Published in final edited form as:

J Neurovirol. 2014 August ; 20(4): 315–331. doi:10.1007/s13365-014-0258-2.

Glutamate Metabolism and HIV-Associated Neurocognitive Disorders

Fabián J. Vázquez-Santiago¹, Richard J. Noel Jr.², James T. Porter³, and Vanessa Rivera-Amill^{1,*}

¹Department of Microbiology, Ponce School of Medicine and Health Sciences, Ponce, PR 00716

²Department of Biochemistry, Ponce School of Medicine and Health Sciences, Ponce, PR 00716

³Department of Pharmacology, Ponce School of Medicine and Health Sciences, Ponce, PR 00716

Abstract

HIV-1 infection can lead to neurocognitive impairment collectively known as HIV-Associated Neurocognitive Disorders (HAND). Although combined antiretroviral treatment (cART) has significantly ameliorated HIV's morbidity and mortality, persistent neuroinflammation and neurocognitive dysfunction continue. This review focuses on the current clinical and molecular evidence of the viral and host factors that influence glutamate-mediated neurotoxicity and neuropathogenesis as an important underlying mechanism during the course of HAND development. In addition, discusses potential pharmacological strategies targeting the glutamatergic system that may help prevent and improve neurological outcomes in HIV-1 infected subjects.

Keywords

HIV; neuropathogenesis; HAND; glutamate; astrocytes

INTRODUCTION

HIV-1 invades the central nervous system (CNS) very early after the initial infection and establishes an infection in non-neuronal brain cells (Figure 1) (Carroll-Anzinger and Al-Harhi, 2006; Li *et al*, 2011; Schnell *et al*, 2011; Valcour *et al*, 2012), which infection may ultimately result in the development of HIV-associated neurocognitive disorder (HAND) (Heaton RK, 2011; Moore *et al*, 2011). CNS infection is believed to originate in the peripheral trafficking of activated infected cells such as monocyte-derived macrophages (MDM) and lymphocytes, both of which reach the CNS through meningeal and parenchymal tissues (Lamers *et al*, 2011). The “Trojan Horse Hypothesis” model states that, in HIV⁺ subjects, the loss of blood brain barrier (BBB) integrity allows infected cells to

*To whom correspondence and reprint request should be addressed at Department of Microbiology, Ponce School of Medicine, Ponce PR 00732-7004, Tel. (787) 840-2575; Fax (787) 841-5150; vrivera@psm.edu.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

enter the CNS. HIV released from the infected cells causes infection in brain tissues resulting in inflammation, neuronal damage and the establishment of central reservoirs (Ghafouri *et al*, 2006; Kaul, 2009; Lamers *et al*, 2011).

The HIV-related neurocognitive complications comprise three conditions: asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-1 associated dementia (HAD) (Antinori *et al*, 2007). In each category, the neuropathological correlate HIV-1 encephalitis (HIVE) may accompany the onset of disease (Cherner *et al*, 2007) and, in some cases, promote a worse neurological outcome. The widespread use of combined antiretroviral therapy (cART) has resulted in a higher prevalence of the milder forms of HAND (ANI, MND). The basis for the persistence of the milder forms of cognitive impairment remains unclear but suggests that HIV-associated neurodegenerative pathology persists in the brain despite cART. This means that the impact of neurologic comorbidity on HIV-1-infected individuals has become a significant clinical challenge: HIV⁺ individuals are living longer and the mental deficits that characterize HAND often interfere with the cognitively demanding activities found in everyday life (Heaton *et al*, 2004; Hinkin *et al*, 2004). In addition, HAND is associated with an increased risk for early mortality independent of clinical predictors (Antinori *et al*, 2007; Cherner *et al*, 2007; Everall *et al*, 2009; Vivithanaporn *et al*, 2010). The etiology for the development of neuropathologic manifestations in HAND include marked neuronal death, loss of synaptic plasticity, improper neurotransmitter balance and loss of metabolic functions that results from chronic pro-inflammatory states and immunologic failure (Kaul, 2008; Kaul, 2009). Current research indicates complex regulatory dynamics and concerted roles of the CNS glutamatergic system in neurological disorders. This has stimulated research into the molecular mechanisms underlying glutamate regulation in the neuropathogenic basis of HAND (Kaul, 2009; Potter *et al*, 2013). A genome-wide screening of transcriptional regulation in the brain tissue of HIV⁺ neurocognitively impaired subjects with and without HIVE revealed that there are 2 patterns of differential gene expression in HAND (Gelman *et al*, 2012). Gene expression in HAND subjects with HIVE is characterized by the upregulation of inflammatory genes and the downregulation of neuronal transcripts, whereas in subjects without HIVE, the pattern of gene expression is characterized by a lack of neuronally expressed changes in the neocortex and the upregulation of endothelial cell-type transcripts, suggesting that more than one pathophysiological process occurs in HAND (Gelman *et al*, 2012).

The use of cART has proven to be effective in improving neurocognitive function in patients with HAND (Bogoch *et al*, 2011; Cysique *et al*, 2009). The cART regimen aims towards controlling systemic viremia and inflammation (Gendelman *et al*, 1998), improving immunological function, and preventing extensive CNS inflammation (Spudich *et al*, 2011), resulting in increased in life expectancy and decreased HIV-related neurological complications (Parsons *et al*, 2006). Although cART does not completely eradicate viral-induced neurologic dysfunction, it has proven considerably effective in ameliorating further CNS progression by preventing neuronal death and decreasing CNS viral titers (Gendelman *et al*, 1998; Xia *et al*, 2011).

While multiple mechanisms are likely at play during the development and progression of HAND, in this review we will place a special emphasis on the evidence which indicates that astrocytes play a significant role in such development and progression. Astrocytes are the primary CNS cells that mediate and contribute physiologic and pathophysiologic regulation within the brain and thus represent both causative and consequential players during various neurologic disease processes. Astrocytes in HAND development and progression have been previously regarded as being secondary players of the mechanisms driving the neuropathology in HIV-1 infection. However, in this review, we will emphasize both the direct and the indirect data that highlight key aspects of astrocytes as important modulators of HIV-1 neuropathology, which data may provide novel targets and new insights for the development of adjunctive pharmacological treatment for ameliorating HAND.

HAND and inflammatory mediators

HIV-1 infection of the CNS is associated with neuroinflammation and immune activation that lead to neuronal injury. HIV⁺ subjects with neurologic impairments display higher levels of inflammatory markers in CSF and plasma than do individuals who do not have HIV or those who are HIV⁺ but who are without any kind of neurological burden. The pro-inflammatory cytokine profile in the CSF and brains of HIV⁺ subjects with neurological impairments is characterized by elevated glutamate-responsive factors, i.e., tumor necrosis factor alpha (TNF- α) (Meeker *et al*, 2011), interleukin-1 beta (IL-1 β), inducible nitric oxide synthase (iNOS) (Zhao *et al*, 2001), IFN- γ (Meeker *et al*, 2011; Shapshak *et al*, 2004), and IFN- γ -inducible protein CXCL10 (formerly known as IP-10) (Peluso *et al*, 2013). Changes in the levels of neuronal and inflammatory markers in the CSF of HIV⁺ subjects have been detected within 3 months of diagnosis, indicating that neuronal inflammation and injury are early events in HIV infection (Peluso *et al*, 2013). Treatment naïve HAND subjects with advanced stages of immunodeficiency exhibited high CSF concentrations of interleukin-6 (IL-6) and macrophage inflammatory protein 1 β (MIP-1 β) as compared to treatment naïve HIV⁺ subjects with advanced disease but without HAND (Airoldi *et al*, 2012). This pro-inflammatory profile in the CNS is not specifically targeted or improved by cART, as the viral loads in plasma and CSF were decreased after antiretroviral treatment initiation, yet the levels of the pro-inflammatory cytokines remained high even after 12 weeks on antiretroviral therapy (Airoldi *et al*, 2012). The cellular sources of pro-inflammatory cytokines most likely arise from infiltrating MDM and microglia, although the persistence of the proinflammatory profile indicates that other factors may also be at play throughout the course of CNS disease (Figure 1).

HIV⁺ subjects with neuroinflammation (HIV encephalitis, HIVE) are also subject to dysregulation of gene-expression patterns. In post-mortem tissues from HIVE patients, the detection of hippocampal neuroinflammation was accompanied by an increase in glial fibrillary acidic protein (GFAP) immunoreactivity (Anthony *et al*, 2005), suggesting that astrocytes have an active role in the modulation of the disease. Other genes that have been found to be dysregulated include: upregulation of the signal transducer and activator of transcription gene (STAT-1), downregulation of the ionotropic glutamate receptor (NMDAR), and downregulation of the microtubule-associated protein 2 (MAP-2) neuronal marker (Masliah *et al*, 2004), some of which are known to participate in neuronal and glial

glutamate metabolism. The findings of increased GFAP and decreased MAP-2 immunoreactivity (Masliah *et al*, 2004) correlated best with IFN-related genes, suggesting that interferons play an important role in the neuropathology. In other cases, subjects who displayed the classical neuropathological hallmarks of diffuse microglia activation, reactive gliosis, and white matter pallor presented a significant loss in the expression of excitatory amino acid transporter (EAAT-2) and upregulation of IL-1 β and TNF- α .

Chronic immune activation and failure to control neuroinflammation promote greater CNS toxicity in HAND partially by modulating the glutamate-glutamine cycle (described below, Figure 2). The pro-inflammatory cytokines TNF- α (Pascual *et al*, 2012; Ye *et al*, 2013; Zou and Crews, 2005), interleukin-1 β (IL-1 β), macrophage inflammatory protein 2 gamma (MIP-2 γ) (Fang *et al*, 2012), and interferon (both IFN- α and IFN- γ) (Lee *et al*, 2013; Li *et al*, 2011) have been demonstrated to increase glutamate-induced neuronal death and potentiate astrocytic and microglial excitotoxicity. By facilitating viral replication and the expression of viral antigens within microglia and astrocytes (Carroll-Anzinger and Al-Harhi, 2006), TNF- α and IL-1 β both play major roles in the pathogenic and neurodegenerative processes seen in the brains of HIV-1-infected individuals (Brabers and Nottet, 2006; Tornatore *et al*, 1991). Post-mortem brains with HIVE present elevated levels of TNF- α , IFN- α , IFN- γ , and IL-1 β in the prefrontal cortices, which levels may render greater permissiveness for the productive infection of astrocytes seen in the most severe case of encephalitis (Anthony *et al*, 2005; Masliah *et al*, 2004; Xing *et al*, 2009). Additional investigation to uncover the broad repertoire of neurotoxic factors released by IFN- γ -stimulated human astrocytes revealed that glutamate neurotoxicity may act through the IFN γ R-STAT3 pathway (Hashioka *et al*, 2011). Human astrocytes stimulated with IFN- γ generate and release neurotoxic factors similar to reactive microglia, including arachidonic acid, glutamate, and ROS. Thus activated astrocytes may act to amplify and potentiate the neurotoxic effects exerted by activated microglia (Lee *et al*, 2013). Furthermore, neonatal murine astrocytes stimulated with TNF- α for 24 hours overexpressed MIP-2 γ , resulting in the downregulation of EAAT-2 (orthologous with rodent glutamate transporter, GLT-1) mRNA and protein levels. MIP-2 γ was not directly toxic to neurons in culture, but rather the accompanying decrease of GLT-1 and its activity resulted in a loss of neuroprotection from high extracellular glutamate (Fang *et al*, 2012). Interferon- γ -inducible peptide-10 (CXCL10) is another neurotoxic chemokine that is elevated in during HIV infection across a broad range of clinical presentations (Asensio *et al*, 2001; Cinque *et al*, 2005). Human astrocytes either infected with HIV-1 BaL or NL4.3 isolates or exposed to TNF- α or IFN- γ upregulate CXCL10 mRNA and protein levels. The combination of these pro-inflammatory cytokines (TNF- α /IFN- γ) and HIV infection further promotes CXCL10 production and enhances glutamate release in cultured human astrocytes via pro-apoptotic and inflammatory pathways involving signaling by JAK, STATs, MAPK, and NF- κ B (Williams *et al*, 2009). These results indicate that cytokines are primarily involved in regulating CNS inflammatory processes and neuronal toxicity by directly affecting the protection to neurons provided by astrocytes.

Glutamate release is subject to calcium regulation, and CD38 is an enzyme in astrocytes involved in intracellular calcium signaling. CD38 upregulation was demonstrated by

immunohistochemical analysis in brain tissue from HIVE patients (Kou *et al*, 2009). Activated astrocytes increase CD38 expression after IL-1 β treatment, and this upregulation is mediated by the MAPK and NF- κ B signaling cascades (Mamik *et al*, 2011). Thus the resulting overexpression of CD38 partially occurred because of the evoked glutamate release, which was in turn caused by elevated Ca²⁺ influx (Bruzzone *et al*, 2004). Further dissections of Ca²⁺-dependent signaling mechanisms have revealed a role for CXCR4-axis-mediated glutamate neurotoxicity as an important modulator between glia-glia and glia-neuron communication system. The stimulation of GFAP⁺ astrocytes expressing CXCR4 with the CXCR4 natural ligand, CXCL12, required the presence of TNF- α to evoke glutamate release. Pharmacological blockage with the non-competitive CXCR4 inhibitor, AMD3100, abrogated the CXCR4 signaling pathways, preventing glutamate release (Bezzi *et al*, 2001). Summed together, the resulting astrocyte dysfunction enhances glutamate-related damage as a result of the persistent and unresolved local CNS inflammatory processes that occurs in patients with HAND, especially in those subjects with HIVE.

