new wave of animal modelling to explore this hypothesis further.

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Declaration of conflict of interest

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Figure legend

Figure 1: A simplified schematic of the tripartite synapse illustrating the Glutamate-Glutamine cycle **(A)** under normal circumstances and **(B)** in the presence of AD pathology-associated molecules such as Aβ oligomers and proinflammatory cytokines. **(A)** In homeostatic conditions, glutamate is taken up into astrocytes via GLT-1, converted to glutamine by GS, and transported back to the presynaptic terminal via SNAT proteins. Glutamine is then converted back to glutamate, ready to be released upon neuronal stimulation. **(B)** In AD, astrocytes undergo structural and functional transformation through a process called astrogliosis. These transformations lead to reduced expression of Glutamate Glutamine cycle associated proteins such as GLT-1 and GS, thus decreasing the rate of extracellular glutamate uptake and glutamine synthesis. In addition, astrogliosis results in the release of proinflammatory cytokines such as IL-β, and gliotransmitters such as glutamate, into the synaptic cleft. Together, this alters the fidelity of the glutamate-glutamine cycle, resulting in accumulation of extracellular glutamate in the synaptic cleft, and potentially leading to synaptic hyperexcitability and increases in seizure susceptibility

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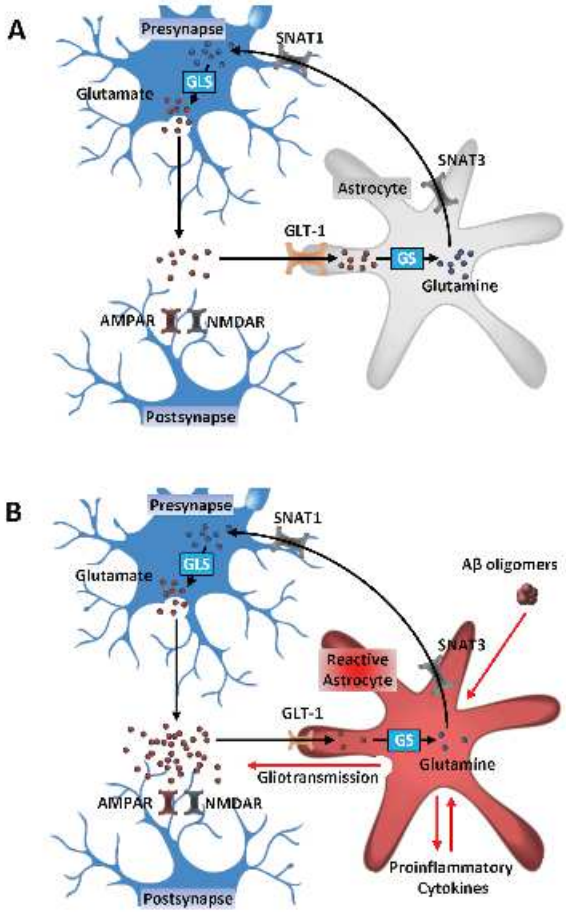

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