The dysregulation of gene expression patterns is affected not only by the neuroinflammation process itself but age-dependent factors may also be at play. Immunohistochemical analyses from 83 HIV⁺ individuals to examine the differences between young vs. aged subjects showed a marked GFAP and Iba-1 increase while MAP-2 was decreased in brain tissue from aged HIV⁺ subjects with HIVE as compared to young HIV⁺ subjects with HIVE (Fields *et al*, 2013). However, both aged and young HIV subjects with HIVE exhibited increased GFAP and Iba-1 immunostaining and decreased MAP-2 as compared to subjects without HIVE. In addition, there were also differences in the levels of autophagy related proteins in brain tissues samples from young and aged HIVE subjects (Fields *et al*, 2013). As HIV becomes a more manageable chronic disease, the list of co-morbidities increases and points to the importance of characterizing the differences in brain-related functions among young and aged HIV⁺ individuals.

The glutamate-glutamine cycle and HAND

In the CNS, the amino acid glutamate is the major excitatory neurotransmitter and its release mediates Ca²⁺-dependent signaling events through the binding and activation of ionotropic N-methyl-D-aspartate receptors (NMDA) (Fu *et al*, 2013; Malarkey and Parpura, 2008). Under physiological conditions, neurons are incapable of producing glutamate *de novo* and thus receive glutamine as the non-toxic precursor for synthesizing glutamate, which is required for post-synaptic excitatory neurotransmission (Hertz, 2011; Pardo *et al*, 2011). Neuronal glutamate is produced from glutamine by the catalytic activity of mitochondrial-bound glutaminase type 1 (GLS-1) (Holcomb *et al*, 2000; Ye *et al*, 2013). The extracellular release of glutamate exerts its excitatory activity upon Ca²⁺-mediated activation of NMDA receptors embedded within the synaptic dendrites of glutamatergic neurons (Fu *et al*, 2013; Tavares *et al*, 2002). The removal of extracellular glutamate from the post-synaptic cleft (Figure 3) occurs via reuptake through the high-affinity Na⁺-dependent excitatory amino acid transporters 1 and 2 (EAAT-1 and EAAT-2), which are expressed at the plasma membrane surface of astrocytes and microglia (Lehmann *et al*, 2009; Qian *et al*, 2011). Thereafter, glutamate may either be amidated by the catalytic action of glutamine synthetase (GS) to replenish the intracellular pools of glutamine (Fernandes *et al*, 2010) or deamidated

by glutamate dehydrogenase (GDH) to produce α -ketoglutarate and other amino acid intermediates for the oxidative pathway of energy production (Dadsetan *et al*, 2011; Skytt *et al*, 2012). A summary of the genes and proteins involved in the glutamate-glutamine cycle is provided in Table 1.

Glutamate transmission and metabolism are affected in many neurological diseases, including Alzheimer's disease (Ferrarese *et al*, 2001), Parkinson's disease (Plaitakis *et al*, 2010), amyotrophic lateral sclerosis, epilepsy (Eid *et al*, 2013a; Eid *et al*, 2013b), and HIV-1-associated neurocognitive disorders (Cohen *et al*, 2010; Gongvatana *et al*, 2013; Lentz *et al*, 2009). Whether glutamate levels in CSF or plasma represents a useful indicator of HIV-associated excitotoxicity remains unclear. Initial studies to examine the potential role of glutamate in chronic neurodegenerative syndromes as a result of a retroviral infection were conducted in the LP-BM5 leukemia retrovirus mixture mouse model (Espey *et al*, 1998). The LP-BM5 infected mice develop an immunodeficiency syndrome similar to human AIDS (Kustova *et al*, 1997) and glutamate concentrations in the CSF of LP-BM5 infected mice were found to be elevated (Espey *et al*, 1998). However, analysis of human CSF revealed no correlation between the concentration of glutamate and either HIV-1 infection or HAD (Espey *et al*, 1999). To the contrary, other studies found increased glutamate levels both in the CSF and plasma of HIV-infected patients and the glutamate levels positively correlated with the degree of dementia and brain atrophy (Ferrarese *et al*, 2001; Ferrarese *et al*, 1997). The difference in the results was later addressed by both groups and it was recognized that the discrepancy could be due to methodological differences (Espey *et al*, 2002). Nonetheless, it is widely accepted that brain metabolite dysregulation occurs early in HIV infection (Sailasuta *et al*, 2012) and persists in the setting of chronic and stable disease (Harezlak *et al*, 2011). Clinical studies using magnetic resonance spectroscopy (MRS) to evaluate metabolite levels in early versus chronic cognitively normal HIV⁺ subjects showed a marked reduction in the glutamate levels of HIV⁺ subjects as compared to control (Lentz *et al*, 2009; Sailasuta N, 2009). These studies suggest that HIV causes neuronal dysfunction soon after infection and may represent a key target for early intervention to reduce neurocognitive impairments among HIV⁺ patients.

The underlying mechanism that leads to neuropathology is somewhat unclear but it is likely to include the peripheral infiltration of HIV-1—infected MDM and subsequent infection of astrocytes and resident microglia. An examination of brain tissue samples from 589 HIV⁺ subjects revealed that approximately 17% of the cases exhibited parenchymal brain pathology characterized by HIVE, leukoencephalopathy and microglial nodular encephalitis (Everall *et al*, 2009). Astroglial alterations have been observed in *in vitro* and *in vivo* using animal models and postmortem tissue from human AIDS patients (Persidsky *et al*, 2001; Wyss-Coray *et al*, 1996). The localization of astrocytic HIV-1 infection in HAND patients has been identified as occurring within the deep tissues of the frontal lobe regions, wherein HAND cases with accompanying encephalitis had a greater frequency and wider anatomical distribution of infected and activated astrocytes (Churchill *et al*, 2009; Thompson *et al*, 2004), thereby suggesting that the extent of astrocytic infection and dysfunction contributes to HIV neuropathogenesis.

Role of glia in glutamate-glutamine dysregulation in HAND

Glial cells comprise a diverse population of non-neuronal cells including astrocytes, oligodendrocytes and microglia that reside within the brain and spinal cord tissues. These cells actively participate in the neuroinflammatory events that occur in various conditions by changing their phenotype to a more active state called reactive gliosis. Microglial cells are involved in a variety of functions under physiological and pathophysiological conditions. They express differentiation markers of the monocyte/macrophage lineage such as CD14 and CD16 (Fischer-Smith *et al*, 2004) and participate in the processes of localized CNS immune response, local tissue repair and scar formation and the removal of surrounding cellular debris. These cells regulate neuroinflammation by secreting anti- and pro-inflammatory cytokines in response to neuronal alterations or neuronal death. Microglial cells have been extensively characterized as key players in mediating the neuropathogenesis of HAND (Bezzi *et al*, 2001; Brabers and Nottet, 2006; Xing *et al*, 2009). Astrocytes, on the other hand, comprise the major cell type population within white and grey matters and perform an array of specialized functions providing anatomical networks and physiological support to regional neurons (Zou *et al*, 2010). Astrocytes are responsible for the metabolic processing of the amino acids and carbohydrates that are necessary to maintain the balance of antioxidants (Almeida *et al*, 2002; Lee *et al*, 2010a), the generation of energy supply (Kreft *et al*, 2012), the intercellular exchange of metabolic precursors (Hertz, 2011; Maciejewski and Rothman, 2008; Pardo *et al*, 2011), and regulate neuroinflammatory responses (Lee *et al*, 2013; Xu *et al*, 2009).

Although some of the complex regulatory mechanisms employed by astrocytes and microglia to maintain glutamate homeostasis have yet to be dissected, HIV-1 cell culture and animal models have granted a better understanding about the contributing roles of glutamate metabolism and altered glutamate neurotransmission during the course of HIV neuropathology. *In vitro* studies dissecting the underlying mechanisms of astrocytic toxicity have uncovered that, despite the low percentage, infected cells astrocytes produce toxicity that promotes apoptosis in uninfected, neighboring astrocytes. The apoptotic signal transits via gap junctions to the uninfected cells (Eugenin and Berman, 2007). This novel mechanism of bystander toxicity coordinated by gap junctions has been demonstrated also *in vivo* using an animal model of accelerated NeuroAIDS, SIV-infected macaques (Eugenin *et al*, 2011). These data indicate that a few infected astrocytes are capable of triggering bystander cell dysfunction, endothelial apoptosis and blood-brain-barrier (BBB) compromise.

Neurons, while not directly infected by HIV-1, are susceptible to the indirect neurotoxic damage induced by cytokine-stimulated or HIV-infected nearby cells (Kovalevich and Langford, 2012). For example, the microglial release of TNF- α induces the downregulation of astrocytic EAAT-2 (Tilleux *et al*, 2009) and potentiates glutamate exocytosis via mechanisms involving oxidative damage and the nuclear translocation of NF- κ B (Zou and Crews, 2005). Activated microglial cells trigger the astrocytic release of glutamate to modulate excitatory transmission (Pascual *et al*, 2012; Patton *et al*, 2000) and also to decrease glutamate uptake (Takaki *et al*, 2012). Experimental data from a mixed culture of astrocytes, microglia, and neurons demonstrated that the microglial response to

inflammation resulted in a significant release of L-glutamate. This led to a rise in intracellular glutamate in astrocytes and a down-regulation of the glutamate aspartate transporter (GLAST/EAAT-1) expression and function, which further increased extracellular glutamate concentration (Takaki *et al*, 2012). The prevention of glutamate release from microglia by carbenoxolone abrogated the response in astrocytes, indicating that under conditions of neuroinflammation, the interplay between microglia and astrocytes contributes to the disruption of the extracellular glutamate homeostasis (Takaki *et al*, 2012). Interestingly, previous studies aimed at unraveling the mechanisms of EAAT-1 regulation in human astrocytes have demonstrated that TNF- α diminishes EAAT-1 promoter activity after 24 hours of treatment (Kim *et al*, 2003), thus suggesting that the loss of EAAT-1 may accompany the early stages of neuroinflammation *in vivo*. In sum, these observations indicate that the inflammatory-driven environment seen in HAND patients plays a major role in the glutamate-glutamine cycle regulation, and thus glial-neuronal communication is sensitive to the presence of inflammatory mediators.

Proton magnetic resonance spectroscopy (^1H -MRS) has been used to monitor *in vivo* and *ex vivo* changes in brain metabolites. Several groups that use the non-human primate model of HIV/AIDS have applied ^1H -MRS to evaluate brain metabolite changes associated with SIV infection and their studies have revealed metabolic abnormalities similar to those observed in HIV-infected human brains (Bossuet *et al*, 2004; Cloak *et al*, 2011; Greco *et al*, 2004; Kim *et al*, 2005; Lentz *et al*, 2008; Ratai *et al*, 2009). Cynomolgus macaques that remain asymptomatic after infection with a pathogenic SIVmac251 isolate, demonstrate elevated glutamate levels in their prefrontal cortices and caudate putamen even with minimal CSF viral burden (Bossuet *et al*, 2004). These regions are responsible for executive functions, decision-making, social behavior, motor movements, learning, and memory. One third of the infected animals in that study, which focused on infected animals without progression, had detectable astrogliosis and microglial inflammation shown by increased GFAP and CD68 immunoreactivity by the first year of infection, even without apparent major neurologic complications. This gliosis eventually came to affect 50% of the chronically infected macaques (ranging from 24–83 months). The data suggest that even in individuals with asymptomatic disease, neurological inflammation and reactive gliosis in the putamen and prefrontal cortex may result in excess glutamate levels in those brain regions (Bossuet *et al*, 2004). Changes in brain metabolites have been detected shortly after infection and correlate with profound astrogliosis as well as viremia (Greco *et al*, 2004; Kim *et al*, 2005). To explore the changes in the environment of the glutamatergic synapses, the expression of the EAATs in the putamen of macaques infected with either neurotropic SIVmac251 or SIVmac239 was analyzed (Meisner *et al*, 2008). Macaques that progressed to AIDS exhibited a greater loss in EAAT-1 and EAAT-2 expression as compared to asymptomatic and control macaques (Meisner *et al*, 2008). This progressive loss of EAAT-1 and EAAT-2 was associated with increased microglial activation and subsequent alteration of astrocytic function (Meisner *et al*, 2008). Astrocytes are the main cell type expressing these 2 transporters, thus under conditions that alter the expression of the transporters (i.e. inflammation) astrocytes cease their neuroprotective activities and contribute to glutamate-mediated injury.

In addition to the improper removal of glutamate from the extracellular space and the decreased intracellular turnover to glutamine during inflammatory responses, excessive neuronal and glial production of glutamate may be an important mechanism in glutamate-evoked toxicity (Ye *et al*, 2013). The upregulation and secretion of mitochondrial-bound glutaminase type 1 (GLS1) has been demonstrated to occur using HIV-1 infection models of both primary human microglia and monocyte-derived macrophages (MDM) (Erdmann *et al*, 2009; Erdmann *et al*, 2006; Huang *et al*, 2011; Pais *et al*, 2008; Tian *et al*, 2008; Zhao *et al*, 2004; Zhao *et al*, 2012). The contribution of MDM-associated neurodegeneration in encephalitis became apparent when SCID HIVE mice exhibited behavioral and memory deficits during the Morris Water Maze test (Zink *et al*, 2002). These deficits, were attributed both to the loss of hippocampal expression of neuronal markers (synaptophysin, MAP-2) and to the inflammatory processes mediated by the presence of infected MDM (Anderson *et al*, 2003; Zink *et al*, 2002). The *in vivo* contribution of viral infection to glutamate production has been studied by the intracranial injection of human MDM infected with HIV-1_{ADA} into a severe combined immunodeficiency mouse model of HIV-1 encephalitis (SCID HIVE) (Ye *et al*, 2013). Notably, astrogliosis and inflammatory cytokines are evident after MDM inoculation (Persidsky *et al*, 1996). Moderate HIV-1 infection within human MDM significantly increased the expression of GLS1 in neurons and led to increased glutamate production in areas of neuroinflammation, suggesting a role for glutaminase in tissue pathology (Ye *et al*, 2013). The up-regulation of glutaminase gene expression is mediated by the phosphorylation of STAT1 and its subsequent binding to the glutaminase promoter (Zhao *et al*, 2012). The relevance of this observation has been corroborated in post-mortem brain tissues (Ye *et al*, 2013) as well as by HIVE subjects from the NNTC cohort who had significant increases in STAT-1 and IFN- γ mRNA levels, which occurred in parallel with a loss of glutamate receptor signaling (Gelman *et al*, 2012). In addition to the modulating effects of HIV-1 infection or viral gene products on glutamate homeostasis, glutamate itself can decrease EAAT-1 and EAAT-2 expression when elevated in the extracellular space, promoting its own neurotoxic build-up in a manner that is independent of mGluR activation (Lehmann *et al*, 2009). These observations suggest that not only may viral proteins act to release excitotoxic levels of glutamate and to diminish reuptake capacity by glial cells and neurons, but also that the extracellular accumulation of glutamate due to failure in its removal can help sustain further neuronal injury.

HIV gp120 impairs the glutamate-glutamine cycle

HAND is thought to result from the complex interaction of both viral and host factors including direct and indirect mechanisms of neurotoxicity. One of the viral neurotoxins implicated as an important factor driving the neuropathogenesis of HAND is the HIV-1 envelope glycoprotein gp120. Besides its well-known role in cellular binding via the CD4 receptor and coreceptors CXCR4 and CCR5, gp120 also promotes an array of detrimental alterations that may lead to the neurodegenerative processes observed in HAND. Gp120 mediates neurotoxicity via multiple pathways leading to cellular death and the release of soluble excitotoxic mediators. There is both *in vitro* and *in vivo* evidence of gp120's ability to cause neurotoxicity. Increased immunoreactivity to GFAP and protein kinase C (PKC) occurs in gp120-treated astrocytes, in brains of gp-120 transgenic mice, and in brains from humans with HIVE (Wyss-Coray *et al*, 1996). Early *in vitro* experiments showed that

astrocytic expression of the EAAT-2 glutamate transporter was reduced rapidly upon exposure to gp120. This loss was accompanied by a reduction in glutamate uptake activity (Patton *et al*, 2000; Vesce *et al*, 1997; Wang *et al*, 2003). Even though it is widely accepted that gp120 triggers neurotoxicity by directly affecting both glutamate uptake and release, the effect of gp120 in modulating CNS glutamate metabolism particularly in astrocytes continues to be an important area of research.

Increased oxidative stress is also suggested to play an important role in the neuropathogenesis of HAND (Nath, 2002; Turchan *et al*, 2003) as glutamate toxicity may result from glutathione depletion and oxidative stress (Reviewed in: (Gras *et al*, 2006). HIV-1 gp120 impairs the endogenous cellular antioxidant defense and increases reactive oxygen species formation (Reddy *et al*, 2012). For instance, gp120 reduced the concentration of extracellular glutamine as well as the expression of glutamine synthetase (GS) and these effects were accompanied by overproduction of free radicals and lipid peroxidation (Visalli *et al*, 2007). The downregulation of GS could be prevented by the antioxidant N-acetylcysteine (NAC) suggesting that an oxidative environment was the contributing factor. The antidepressant tianeptine, that interferes with the effects on glutamatergic transmission (Piroli *et al*, 2013; Reagan *et al*, 2004; Svenningsson *et al*, 2007) and decreases the production of inflammatory cytokines (Castanon *et al*, 2004; Mutlu *et al*, 2012), increased cell viability and prevented the loss of astrocytic GS levels caused by gp120. Further examination of the mechanism of protection of tianeptine revealed that it prevented the activation of NF- κ B by controlling the levels of I κ B- α and thus in turn modulating the expression of inducible nitric oxide synthase (iNOS) and the stabilization of constitutive nitric oxide synthase (cNOS) (Janda *et al*, 2011). Collectively, these data demonstrate that HIV-mediated glutamate toxicity results from the combination of persistent inflammatory stimuli, unresolved oxidative damage, and direct damage by viral proteins (i.e. gp120). Although in all likelihood this appears to be the case, whether additional mechanisms that lead to neurotoxicity are at play remains to be elucidated. Understanding the relationships between neuroinflammation, neurotoxicity and HAND disease progression is critical to elucidate the underlying mechanism and to the development of therapeutic modalities for the treatment of HAND.

Amelioration of virally-induced glutamate excitotoxicity as a therapeutic approach

Although cART has successfully eradicated the most severe forms of HAND through the beneficial properties of reducing viral replication and the associated inflammatory responses (Anthony *et al*, 2005; Gendelman *et al*, 1998), additional therapeutic modalities directed at glutamate transmission and metabolism during HIV-1 infection are highly desired to alleviate glutamate-related toxicity in the CNS (Ferrarese *et al*, 2001; Sailasuta N, 2009). The prevention and rapid resolution of the residual inflammatory responses seen in HAND and HIVE is a major goal in mitigating the neurocognitive deficits in HIV⁺ subjects (Meeker *et al*, 2011; Peluso *et al*, 2013; Sailasuta *et al*, 2012), and, therefore, the use of anti-inflammatory drugs to reduce the neuroinflammatory effects caused by glial cells (Lee *et al*, 2013) may, at this point, be the most straightforward pharmacological regimen available for use during HIV-1 CNS infection.

The search for anti-inflammatory agents able to act within the CNS for use during HAND and HIVE has uncovered the effectiveness of 2 compounds as inhibitors of pro-inflammatory cytokine production: BB-882, an inhibitor of platelet-activating factor, and BB-1101, an inhibitor of both matrix metalloproteinase (MMP) and TNF- α release. In the SCID HIVE mouse model, the extent of widespread astrogliosis and microglia-associated inflammation is significantly reduced with the combined use of the anti-inflammatory drugs BB-882 and BB-1101 (Persidsky *et al*, 2001). Moreover, these anti-inflammatory agents reduce TNF- α expression in brain tissues and are thought to prevent further neuropathology by overcoming the inhibition of glutamate reuptake (Carmen *et al*, 2009; Persidsky *et al*, 2001). In terms of HIVE, anti-inflammatory agents that can successfully cross the BBB and neutralizing antibodies against pro-inflammatory cytokines may be useful tools for preventing the peripheral infiltration of HIV-1-infected CD4⁺ cells. Not only did the use of antibodies against IFN- α improve the pathological markers of astrogliosis and microgliosis but also the resulting neutralization was reflected in improved learning and working memory in the SCID HIVE mouse model (Sas *et al*, 2009). Whether the use of anti-inflammatory agents in combination with cART proves to be an important adjunctive therapy for diminishing glial-mediated inflammatory processes in HIVE and HAND in humans remains to be elucidated.

Another pathological mechanism in HIV-1-induced neuronal damage involves NMDAR activation (Kaul, 2008). *In vitro* studies have revealed that conditioned medium from HIV_{ADA}-infected or LPS-activated MDM damages primary cortical neurons because of the elevation of glutamate levels (Jiang *et al*, 2001). Extracellular glutamate binds to and stimulates ionotropic NMDAR to increase Ca²⁺ influx, which causes activation of CaMKII (Mayadevi *et al*, 2012) and subsequently leads to membrane depolarization and postsynaptic excitatory mechanisms. The neurotoxicity caused by glutamate ensues when the overstimulation of NMDARs is accompanied with a high enough elevation of intracellular Ca²⁺ levels that results in Ca²⁺-dependent mechanisms for cellular apoptosis (Lee *et al*, 2010b). Interestingly, an increase in neuronal susceptibility to soluble neurotoxins from HIV-infected MDM supernatant correlates the increased expression of the NR2A and NR2B subunits (O'Donnell *et al*, 2006), thus not only evidencing that the activation of NMDARs plays a central role in neuronal apoptosis during HIV-1 CNS infection but also that the protein expression patterns of the NMDAR subunits may determine the magnitude of neuronal death triggered by glutamate.

Pharmacological antagonists of the NMDAR such as dizocilpine (MK801) and amino-5-phosphonovaleric acid (AP-5) provide neuroprotective support against neuronal apoptosis after treatment with conditioned media from HIV-1-infected MDM (Chen *et al*, 2002). Besides the non-competitive blockage of glutamate stimulation on astrocytic or neuronal NMDRs (Lee *et al*, 2010b), MK801 has been shown to mediate some of its protection *in vitro* through various mechanisms of action, including the prevention of NR2B subunit cleavage (O'Donnell *et al*, 2006), inhibition of synaptosomal glutamate release (Tavares *et al*, 2002), and inhibition of NMDAR-activated Ca²⁺ influx (Lee *et al*, 2010b) after stimulation with quinolinic acid. In addition, MK801 prevented the loss of dendritic length in neuronal cultures after the addition of increasing doses of IFN- α (Sas *et al*, 2009).

Therefore, future clinical and pharmacological studies should consider and evaluate the use of MK801 as powerful and promising tool against the pathological processes in HAND and HIVE. AP-5 also inhibits NMDARs, and its effects are similar to those of the MK801. However, its use has not been studied *in vivo* and yet remains to be evaluated as a candidate drug for HIV-1 treatment. On the other hand, memantine, an NMDAR antagonist, has a slightly different mechanism of action (Parsons *et al*, 2007) than does MK801. In animal studies, memantine has been shown to protect from the neurotoxic effects of HIV-1_{ADA}-infected MDM when injected into the caudate and putamen regions of the SCID HIVE mouse model (Anderson *et al*, 2004). Measurements of synaptic transmission and synaptic plasticity in SCID HIVE mice indicated that those receiving memantine exhibit improvements in synaptic function (Anderson *et al*, 2004). Memantine has also been used in clinical trials and was determined to be safe and well-tolerated by HIV-infected subjects with cognitive impairment in a phase II 20-week study (Schifitto *et al*, 2007) as well as in the 48-week extension study (Zhao *et al*, 2010). Although no significant differences were observed in cognitive performance during the 20-week study, the magnetic resonance spectroscopy analyses suggest that memantine may ameliorate neuronal metabolism and thus may help stabilize or prevent neuronal injury (Schifitto *et al*, 2007). However, the 48-week extension study was inconclusive due to inherent study limitations and thus the question remains in terms of the efficacy of memantine for the treatment of HIV-associated cognitive impairment (Zhao *et al*, 2010). These results highlight the need for longer studies to evaluate the full potential of neuroprotective agents.

Curcumin and chemically related curcuminoids are derived from the turmeric root and have various cytoprotective attributes, including protective glutamate excitotoxicity (Wang *et al*, 2008), antioxidant capacity, anti-inflammatory properties, and anti-apoptotic functions (Khanna *et al*, 2009). Some mechanisms whereby curcumin exerts its neuroprotective effects occur through the reduction of ROS within the hippocampus (Tang *et al*, 2009) and the reduction of Ca²⁺-dependent and -independent activities of calcium/calmodulin-dependent protein kinase (CaMKII) (Mayadevi *et al*, 2012), which enzyme activity constitutes a downstream signaling component after the glutamate-NMDA stimulatory response. Curcumin has been shown to protect cortical neurons from caspase-3-dependent apoptosis when the cells are exposed to conditioned media from microglia stimulated with gp120 variable region 3 peptide (gp120 V3) (Guo *et al*, 2013). Moreover, curcumin diminishes neurotoxic effects by reducing microglial ROS formation, decreasing TNF- α secretion, and decreasing MCP-1 production, which in turn prevents neuronal apoptosis. Rats intracranially injected with gp120 V3 and that received curcumin were more successful in completing the Morris Water Maze than the gp120 V3 group without curcumin (Tang *et al*, 2009). Hippocampal histological and electrophysiological findings respectively showed the conserved anatomical integrity of the CA1 and CA3 subregions and an increase in the magnitude of LTP in curcumin-treated rats (Tang *et al*, 2009). Together these findings provide support for the search of pharmacological modulation directed towards the glutamate-NMDAR-axis to help modulate neuronal dysfunction and loss, as well as some of the HIV-1-related neurocognitive dysfunctions.

According to various groups, blocking the enzymatic activity of glutaminase (Erdmann *et al*, 2009; Erdmann *et al*, 2006; Ye *et al*, 2013; Zhao, 2004; Zhao L, 2012) or promoting the conversion of glutamate to glutamine via GS (Janda *et al*, 2011; Kleinkauf-Rocha *et al*, 2013; Muscoli *et al*, 2005; Visalli *et al*, 2007) may be an alternative strategy in preventing HIV-1 neuropathology. ROS and RNS are known to influence glutamate handling, as antioxidant compounds are able to restore glutamatergic homeostasis that may result from viral replication or protein-mediated oxidative effects on CNS cultures and murine models of HIV-1. Therefore, the modulation of oxidative stress may be beneficial in ameliorating neurological diseases. Pharmacological tools with mechanisms of actions that involve increased antioxidant functions have been shown to lessen the neurotoxic effects of HIV-1 infection or viral products. For example, the use of the powerful antioxidant NAC rescues the astrocytic loss of GS activity and reduces glutamate content in cell cultures when exposed to μ molar doses of recombinant HIV-1 gp120 (Visalli *et al*, 2007). Despite the need to unravel molecular mechanisms whereby the antioxidant/oxidant balance correlates with glutamate dysregulation, in a similar fashion that involves overcoming oxidative stress levels, the clinically approved antidepressant tianeptine also shows potential for reducing the development of the neurotoxic effects of HIV CNS pathology. Tianeptine protects astrocytic cultures from the pro-apoptotic and neurodegenerative effects of HIV-1 gp120 IIIB through a mechanism that involves the inactivation of NF- κ B, suppression of iNOS, and stabilization of cNOS, which final process results in reduced nitric oxide levels (Janda *et al*, 2011). Similar findings show the involvement of iNOS and GS pathway as a link between glutamate-mediated insults and the response to inflammatory stimulus by pro-inflammatory cytokines (Muscoli *et al*, 2005). In this case the pharmacological inhibition of iNOS during NMDAR stimulation induces neuroprotective properties by favoring glutamate conversion to glutamine through the activity of astroglial GS (Muscoli *et al*, 2005) and subsequently decreasing glutamate levels in the extracellular space. However, the therapeutic efficacy of tianeptine in ameliorating the severity of HIV-1 neurocognitive decline and improving behavioral and memory deficits in animal models remains to be demonstrated. Lipoic acid constitutes an important cofactor for lipid biosynthesis that has ROS-scavenging capacity. In C6 rat astrocytes, lipoic acid has also been shown to increase GS activity, enhance extracellular glutamate reuptake, and increase endogenous glutathione content and antioxidant capacity (Kleinkauf-Rocha *et al*, 2013) and thus may potentially provide modulatory functions towards glutamate metabolism during CNS HIV-1 infection.

The modulation of signaling pathways, which triggers the induction of glutamate-producing enzymes, such as glutaminase 1 and 2, provides alternative targets for reducing further neurotoxicity. The suppression of glutamate production in HIV-1-infected MDM and microglia can be circumvented by the inhibition of STAT-1 phosphorylation (Zhao L, 2012) and may represent one of the most important means to reduce the production of extracellular glutamate. In primary neurons from newborn mice, polyphenol epigallocatechin-3-gallate (which is derived from green tea extracts), abolished HIV-1 gp120- or Tat-induced neuronal toxicity by interfering with STAT-1/JAK phosphorylation (Giunta *et al*, 2006). In this study, the involvement of STAT-1/JAK signaling was further implicated since neither neurons from STAT-1 knockout mice nor the inhibition of JAK1 responded to IFN- γ -enhanced neurotoxic damage induced by gp120 or Tat (Giunta *et al*, 2006). Besides the use of

pharmacological strategies aimed at diminishing glutamate production, the enhancement of microglial and astroglial extracellular glutamate clearance is another approach for decreasing worsening of HAND and HIVE. One of the most promising neuromodulatory compounds to use during HIV-1 infection is the antibiotic ceftriaxone. Ceftriaxone is a third generation cephalosporin antibiotic that has neuroprotective effects against glutamate overexcitation by enhancing extracellular glutamate clearance (Lee *et al*, 2008) and has been used for reestablishing hippocampal function by restoring glutamate homeostasis (Yang *et al*, 2013). It has been shown to induce EAAT-2 protein expression in primary human fetal astrocytes by promoting both the nuclear translocation binding of NF- κ B to the -282 position of the EAAT-2 promoter and nuclear transcription (Lee *et al*, 2008).

The search for pharmacological compounds may not only have uncovered new mechanisms for understanding the aspects of HIV-related neuropathology that is associated with defects in glutamate metabolism but also have led to the identification of novel ways of modifying and exploiting divergent pathways of its inter- and intra-cellular neurotransmission. In sum, the focus of future research should be placed on eradicating the neuropathology associated with HAND.

CONCLUDING REMARKS

HIV-1 infection can lead to neurocognitive impairment and neurodysfunction. Even after cART initiation, neuroinflammation and neurocognitive dysfunction persist in the setting of chronic HIV infection for reasons that are not entirely clear. The purpose of this review was to summarize the underlying mechanisms of the glutamate/glutamine cycle and its role in the development of HIV-1 Associated Neurocognitive Disorders. There are several different mechanisms by which HIV-1 can mediate the development of HAND; however there are issues that remain unsolved. It will be necessary to determine whether the same mechanisms that operate in *in vitro* studies operate under physiological conditions during the development of HAND. Alternatively, all proposed mechanisms could operate at the same time, but each contributing a different portion to the total amount of pathologies observed.

In sum, the underlying mechanisms of glutamate-induced neurotoxicity in HAND are complex, as shown by experimental CNS HIV-1 models and as seen in the autopsies of brain specimens (Xing *et al*, 2009; Zhao *et al*, 2001). Glutamate establishes an important link between amino acid and energy metabolism, as defects in these pathways are observed in other neurological diseases. Neuronal and glial glutamate metabolism comprises an important system capable of ameliorating the neurological complications observed in HAND, unless disrupted. In addition, the metabolic compartmentalization of glutamate within cellular organelles serves a number of purposes; it maintains antioxidant balance by increasing glutathione levels (Zhang and Forman, 2012) and it acts as an amino acid precursor that is required for protein synthesis. The neurocognitive deficits in HIV-1 subjects are associated with disrupted glutamate levels within the cells of the nervous tissues (Ernst *et al*, 2010) and lower antioxidant defense (Zhao *et al*, 2001). Both viral products and inflammatory cytokines trigger the increase in oxidative damage and the impaired glial regulation (or use maintenance) of glutamate as a consequence of improper immune responses. Although neuroinflammation and immunological failure both play decisive roles

in HIV-1 CNS disease severity in various ways, glutamate-mediated neuronal apoptosis and glial dysfunction appear to be at the center of neuropathological development. Therefore, future research must more closely scrutinize the modulatory factors of CNS glutamate metabolism and neurotransmission for the development of effective therapies that help ameliorate the neurotoxic burden by the imbalance of CNS glutamate-responsive activities.

Acknowledgments

This work was supported by NCRR-RCMI (RR003050)/NIMHD (G12-MD007579) and the NIGMS RISE grant R25GM082406. We also acknowledge the support of RCMI Publications Office.

References

- Airolidi M, Bandera A, Trabattoni D, Tagliabue B, Arosio B, Soria A, Rainone V, Lapadula G, Annoni G, Clerici M, Gori A. Neurocognitive impairment in HIV-infected naive patients with advanced disease: the role of virus and intrathecal immune activation. *Clin Dev Immunol*. 2012; 2012:467154. [PubMed: 22489249]
- Almeida A, Delgado-Esteban M, Bolanos JP, Medina JM. Oxygen and glucose deprivation induces mitochondrial dysfunction and oxidative stress in neurones but not in astrocytes in primary culture. *J Neurochem*. 2002; 81:207–17. [PubMed: 12064468]
- Anderson ER, Boyle J, Zink WE, Persidsky Y, Gendelman HE, Xiong H. Hippocampal synaptic dysfunction in a murine model of human immunodeficiency virus type 1 encephalitis. *Neuroscience*. 2003; 118:359–69. [PubMed: 12699772]
- Anderson ER, Gendelman HE, Xiong H. Memantine protects hippocampal neuronal function in murine human immunodeficiency virus type 1 encephalitis. *J Neurosci*. 2004; 24:7194–8. [PubMed: 15306653]
- Anthony IC, Ramage SN, Carnie FW, Simmonds P, Bell JE. Influence of HAART on HIV-related CNS disease and neuroinflammation. *J Neuropathol Exp Neurol*. 2005; 64:529–36. [PubMed: 15977645]
- Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, Clifford DB, Cinque P, Epstein LG, Goodkin K, Gisslen M, Grant I, Heaton RK, Joseph J, Marder K, Marra CM, McArthur JC, Nunn M, Price RW, Pulliam L, Robertson KR, Sacktor N, Valcour V, Wojna VE. Updated research nosology for HIV-associated neurocognitive disorders. *Neurology*. 2007; 69:1789–99. [PubMed: 17914061]
- Asensio VC, Maier J, Milner R, Boztug K, Kincaid C, Moulard M, Phillipson C, Lindsley K, Krucker T, Fox HS, Campbell IL. Interferon-independent, human immunodeficiency virus type 1 gp120-mediated induction of CXCL10/IP-10 gene expression by astrocytes in vivo and in vitro. *J Virol*. 2001; 75:7067–77. [PubMed: 11435587]
- Baird FE, Pinilla-Tenas JJ, Ogilvie WL, Ganapathy V, Hundal HS, Taylor PM. Evidence for allosteric regulation of pH-sensitive System A (SNAT2) and System N (SNAT5) amino acid transporter activity involving a conserved histidine residue. *Biochem J*. 2006; 397:369–75. [PubMed: 16629640]
- Bezzi P, Domercq M, Brambilla L, Galli R, Schols D, De Clercq E, Vescovi A, Bagetta G, Kollias G, Meldolesi J, Volterra A. CXCR4-activated astrocyte glutamate release via TNF α : amplification by microglia triggers neurotoxicity. *Nat Neurosci*. 2001; 4:702–10. [PubMed: 11426226]
- Bogoch, Davis BT, Venna N. Reversible dementia in a patient with central nervous system escape of human immunodeficiency virus. *J Infect*. 2011; 63:236–9. [PubMed: 21658774]
- Bossuet C, Vaufray F, Conde F, Chretien F, Pichon J, Hantraye P, Le Grand R, Dormont D, Gras G. Up-regulation of glutamate concentration in the putamen and in the prefrontal cortex of asymptomatic SIVmac251-infected macaques without major brain involvement. *J Neurochem*. 2004; 88:928–38. [PubMed: 14756814]
- Brabers NA, Nottet HS. Role of the pro-inflammatory cytokines TNF- α and IL-1 β in HIV-associated dementia. *Eur J Clin Invest*. 2006; 36:447–58. [PubMed: 16796601]

- Bruzzone S, Verderio C, Schenk U, Fedele E, Zocchi E, Matteoli M, De Flora A. Glutamate-mediated overexpression of CD38 in astrocytes cultured with neurones. *J Neurochem*. 2004; 89:264–72. [PubMed: 15030411]
- Burkhalter J, Fiumelli H, Erickson JD, Martin JL. A critical role for system A amino acid transport in the regulation of dendritic development by brain-derived neurotrophic factor (BDNF). *J Biol Chem*. 2007; 282:5152–9. [PubMed: 17179157]
- Buschdorf JP, Li Chew L, Zhang B, Cao Q, Liang FY, Liou YC, Zhou YT, Low BC. Brain-specific BNIP-2-homology protein Caytaxin relocalises glutaminase to neurite terminals and reduces glutamate levels. *J Cell Sci*. 2006; 119:3337–50. [PubMed: 16899818]
- Carmen J, Rothstein JD, Kerr DA. Tumor necrosis factor- α modulates glutamate transport in the CNS and is a critical determinant of outcome from viral encephalomyelitis. *Brain Res*. 2009; 1263:143–54. [PubMed: 19368827]
- Carroll-Anzinger D, Al-Harthi L. Gamma interferon primes productive human immunodeficiency virus infection in astrocytes. *J Virol*. 2006; 80:541–4. [PubMed: 16352578]
- Castanon N, Medina C, Mormede C, Dantzer R. Chronic administration of tianeptine balances lipopolysaccharide-induced expression of cytokines in the spleen and hypothalamus of rats. *Psychoneuroendocrinology*. 2004; 29:778–90. [PubMed: 15110927]
- Chen W, Sulcove J, Frank I, Jaffer S, Ozdener H, Kolson DL. Development of a human neuronal cell model for human immunodeficiency virus (HIV)-infected macrophage-induced neurotoxicity: apoptosis induced by HIV type 1 primary isolates and evidence for involvement of the Bcl-2/Bcl-xL-sensitive intrinsic apoptosis pathway. *J Virol*. 2002; 76:9407–19. [PubMed: 12186923]
- Cherner M, Cysique L, Heaton RK, Marcotte TD, Ellis RJ, Masliah E, Grant I, Group H. Neuropathologic confirmation of definitional criteria for human immunodeficiency virus-associated neurocognitive disorders. *J Neurovirol*. 2007; 13:23–8. [PubMed: 17454445]
- Churchill MJ, Wesselingh SL, Cowley D, Pardo CA, McArthur JC, Brew BJ, Gorry PR. Extensive astrocyte infection is prominent in human immunodeficiency virus-associated dementia. *Ann Neurol*. 2009; 66:253–8. [PubMed: 19743454]
- Cinque P, Bestetti A, Marenzi R, Sala S, Gisslen M, Hagberg L, Price RW. Cerebrospinal fluid interferon-gamma-inducible protein 10 (IP-10, CXCL10) in HIV-1 infection. *J Neuroimmunol*. 2005; 168:154–63. [PubMed: 16091292]
- Cloak CC, Chang L, O'Neil SP, Ernst TM, Anderson DC, Donahoe RM. Neurometabolite abnormalities in simian immunodeficiency virus-infected macaques with chronic morphine administration. *J Neuroimmune Pharmacol*. 2011; 6:371–80. [PubMed: 20938808]
- Cohen RA, Harezlak J, Gongvatana A, Buchthal S, Schifitto G, Clark U, Paul R, Taylor M, Thompson P, Tate D, Alger J, Brown M, Zhong J, Campbell T, Singer E, Daar E, McMahon D, Tso Y, Yiannoutsos CT, Navia B, Consortium HIVN. Cerebral metabolite abnormalities in human immunodeficiency virus are associated with cortical and subcortical volumes. *J Neurovirol*. 2010; 16:435–44. [PubMed: 20961212]
- Cysique LA, Vaida F, Letendre S, Gibson S, Cherner M, Woods SP, McCutchan JA, Heaton RK, Ellis RJ. Dynamics of cognitive change in impaired HIV-positive patients initiating antiretroviral therapy. *Neurology*. 2009; 73:342–8. [PubMed: 19474412]
- Dadsetan S, Bak LK, Sorensen M, Keiding S, Vilstrup H, Ott P, Leke R, Schousboe A, Waagepetersen HS. Inhibition of glutamine synthesis induces glutamate dehydrogenase-dependent ammonia fixation into alanine in co-cultures of astrocytes and neurons. *Neurochem Int*. 2011; 59:482–8. [PubMed: 21397649]
- Eid T, Lee TS, Wang Y, Perez E, Drummond J, Lauritzen F, Bergersen LH, Meador-Woodruff JH, Spencer DD, de Lanerolle NC, McCullumsmith RE. Gene expression of glutamate metabolizing enzymes in the hippocampal formation in human temporal lobe epilepsy. *Epilepsia*. 2013a; 54:228–38. [PubMed: 23384343]
- Eid T, Tu N, Lee TS, Lai JC. Regulation of astrocyte glutamine synthetase in epilepsy. *Neurochem Int*. 2013b
- Erdmann N, Tian C, Huang Y, Zhao J, Herek S, Curthoys N, Zheng J. In vitro glutaminase regulation and mechanisms of glutamate generation in HIV-1-infected macrophage. *J Neurochem*. 2009; 109:551–61. [PubMed: 19222703]

- Erdmann NB, Whitney NP, Zheng J. Potentiation of Excitotoxicity in HIV-1 Associated Dementia and the Significance of Glutaminase. *Clin Neurosci Res.* 2006; 6:315–328. [PubMed: 18059978]
- Ernst T, Jiang CS, Nakama H, Buchthal S, Chang L. Lower brain glutamate is associated with cognitive deficits in HIV patients: a new mechanism for HIV-associated neurocognitive disorder. *J Magn Reson Imaging.* 2010; 32:1045–53. [PubMed: 21031507]
- Espey MG, Basile AS, Heaton RK, Ellis RJ. Increased glutamate in CSF and plasma of patients with HIV dementia. *Neurology.* 2002; 58:1439. author reply 1439–40. [PubMed: 12011305]
- Espey MG, Ellis RJ, Heaton RK, Basile AS. Relevance of glutamate levels in the CSF of patients with HIV-1-associated dementia complex. *Neurology.* 1999; 53:1144–5. [PubMed: 10496286]
- Espey MG, Kustova Y, Sei Y, Basile AS. Extracellular glutamate levels are chronically elevated in the brains of LP-BM5-infected mice: a mechanism of retrovirus-induced encephalopathy. *J Neurochem.* 1998; 71:2079–87. [PubMed: 9798933]
- Eugenin EA, Berman JW. Gap junctions mediate human immunodeficiency virus-bystander killing in astrocytes. *J Neurosci.* 2007; 27:12844–50. [PubMed: 18032656]
- Eugenin EA, Clements JE, Zink MC, Berman JW. Human immunodeficiency virus infection of human astrocytes disrupts blood-brain barrier integrity by a gap junction-dependent mechanism. *J Neurosci.* 2011; 31:9456–65. [PubMed: 21715610]
- Everall I, Vaida F, Khanlou N, Lazzaretto D, Achim C, Letendre S, Moore D, Ellis R, Cherner M, Gelman B, Morgello S, Singer E, Grant I, Masliah E, National Neuro ATC. Cliniconeuropathologic correlates of human immunodeficiency virus in the era of antiretroviral therapy. *J Neurovirol.* 2009; 15:360–70. [PubMed: 20175693]
- Fang J, Han D, Hong J, Tan Q, Tian Y. The chemokine, macrophage inflammatory protein-2gamma, reduces the expression of glutamate transporter-1 on astrocytes and increases neuronal sensitivity to glutamate excitotoxicity. *J Neuroinflammation.* 2012; 9:267. [PubMed: 23234294]
- Fernandes SP, Dringen R, Lawen A, Robinson SR. Neurones express glutamine synthetase when deprived of glutamine or interaction with astrocytes. *J Neurochem.* 2010; 114:1527–36. [PubMed: 20557426]
- Ferrarese C, Aliprandi A, Tremolizzo L, Stanzani L, De Micheli A, Dolara A, Frattola L. Increased glutamate in CSF and plasma of patients with HIV dementia. *Neurology.* 2001; 57:671–5. [PubMed: 11524477]
- Ferrarese C, Riva R, Dolara A, De Micheli A, Frattola L. Elevated glutamate in the cerebrospinal fluid of patients with HIV dementia. *JAMA.* 1997; 277:630. [PubMed: 9039879]
- Fields J, Dumaop W, Rockenstein E, Mante M, Spencer B, Grant I, Ellis R, Letendre S, Patrick C, Adame A, Masliah E. Age-dependent molecular alterations in the autophagy pathway in HIVE patients and in a gp120 tg mouse model: reversal with beclin-1 gene transfer. *J Neurovirol.* 2013; 19:89–101. [PubMed: 23341224]
- Fischer-Smith T, Croul S, Adeniyi A, Rybicka K, Morgello S, Khalili K, Rappaport J. Macrophage/microglial accumulation and proliferating cell nuclear antigen expression in the central nervous system in human immunodeficiency virus encephalopathy. *Am J Pathol.* 2004; 164:2089–99. [PubMed: 15161643]
- Fu W, Ruangkittisakul A, Mactavish D, Baker GB, Ballanyi K, Jhamandas JH. Activity and metabolism-related Ca(2+) and mitochondrial dynamics in co-cultured human fetal cortical neurons and astrocytes. *Neuroscience.* 2013; 250:520–35. [PubMed: 23876319]
- Gelman BB, Chen T, Lisinicchia JG, Soukup VM, Carmical JR, Starkey JM, Masliah E, Commens DL, Brandt D, Grant I, Singer EJ, Levine AJ, Miller J, Winkler JM, Fox HS, Luxon BA, Morgello S, National Neuro ATC. The National NeuroAIDS Tissue Consortium brain gene array: two types of HIV-associated neurocognitive impairment. *PLoS One.* 2012; 7:e46178. [PubMed: 23049970]
- Gendelman HE, Zheng J, Coulter CL, Ghorpade A, Che M, Thylin M, Rubocki R, Persidsky Y, Hahn F, Reinhard J Jr, Swindells S. Suppression of inflammatory neurotoxins by highly active antiretroviral therapy in human immunodeficiency virus-associated dementia. *J Infect Dis.* 1998; 178:1000–7. [PubMed: 9806027]
- Ghafari M, Amini S, Khalili K, Sawaya BE. HIV-1 associated dementia: symptoms and causes. *Retrovirology.* 2006; 3:28. [PubMed: 16712719]

- Giunta B, Obregon D, Hou H, Zeng J, Sun N, Nikolic V, Ehrhart J, Shytle D, Fernandez F, Tan J. EGCG mitigates neurotoxicity mediated by HIV-1 proteins gp120 and Tat in the presence of IFN-gamma: role of JAK/STAT1 signaling and implications for HIV-associated dementia. *Brain Res.* 2006; 1123:216–25. [PubMed: 17078933]
- Gongvatana A, Harezlak J, Buchthal S, Daar E, Schifitto G, Campbell T, Taylor M, Singer E, Algers J, Zhong J, Brown M, McMahon D, So YT, Mi D, Heaton R, Robertson K, Yiannoutsos C, Cohen RA, Navia B, Consortium HIVN. Progressive cerebral injury in the setting of chronic HIV infection and antiretroviral therapy. *J Neurovirol.* 2013; 19:209–18. [PubMed: 23613008]
- Gras G, Porcheray F, Samah B, Leone C. The glutamate-glutamine cycle as an inducible, protective face of macrophage activation. *J Leukoc Biol.* 2006; 80:1067–75. [PubMed: 16912070]
- Greco JB, Westmoreland SV, Ratai EM, Lentz MR, Sakaie K, He J, Sehgal PK, Masliah E, Lackner AA, Gonzalez RG. In vivo ¹H MRS of brain injury and repair during acute SIV infection in the macaque model of neuroAIDS. *Magn Reson Med.* 2004; 51:1108–14. [PubMed: 15170829]
- Guo L, Xing Y, Pan R, Jiang M, Gong Z, Lin L, Wang J, Xiong G, Dong J. Curcumin protects microglia and primary rat cortical neurons against HIV-1 gp120-mediated inflammation and apoptosis. *PLoS One.* 2013; 8:e70565. [PubMed: 23936448]
- Harezlak J, Buchthal S, Taylor M, Schifitto G, Zhong J, Daar E, Alger J, Singer E, Campbell T, Yiannoutsos C, Cohen R, Navia B, Consortium HIVN. Persistence of HIV-associated cognitive impairment, inflammation, and neuronal injury in era of highly active antiretroviral treatment. *AIDS.* 2011; 25:625–33. [PubMed: 21297425]
- Hashioka S, Klegeris A, Qing H, McGeer PL. STAT3 inhibitors attenuate interferon-gamma-induced neurotoxicity and inflammatory molecule production by human astrocytes. *Neurobiol Dis.* 2011; 41:299–307. [PubMed: 20888416]
- Heaton RKFD, Ellis RJ, McCutchan JA, Letendre SL, LeBlanc S, Corkran SH, Duarte NA, Clifford DB, Woods SP, Collier AC, Marra CM, Morgello S, Rivera Mindt M, Taylor MJ, Marcotte TD, Hampton Atkinson J, Wolfson T, Gelman BB, McArthur JC, Simpson DM, Abramson I, Gamst A, Fennema-Notestine C, Jernigan TL, Wong J, Grant I. the CHARTER and HNRC Groups. HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. *Journal of Neurovirology.* 2011; 17:3– 16. [PubMed: 21174240]
- Heaton RK, Marcotte TD, Mindt MR, Sadek J, Moore DJ, Bentley H, McCutchan JA, Reicks C, Grant I, Group H. The impact of HIV-associated neuropsychological impairment on everyday functioning. *J Int Neuropsychol Soc.* 2004; 10:317–31. [PubMed: 15147590]
- Hertz L. Brain glutamine synthesis requires neuronal aspartate: a commentary. *J Cereb Blood Flow Metab.* 2011; 31:384–7. [PubMed: 21063427]
- Hinkin CH, Hardy DJ, Mason KI, Castellon SA, Durvasula RS, Lam MN, Stefaniak M. Medication adherence in HIV-infected adults: effect of patient age, cognitive status, and substance abuse. *AIDS.* 2004; 18(Suppl 1):S19–25. [PubMed: 15075494]
- Holcomb T, Taylor L, Trohkimoinen J, Curthoys NP. Isolation, characterization and expression of a human brain mitochondrial glutaminase cDNA. *Brain Res Mol Brain Res.* 2000; 76:56–63. [PubMed: 10719215]
- Huang Y, Zhao L, Jia B, Wu L, Li Y, Curthoys N, Zheng JC. Glutaminase dysregulation in HIV-1-infected human microglia mediates neurotoxicity: relevant to HIV-1-associated neurocognitive disorders. *J Neurosci.* 2011; 31:15195–204. [PubMed: 22016553]
- Janda E, Visalli V, Colica C, Aprigliano S, Musolino V, Vadala N, Muscoli C, Sacco I, Iannone M, Rotiroti D, Spedding M, Mollace V. The protective effect of tianeptine on Gp120-induced apoptosis in astroglial cells: role of GS and NOS, and NF-kappaB suppression. *Br J Pharmacol.* 2011; 164:1590–9. [PubMed: 21175585]
- Jiang ZG, Piggee C, Heyes MP, Murphy C, Quearry B, Bauer M, Zheng J, Gendelman HE, Markey SP. Glutamate is a mediator of neurotoxicity in secretions of activated HIV-1-infected macrophages. *J Neuroimmunol.* 2001; 117:97–107. [PubMed: 11431009]
- Jones HN, Jansson T, Powell TL. IL-6 stimulates system A amino acid transporter activity in trophoblast cells through STAT3 and increased expression of SNAT2. *Am J Physiol Cell Physiol.* 2009; 297:C1228–35. [PubMed: 19741197]

- Juge N, Gray JA, Omote H, Miyaji T, Inoue T, Hara C, Uneyama H, Edwards RH, Nicoll RA, Moriyama Y. Metabolic control of vesicular glutamate transport and release. *Neuron*. 2010; 68:99–112. [PubMed: 20920794]
- Kaul M. HIV's double strike at the brain: neuronal toxicity and compromised neurogenesis. *Front Biosci*. 2008; 13:2484–94. [PubMed: 17981728]
- Kaul M. HIV-1 associated dementia: update on pathological mechanisms and therapeutic approaches. *Curr Opin Neurol*. 2009; 22:315–20. [PubMed: 19300249]
- Khanna S, Park HA, Sen CK, Golakoti T, Sengupta K, Venkateswarlu S, Roy S. Neuroprotective and antiinflammatory properties of a novel demethylated curcuminoid. *Antioxid Redox Signal*. 2009; 11:449–68. [PubMed: 18724833]
- Kim JP, Lentz MR, Westmoreland SV, Greco JB, Ratai EM, Halpern E, Lackner AA, Masliah E, Gonzalez RG. Relationships between astrogliosis and 1H MR spectroscopic measures of brain choline/creatine and myo-inositol/creatine in a primate model. *AJNR Am J Neuroradiol*. 2005; 26:752–9. [PubMed: 15814917]
- Kim SY, Choi SY, Chao W, Volsky DJ. Transcriptional regulation of human excitatory amino acid transporter 1 (EAAT1): cloning of the EAAT1 promoter and characterization of its basal and inducible activity in human astrocytes. *J Neurochem*. 2003; 87:1485–98. [PubMed: 14713304]
- Kleinkauf-Rocha J, Bobermin LD, de Machado PM, Goncalves CA, Gottfried C, Quincozes-Santos A. Lipoic acid increases glutamate uptake, glutamine synthetase activity and glutathione content in C6 astrocyte cell line. *Int J Dev Neurosci*. 2013; 31:165–70. [PubMed: 23286972]
- Kou W, Banerjee S, Eudy J, Smith LM, Persidsky R, Borgmann K, Wu L, Sakhuja N, Deshpande MS, Walseth TF, Ghorpade A. CD38 regulation in activated astrocytes: implications for neuroinflammation and HIV-1 brain infection. *J Neurosci Res*. 2009; 87:2326–39. [PubMed: 19365854]
- Kovalevich J, Langford D. Neuronal toxicity in HIV CNS disease. *Future Virol*. 2012; 7:687–698. [PubMed: 23616788]
- Kreft M, Bak LK, Waagepetersen HS, Schousboe A. Aspects of astrocyte energy metabolism, amino acid neurotransmitter homeostasis and metabolic compartmentation. *ASN Neuro*. 2012; 4
- Kustova Y, Espey MG, Sei Y, Basile AS. Regional decreases [corrected] in AMPA receptor density in mice infected with the LP-BM5 murine leukemia virus. *Neuroreport*. 1997; 8:1243–7. [PubMed: 9175122]
- Lamers SL, Gray RR, Salemi M, Huysentruyt LC, McGrath MS. HIV-1 phylogenetic analysis shows HIV-1 transits through the meninges to brain and peripheral tissues. *Infect Genet Evol*. 2011; 11:31–7. [PubMed: 21055482]
- Lee M, Cho T, Jantarotnotai N, Wang YT, McGeer E, McGeer PL. Depletion of GSH in glial cells induces neurotoxicity: relevance to aging and degenerative neurological diseases. *FASEB J*. 2010a; 24:2533–45. [PubMed: 20228251]
- Lee M, McGeer E, McGeer PL. Neurotoxins released from interferon-gamma-stimulated human astrocytes. *Neuroscience*. 2013; 229:164–75. [PubMed: 23098801]
- Lee MC, Ting KK, Adams S, Brew BJ, Chung R, Guillemin GJ. Characterisation of the expression of NMDA receptors in human astrocytes. *PLoS ONE*. 2010b; 5:e14123. [PubMed: 21152063]
- Lee SG, Su ZZ, Emdad L, Gupta P, Sarkar D, Borjabad A, Volsky DJ, Fisher PB. Mechanism of ceftriaxone induction of excitatory amino acid transporter-2 expression and glutamate uptake in primary human astrocytes. *J Biol Chem*. 2008; 283:13116–23. [PubMed: 18326497]
- Lehmann C, Bette S, Engele J. High extracellular glutamate modulates expression of glutamate transporters and glutamine synthetase in cultured astrocytes. *Brain Res*. 2009; 1297:1–8. [PubMed: 19728998]
- Lentz MR, Kim WK, Lee V, Bazner S, Halpern EF, Venna N, Williams K, Rosenberg ES, Gonzalez RG. Changes in MRS neuronal markers and T cell phenotypes observed during early HIV infection. *Neurology*. 2009; 72:1465–72. [PubMed: 19398702]
- Lentz MR, Lee V, Westmoreland SV, Ratai EM, Halpern EF, Gonzalez RG. Factor analysis reveals differences in brain metabolism in macaques with SIV/AIDS and those with SIV-induced encephalitis. *NMR Biomed*. 2008; 21:878–87. [PubMed: 18574793]

- Li W, Henderson LJ, Major EO, Al-Harhi L. IFN-gamma mediates enhancement of HIV replication in astrocytes by inducing an antagonist of the beta-catenin pathway (DKK1) in a STAT 3-dependent manner. *J Immunol.* 2011; 186:6771–8. [PubMed: 21562161]
- Maciejewski PK, Rothman DL. Proposed cycles for functional glutamate trafficking in synaptic neurotransmission. *Neurochem Int.* 2008; 52:809–25. [PubMed: 18006192]
- Malarkey EB, Parpura V. Mechanisms of glutamate release from astrocytes. *Neurochem Int.* 2008; 52:142–54. [PubMed: 17669556]
- Mamik MK, Banerjee S, Walseth TF, Hirte R, Tang L, Borgmann K, Ghorpade A. HIV-1 and IL-1 β regulate astrocytic CD38 through mitogen-activated protein kinases and nuclear factor-kappaB signaling mechanisms. *J Neuroinflammation.* 2011; 8:145. [PubMed: 22027397]
- Masliah E, Roberts ES, Langford D, Everall I, Crews L, Adame A, Rockenstein E, Fox HS. Patterns of gene dysregulation in the frontal cortex of patients with HIV encephalitis. *J Neuroimmunol.* 2004; 157:163–75. [PubMed: 15579294]
- Mayadevi M, Sherin DR, Keerthi VS, Rajasekharan KN, Omkumar RV. Curcumin is an inhibitor of calcium/calmodulin dependent protein kinase II. *Bioorg Med Chem.* 2012; 20:6040–7. [PubMed: 22989913]
- Meeker RB, Poulton W, Markovic-Plese S, Hall C, Robertson K. Protein changes in CSF of HIV-infected patients: evidence for loss of neuroprotection. *J Neurovirol.* 2011; 17:258–73. [PubMed: 21556959]
- Meisner F, Neuen-Jacob E, Sopper S, Schmidt M, Schlamme S, Scheller C, Vosswinkel D, Ter Meulen V, Riederer P, Koutsilieri E. Disruption of excitatory amino acid transporters in brains of SIV-infected rhesus macaques is associated with microglia activation. *J Neurochem.* 2008; 104:202–9. [PubMed: 17986224]
- Melo CV, Mele M, Curcio M, Comprido D, Silva CG, Duarte CB. BDNF regulates the expression and distribution of vesicular glutamate transporters in cultured hippocampal neurons. *PLoS One.* 2013; 8:e53793. [PubMed: 23326507]
- Moore DJ, Letendre SL, Morris S, Umlauf A, Deutsch R, Smith DM, Little S, Rooney A, Franklin DR, Gouaux B, Leblanc S, Rosario D, Fennema-Notestine C, Heaton RK, Ellis RJ, Atkinson JH, Grant I. Neurocognitive functioning in acute or early HIV infection. *J Neurovirol.* 2011; 17:50–7. [PubMed: 21165782]
- Muscoli C, Visalli V, Colica C, Nistico R, Palma E, Costa N, Rotiroli D, Nistico G, Mollace V. The effect of inflammatory stimuli on NMDA-related activation of glutamine synthase in human cultured astroglial cells. *Neurosci Lett.* 2005; 373:184–8. [PubMed: 15619540]
- Mutlu O, Gumuslu E, Ulak G, Celikyurt IK, Kokturk S, Kir HM, Akar F, Erden F. Effects of fluoxetine, tianeptine and olanzapine on unpredictable chronic mild stress-induced depression-like behavior in mice. *Life Sci.* 2012; 91:1252–62. [PubMed: 23069580]
- Nath A. Human immunodeficiency virus (HIV) proteins in neuropathogenesis of HIV dementia. *J Infect Dis.* 2002; 186(Suppl 2):S193–8. [PubMed: 12424697]
- O'Donnell LA, Agrawal A, Jordan-Sciutto KL, Dichter MA, Lynch DR, Kolson DL. Human immunodeficiency virus (HIV)-induced neurotoxicity: roles for the NMDA receptor subtypes. *J Neurosci.* 2006; 26:981–90. [PubMed: 16421318]
- Pais TF, Figueiredo C, Peixoto R, Braz MH, Chatterjee S. Necrotic neurons enhance microglial neurotoxicity through induction of glutaminase by a MyD88-dependent pathway. *J Neuroinflammation.* 2008; 5:43. [PubMed: 18844999]
- Pardo B, Rodrigues TB, Contreras L, Garzon M, Llorente-Folch I, Kobayashi K, Saheki T, Cerdan S, Satrustegui J. Brain glutamine synthesis requires neuronal-born aspartate as amino donor for glial glutamate formation. *J Cereb Blood Flow Metab.* 2011; 31:90–101. [PubMed: 20736955]
- Parsons CG, Stoffler A, Danysz W. Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system--too little activation is bad, too much is even worse. *Neuropharmacology.* 2007; 53:699–723. [PubMed: 17904591]
- Parsons TD, Braaten AJ, Hall CD, Robertson KR. Better quality of life with neuropsychological improvement on HAART. *Health Qual Life Outcomes.* 2006; 4:11. [PubMed: 16504114]

- Pascual O, Ben Achour S, Rostaing P, Triller A, Bessis A. Microglia activation triggers astrocyte-mediated modulation of excitatory neurotransmission. *Proc Natl Acad Sci U S A*. 2012; 109:E197–205. [PubMed: 22167804]
- Patton HK, Zhou ZH, Bubien JK, Benveniste EN, Benos DJ. gp120-induced alterations of human astrocyte function: Na(+)/H(+) exchange, K(+) conductance, and glutamate flux. *Am J Physiol Cell Physiol*. 2000; 279:C700–8. [PubMed: 10942720]
- Pawlak J, Brito V, Kuppers E, Beyer C. Regulation of glutamate transporter GLAST and GLT-1 expression in astrocytes by estrogen. *Brain Res Mol Brain Res*. 2005; 138:1–7. [PubMed: 15896872]
- Peluso MJ, Meyerhoff DJ, Price RW, Peterson J, Lee E, Young AC, Walter R, Fuchs D, Brew BJ, Cinque P, Robertson K, Hagberg L, Zetterberg H, Gisslen M, Spudich S. Cerebrospinal fluid and neuroimaging biomarker abnormalities suggest early neurological injury in a subset of individuals during primary HIV infection. *J Infect Dis*. 2013; 207:1703–12. [PubMed: 23460748]
- Persidsky Y, Limoges J, McComb R, Bock P, Baldwin T, Tyor W, Patil A, Nottet HS, Epstein L, Gelbard H, Flanagan E, Reinhard J, Pirruccello SJ, Gendelman HE. Human immunodeficiency virus encephalitis in SCID mice. *Am J Pathol*. 1996; 149:1027–53. [PubMed: 8780406]
- Persidsky Y, Limoges J, Rasmussen J, Zheng J, Gearing A, Gendelman HE. Reduction in glial immunity and neuropathology by a PAF antagonist and an MMP and TNFalpha inhibitor in SCID mice with HIV-1 encephalitis. *J Neuroimmunol*. 2001; 114:57–68. [PubMed: 11240016]
- Piroli GG, Reznikov LR, Grillo CA, Hagar JM, Fadel JR, Reagan LP. Tianeptine modulates amygdalar glutamate neurochemistry and synaptic proteins in rats subjected to repeated stress. *Exp Neurol*. 2013; 241:184–93. [PubMed: 23262120]
- Plaitakis A, Latsoudis H, Kanavouras K, Ritz B, Bronstein JM, Skoula I, Mastorodemos V, Papapetropoulos S, Borompokas N, Zaganas I, Xiromerisiou G, Hadjigeorgiou GM, Spanaki C. Gain-of-function variant in GLUD2 glutamate dehydrogenase modifies Parkinson's disease onset. *Eur J Hum Genet*. 2010; 18:336–41. [PubMed: 19826450]
- Plaitakis A, Metaxari M, Shashidharan P. Nerve tissue-specific (GLUD2) and housekeeping (GLUD1) human glutamate dehydrogenases are regulated by distinct allosteric mechanisms: implications for biologic function. *J Neurochem*. 2000; 75:1862–9. [PubMed: 11032875]
- Potter MC, Figuera-Losada M, Rojas C, Slusher BS. Targeting the Glutamatergic System for the Treatment of HIV-Associated Neurocognitive Disorders. *J Neuroimmune Pharmacol*. 2013; 8:594–607. [PubMed: 23553365]
- Qian Y, Guan T, Tang X, Huang L, Huang M, Li Y, Sun H, Yu R, Zhang F. Astrocytic glutamate transporter-dependent neuroprotection against glutamate toxicity: an in vitro study of maslinic acid. *Eur J Pharmacol*. 2011; 651:59–65. [PubMed: 21118675]
- Ratai EM, Pilkenton SJ, Greco JB, Lentz MR, Bombardier JP, Turk KW, He J, Joo CG, Lee V, Westmoreland S, Halpern E, Lackner AA, Gonzalez RG. In vivo proton magnetic resonance spectroscopy reveals region specific metabolic responses to SIV infection in the macaque brain. *BMC Neurosci*. 2009; 10:63. [PubMed: 19545432]
- Reagan LP, Rosell DR, Wood GE, Spedding M, Munoz C, Rothstein J, McEwen BS. Chronic restraint stress up-regulates GLT-1 mRNA and protein expression in the rat hippocampus: reversal by tianeptine. *Proc Natl Acad Sci U S A*. 2004; 101:2179–84. [PubMed: 14766991]
- Reddy PV, Gandhi N, Samikkannu T, Saiyed Z, Agudelo M, Yndart A, Khatavkar P, Nair MP. HIV-1 gp120 induces antioxidant response element-mediated expression in primary astrocytes: role in HIV associated neurocognitive disorder. *Neurochem Int*. 2012; 61:807–14. [PubMed: 21756955]
- Sailasuta N, Ross W, Ananworanich J, Chalermchai T, DeGruttola V, Lerdum S, Pothisri M, Busovaca E, Ratto-Kim S, Jagodzinski L, Spudich S, Michael N, Kim JH, Valcour V. teams RSp. Change in brain magnetic resonance spectroscopy after treatment during acute HIV infection. *PLoS One*. 2012; 7:e49272. [PubMed: 23229129]
- Sailasuta NSK, Ross B. Evidence of reduced glutamate in the frontal lobe of HIV-seropositive patients. *NMR in Biomedicine*. 2009; 22:326–331. [PubMed: 18988228]
- Sas AR, Bimonte-Nelson H, Smothers CT, Woodward J, Tyor WR. Interferon-alpha causes neuronal dysfunction in encephalitis. *J Neurosci*. 2009; 29:3948–55. [PubMed: 19321791]

- Schifitto G, Navia BA, Yiannoutsos CT, Marra CM, Chang L, Ernst T, Jarvik JG, Miller EN, Singer EJ, Ellis RJ, Kolson DL, Simpson D, Nath A, Berger J, Shriver SL, Millar LL, Colquhoun D, Lenkinski R, Gonzalez RG, Lipton SA. Adult ACTG Teams Consortium HM. Memantine and HIV-associated cognitive impairment: a neuropsychological and proton magnetic resonance spectroscopy study. *AIDS*. 2007; 21:1877–86. [PubMed: 17721095]
- Schnell G, Joseph S, Spudich S, Price RW, Swanstrom R. HIV-1 replication in the central nervous system occurs in two distinct cell types. *PLoS Pathog*. 2011; 7:e1002286. [PubMed: 22007152]
- Shapshak P, Duncan R, Minagar A, Rodriguez de la Vega P, Stewart RV, Goodkin K. Elevated expression of IFN-gamma in the HIV-1 infected brain. *Front Biosci*. 2004; 9:1073–81. [PubMed: 14977530]
- Skytt DM, Klawonn AM, Stridh MH, Pajicka K, Patruss Y, Quintana-Cabrera R, Bolanos JP, Schousboe A, Waagepetersen HS. siRNA knock down of glutamate dehydrogenase in astrocytes affects glutamate metabolism leading to extensive accumulation of the neuroactive amino acids glutamate and aspartate. *Neurochem Int*. 2012; 61:490–7. [PubMed: 22542772]
- Spanaki C, Zaganas I, Kounoupa Z, Plaitakis A. The complex regulation of human glud1 and glud2 glutamate dehydrogenases and its implications in nerve tissue biology. *Neurochem Int*. 2012; 61:470–81. [PubMed: 22658952]
- Spudich S, Gisslen M, Hagberg L, Lee E, Liegler T, Brew B, Fuchs D, Tambussi G, Cinque P, Hecht FM, Price RW. Central nervous system immune activation characterizes primary human immunodeficiency virus 1 infection even in participants with minimal cerebrospinal fluid viral burden. *J Infect Dis*. 2011; 204:753–60. [PubMed: 21844301]
- Svenningsson P, Bateup H, Qi H, Takamiya K, Huganir RL, Spedding M, Roth BL, McEwen BS, Greengard P. Involvement of AMPA receptor phosphorylation in antidepressant actions with special reference to tianeptine. *Eur J Neurosci*. 2007; 26:3509–17. [PubMed: 18088278]
- Takaki J, Fujimori K, Miura M, Suzuki T, Sekino Y, Sato K. L-glutamate released from activated microglia downregulates astrocytic L-glutamate transporter expression in neuroinflammation: the ‘collusion’ hypothesis for increased extracellular L-glutamate concentration in neuroinflammation. *J Neuroinflammation*. 2012; 9:275. [PubMed: 23259598]
- Tang H, Lu D, Pan R, Qin X, Xiong H, Dong J. Curcumin improves spatial memory impairment induced by human immunodeficiency virus type 1 glycoprotein 120 V3 loop peptide in rats. *Life Sci*. 2009; 85:1–10. [PubMed: 19345695]
- Tavares RG, Tasca CI, Santos CE, Alves LB, Porciuncula LO, Emanuelli T, Souza DO. Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes. *Neurochem Int*. 2002; 40:621–7. [PubMed: 11900857]
- Thompson KA, Churchill MJ, Gorry PR, Sterjovski J, Oelrichs RB, Wesselingh SL, McLean CA. Astrocyte specific viral strains in HIV dementia. *Ann Neurol*. 2004; 56:873–7. [PubMed: 15562411]
- Tian C, Erdmann N, Zhao J, Cao Z, Peng H, Zheng J. HIV-infected macrophages mediate neuronal apoptosis through mitochondrial glutaminase. *J Neurochem*. 2008; 105:994–1005. [PubMed: 18088378]
- Tilleux S, Goursaud S, Hermans E. Selective up-regulation of GLT-1 in cultured astrocytes exposed to soluble mediators released by activated microglia. *Neurochem Int*. 2009; 55:35–40. [PubMed: 19428805]
- Tilleux S, Hermans E. Down-regulation of astrocytic GLAST by microglia-related inflammation is abrogated in dibutyryl cAMP-differentiated cultures. *J Neurochem*. 2008; 105:2224–36. [PubMed: 18298666]
- Tornatore C, Nath A, Amemiya K, Major EO. Persistent human immunodeficiency virus type 1 infection in human fetal glial cells reactivated by T-cell factor(s) or by the cytokines tumor necrosis factor alpha and interleukin-1 beta. *J Virol*. 1991; 65:6094–100. [PubMed: 1920627]
- Turchan J, Pocernich CB, Gairola C, Chauhan A, Schifitto G, Butterfield DA, Buch S, Narayan O, Sinai A, Geiger J, Berger JR, Elford H, Nath A. Oxidative stress in HIV demented patients and protection ex vivo with novel antioxidants. *Neurology*. 2003; 60:307–14. [PubMed: 12552050]
- Valcour V, Chalermchai T, Sailasuta N, Marovich M, Lerdlum S, Suttichom D, Suwanwela NC, Jagodzinski L, Michael N, Spudich S, van Griensven F, de Souza M, Kim J, Ananworanich J,

- Group RSS. Central nervous system viral invasion and inflammation during acute HIV infection. *J Infect Dis.* 2012; 206:275–82. [PubMed: 22551810]
- Vesce S, Bezzi P, Rossi D, Meldolesi J, Volterra A. HIV-1 gp120 glycoprotein affects the astrocyte control of extracellular glutamate by both inhibiting the uptake and stimulating the release of the amino acid. *FEBS Lett.* 1997; 411:107–9. [PubMed: 9247152]
- Visalli V, Muscoli C, Sacco I, Sculco F, Palma E, Costa N, Colica C, Rotiroti D, Mollace V. N-acetylcysteine prevents HIV gp 120-related damage of human cultured astrocytes: correlation with glutamine synthase dysfunction. *BMC Neurosci.* 2007; 8:106. [PubMed: 18062818]
- Vivithanaporn P, Heo G, Gamble J, Krentz HB, Hoke A, Gill MJ, Power C. Neurologic disease burden in treated HIV/AIDS predicts survival: a population-based study. *Neurology.* 2010; 75:1150–8. [PubMed: 20739646]
- Wang R, Li YB, Li YH, Xu Y, Wu HL, Li XJ. Curcumin protects against glutamate excitotoxicity in rat cerebral cortical neurons by increasing brain-derived neurotrophic factor level and activating TrkB. *Brain Res.* 2008; 1210:84–91. [PubMed: 18420184]
- Wang Z, Pekarskaya O, Bencheikh M, Chao W, Gelbard HA, Ghorpade A, Rothstein JD, Volsky DJ. Reduced expression of glutamate transporter EAAT2 and impaired glutamate transport in human primary astrocytes exposed to HIV-1 or gp120. *Virology.* 2003; 312:60–73. [PubMed: 12890621]
- Williams R, Dhillon NK, Hegde ST, Yao H, Peng F, Callen S, Chebloune Y, Davis RL, Buch SJ. Proinflammatory cytokines and HIV-1 synergistically enhance CXCL10 expression in human astrocytes. *Glia.* 2009; 57:734–43. [PubMed: 18985732]
- Wyss-Coray T, Masliah E, Toggas SM, Rockenstein EM, Brooker MJ, Lee HS, Mucke L. Dysregulation of signal transduction pathways as a potential mechanism of nervous system alterations in HIV-1 gp120 transgenic mice and humans with HIV-1 encephalitis. *J Clin Invest.* 1996; 97:789–98. [PubMed: 8609236]
- Xia C, Luo D, Yu X, Jiang S, Liu S. HIV-associated dementia in the era of highly active antiretroviral therapy (HAART). *Microbes Infect.* 2011; 13:419–25. [PubMed: 21262373]
- Xing HQ, Hayakawa H, Gelpi E, Kubota R, Budka H, Izumo S. Reduced expression of excitatory amino acid transporter 2 and diffuse microglial activation in the cerebral cortex in AIDS cases with or without HIV encephalitis. *J Neuropathol Exp Neurol.* 2009; 68:199–209. [PubMed: 19151621]
- Xu ZY, Liu HD, Lau LT, Yingge Z, Zhao R, Tong GL, Chan PH, Yu AC. Responses of astrocyte to simultaneous glutamate and arachidonic acid treatment. *Neurochem Int.* 2009; 55:143–50. [PubMed: 19428819]
- Yang J, Li MX, Luo Y, Chen T, Liu J, Fang P, Jiang B, Hu ZL, Jin Y, Chen JG, Wang F. Chronic ceftriaxone treatment rescues hippocampal memory deficit in AQP4 knockout mice via activation of GLT-1. *Neuropharmacology.* 2013; 75C:213–222. [PubMed: 23973312]
- Ye L, Huang Y, Zhao L, Li Y, Sun L, Zhou Y, Qian G, Zheng JC. IL-1 β and TNF- α induce neurotoxicity through glutamate production: a potential role for neuronal glutaminase. *J Neurochem.* 2013; 125:897–908. [PubMed: 23578284]
- Zhang H, Forman HJ. Glutathione synthesis and its role in redox signaling. *Semin Cell Dev Biol.* 2012; 23:722–8. [PubMed: 22504020]
- Zhao J, Lopez AL, Erichsen D, Herek S, Cotter RL, Curthoys NP, Zheng J. Mitochondrial glutaminase enhances extracellular glutamate production in HIV-1-infected macrophages: linkage to HIV-1 associated dementia. *J Neurochem.* 2004; 88:169–80. [PubMed: 14675161]
- Zhao J, Lopez AL, Erichsen D, Herek S, Cotter RL, Curthoys NP, Zheng J. Mitochondrial glutaminase enhances extracellular glutamate production in HIV-1 infected macrophages: Linkage to HIV-1 associated dementia. *Journal of Neurochemistry.* 2004; 88:169–180. [PubMed: 14675161]
- Zhao L, Huang Y, Tian C, Taylor L, Curthoys N, Wang Y, Vernon H, Zheng J. Interferon- α regulates glutaminase 1 promoter through STAT1 phosphorylation: relevance to HIV-1 associated neurocognitive disorders. *PLoS One.* 2012; 7:e32995. [PubMed: 22479354]
- Zhao LHY, Tian C, Taylor L, Curthoys N, Wang Y, Vernon H, Zheng J. Interferon- α Regulates Glutaminase 1 Promoter Through STAT1 Phosphorylation: Relevance to HIV-1 Associated Neurocognitive Disorders. *PLoS ONE.* 2012; 7:e32995. [PubMed: 22479354]

- Zhao ML, Kim MO, Morgello S, Lee SC. Expression of inducible nitric oxide synthase, interleukin-1 and caspase-1 in HIV-1 encephalitis. *J Neuroimmunol.* 2001; 115:182–91. [PubMed: 11282169]
- Zhao Y, Navia BA, Marra CM, Singer EJ, Chang L, Berger J, Ellis RJ, Kolson DL, Simpson D, Miller EN, Lipton SA, Evans SR, Schifitto G. Adult Aids Clinical Trial Group T. Memantine for AIDS dementia complex: open-label report of ACTG 301. *HIV Clin Trials.* 2010; 11:59–67. [PubMed: 20400412]
- Zink WE, Anderson E, Boyle J, Hock L, Rodriguez-Sierra J, Xiong H, Gendelman HE, Persidsky Y. Impaired spatial cognition and synaptic potentiation in a murine model of human immunodeficiency virus type 1 encephalitis. *J Neurosci.* 2002; 22:2096–105. [PubMed: 11896149]
- Zou J, Wang YX, Dou FF, Lu HZ, Ma ZW, Lu PH, Xu XM. Glutamine synthetase down-regulation reduces astrocyte protection against glutamate excitotoxicity to neurons. *Neurochem Int.* 2010; 56:577–84. [PubMed: 20064572]
- Zou JWY, Dou FF, Lü HZ, Ma ZW, Lu PH, Xu XM. Glutamine Synthetase Downregulation Reduces Astrocytes Protection Against Glutamate Excitotoxicity to Neurons. *Neurochemistry Int.* 2010; 56:577– 584.
- Zou JY, Crews FT. TNF alpha potentiates glutamate neurotoxicity by inhibiting glutamate uptake in organotypic brain slice cultures: neuroprotection by NF kappa B inhibition. *Brain Res.* 2005; 1034:11–24. [PubMed: 15713255]

Figure 1. General Mechanism in HIV-1 Neuropathology Development

Systemic HIV-1 infection in CD4⁺ T cells and MDM modulate upregulation of adhesion factors and downregulation in tight junction proteins to facilitate cellular attachment of peripherally activated or infected immune cells to endothelial cells of the blood brain barrier (BBB). Loss of BBB integrity and increase in its permeability allow peripheral circulating CD4⁺ T cells and HIV-1-infected MDM to enter the brain parenchyma. Release of HIV-1 virions from peripherally infected immune cells into the extracellular CNS milieu contributes to infection of resident microglia and local astrocytes. Although, neurons are spared from direct infection by HIV-1, they are susceptible to the resulting damage via secretion of viral toxins and other neurotoxins from activated or infected MDM, microglia and astrocytes. CNS immune activation leads to release of soluble host neurotoxic mediators including pro-inflammatory cytokines, arachidonic acid, matrix metalloproteinases, glutamate and RNS/ROS which in turn lead to indirect neuronal dysfunction, dendritic loss and neuronal death. Likewise, release of viral proteins such as gp120 and Tat further enhance and sustain the inflammatory processes that potentiate and drive neuropathogenesis. Disruption in astroglial and microglial functions by inflammation-driven neurotoxicity may play a pivotal role in the pathological features that include: viral replication, altered BBB integrity, neuronal/glia dysfunction and apoptosis that ultimately ensue in HAND.

Figure 2. Neuron-glia interaction and the glutamate-glutamine cycle in the context of HIV infection in the CNS

Intracellular glutamate is converted to glutamine in astrocytes and is exported to the extracellular space. Glutamine enters glutamatergic neurons as a precursor for the generation of the neuronal glutamate pool, by intracellular neuronal glutaminase (GLS). Glutamate is then released into the post-synaptic cleft. Extracellular glutamate secreted from neurons and/or glia activates NMDA receptors and increase intracellular Ca^{2+} levels to evoke excitatory functions (EPSP). Glutamate transporters (EAAT-1 and EAAT-2) found in the plasma membranes of glial cells mobilize excess glutamate from the extracellular space into

the cell interior. Glutamate may be then metabolically fated for either glutathione synthesis (GSH) for antioxidant function, or deaminated to produce the tricarboxylic acid cycle intermediate: α -ketoglutarate. In the astrocyte, glutamate can be converted to glutamine by the action of the enzyme glutamine synthetase (GS). Several cellular- and viral-derived soluble factors modulate the glutamate-glutamine balance by altering protein expression patterns, catalytic enzyme activity, and transcriptional and translational regulation in astrocytes, microglia and neurons. HIV-1 infection in monocyte-derived macrophages (MDM), microglia and astrocytes increases production and secretion of pro-inflammatory mediators: TNF- α , IL-1 β , IFN- α , and IFN- γ . HIV-1 viral proteins gp120 and Tat are secreted and further evoke the upregulation of inflammatory cytokines, the generation of reactive oxygen/nitrogen species (ROS/RNS), and the activation of transcription and signaling factors NF- κ B, JNK/STAT-1, ERK1/2, and PI3 to promote neurotoxicity. TNF- α promotes Ca²⁺-mediated caspase-dependent apoptosis via NF- κ B activation and phosphorylation of STAT-1 which subsequently leads to nuclear translocation and transcription. Tat and gp120 promote STAT-1 phosphorylation. HIV-1 infection, gp120, TNF- α , and MIP-2 γ downregulate EAAT-2 expression and activity and prevent excess glutamate removal. IFN- α and HIV-1 infection in MDM and microglia promote release of mitochondrial-bound GLS into the cytosol and extracellular space where it can further catalyze glutamine to generate elevated levels of glutamate. Stimulation of iNOS by either, TNF- α , HIV-1 infection or gp120 and generation of high nitric oxide levels (ONOO-) diminishes GS expression and activity thus preventing conversion of glutamate to non-toxic glutamine. Furthermore, involvement of the CXCR4 axis in the neuropathology of HIV-1, triggered by SDF-1 or gp120 binding may further potentiate glutamate-evoked toxicity via ERK1/2 signaling pathway. Failure of these mechanisms leads to sustained neuronal NMDAR activation, Ca²⁺-mediated signaling and ultimately inflammation and cell death.

Figure 3. Neurotransmitter uptake by glial cells (astrocytes, microglia or activated macrophages) is mediated by specific transporters located on the glial cell membrane

In the glial cell glutamate-glutamine cycle, the excess glutamate (Glu) released at the pre-synaptic cleft is transported into the glial cell by the excitatory amino acid transporters 1 and 2 (EAAT 1/2). Glutamate is converted to glutamine by glutamine synthetase (GS).

Glutamine (Gln) is then transported into the extracellular space by N-system amino acid transporter (SNAT1) and is taken up by the neuronal SNAT.

Glutamate in the neuron is generated by the action of the enzyme glutaminase (GLS) in the mitochondrial matrix.

Glutamate is then loaded into synaptic vesicles with the transporter protein, vGLUT1, and

then is released into the synaptic cleft. Not depicted in this pathway are: the uptake of Glu by EAAT3 or metabotropic Glu receptors (mGluRs) in the post-synaptic neuron and the

action of glutamate dehydrogenase in glial cells that links glutamate metabolism with the Krebs cycle by catalyzing the reversible reaction $\alpha\text{-ketoglutarate} + \text{NH}_3 + \text{NADH} \leftarrow \rightarrow \text{Glu} + \text{NAD}^+$.

Table 1

Components of the glutamate-glutamine cycle

Gene (Protein)	Function	Positive Modulators	Negative Modulators	Cellular Localization	References
<i>SLC1A3</i> (Excitatory Amino Acid Transporter-1; EAAT-1)	Sodium-dependent L-glutamate/L-aspartate transmembrane symporter activity	Estrogen dbcAMP ^a EGF ^b Maslinic Acid	DL-TBOA ^c Glutamate	Plasma Membrane	(Takaki <i>et al.</i> , 2012) (Pawlak <i>et al.</i> , 2005) (Kim <i>et al.</i> , 2003) (Tilleux and Hermans, 2008)
<i>SLC1A2</i> (Excitatory Amino Acid Transporter-2; EAAT-2)	Sodium-dependent L-glutamate/L-aspartate transmembrane symporter activity	Estrogen dbcAMP ^a Maslinic Acid	DL-TBOA ^c L-Glutamate HIV-1 gp120 HIV-1 Tat	Plasma Membrane	(Wang <i>et al.</i> , 2003) (Pawlak <i>et al.</i> , 2005) (Qian <i>et al.</i> , 2011)
<i>GLUL</i> (Glutamine Synthetase)	Catalyzes the production of glutamine and GABA ^d	[Glutamine] _{Low} ; Glucocorticoids	Wnt signaling pathway; TNF-α	Cytoplasm/Mitochondria	(Zou J, 2010) (Janda <i>et al.</i> , 2011)
<i>SLC38</i> System A (SNAT1, SNAT2, SNAT4) System N (SNAT3, SNAT5)	Sodium-dependent amino acid transporter	BDNF IL-6 Hypertonic conditions; amino acid deprivation	[H ⁺] _{High} N-methyl-D-glucamine and probably choline	Plasma Membrane	(Baird <i>et al.</i> , 2006) (Burkhalter <i>et al.</i> , 2007) (Jones <i>et al.</i> , 2009)
<i>GLS</i> (Glutaminase; kidney type)	Catalyzes the hydrolysis of glutamine to glutamate and ammonia	IFN- α; phosphate; glutamine	Caytaxin; glutamate; ammonia	Cytoplasm/Mitochondria	(Zhao L, 2012) (Buschdorf <i>et al.</i> , 2006)
<i>SLC17A7/SLC17A6</i> (Vesicular Glutamate Transporter, VGLUT 1/2)	Mediates sodium-dependent uptake of glutamate into synaptic vesicles and may also mediate the transport of inorganic phosphate	[Cl ⁻] _{High} BDNF	Acetoacetate 3-Hydroxybutyrate Pyruvate	Membrane of synaptic vesicles	(Juge <i>et al.</i> , 2010) (Melo <i>et al.</i> , 2013)
<i>GLUD1</i> (Glutamate Dehydrogenase)	Catalyzes the oxidative deamination of glutamate to α-ketoglutarate	ADP, L-Leucine	GTP ATP EGCG ^e Spermidine	Mitochondria	(Platakis <i>et al.</i> , 2000) (Spanaki <i>et al.</i> , 2012)

^a dbcAMP: N(6),2'-O-dibutyryladenosine 3':5' cyclic monophosphate

^b EGF: epidermal growth factor

^cDL-TBOA: dl-threo-β-benzyloxyaspartate

^dGABA: gamma-aminobutyric acid

^eEGCG: epigallocatechin gallate