Glutamate metabolism and recycling at the excitatory synapse in health and neurodegeneration

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1. Introduction

Cellular homeostasis of the neurotransmitter glutamate is essential for normal brain function. Glutamate is not only the main excitatory neurotransmitter of the brain; it also represents a metabolic hub connecting glucose and amino acid metabolism with synaptic transmission (Fig. 1). This crucial amino acid is extensively recycled between neurons and astrocytes in a process known as the glutamate-glutamine cycle (Bak et al., 2006; Hertz, 2013; Hertz and Rothman, 2016). The glutamate-glutamine cycle is an open cycle, meaning that intermediates are recycled. Disruptions in glutamate clearance, leading to neuronal overstimulation and excitotoxicity, have been implicated in several neurodegenerative diseases. Furthermore, the link between brain energy homeostasis and glutamate metabolism is gaining attention in several neurological conditions. In this review, we provide an overview of the dynamics of synaptic glutamate homeostasis and the underlying metabolic processes with a cellular focus on neurons and astrocytes. In particular, we review the recently discovered role of neuronal glutamate uptake in synaptic glutamate homeostasis and discuss current advances in cellular glutamate metabolism in the context of Alzheimer’s disease and Huntington’s disease. Understanding the intricate regulation of glutamate-dependent metabolic processes at the synapse will not only increase our insight into the metabolic mechanisms of glutamate homeostasis, but may reveal new metabolic targets to ameliorate neurodegeneration.

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Glutamate recycling links the cellular homeostasis of glutamate to energy metabolism. Since dysfunctional brain energy metabolism is implicated in several neurodegenerative diseases (Beal, 1992; Bordone et al., 2019; Camandola and Mattson, 2017; Choi, 1988), cellular glutamate metabolism is a growing area of interest in neurodegenerative disease research (Cunnane et al., 2020; Dienel, 2019).

Excitatory glutamatergic transmission consumes the most energy of any signaling processes in the brain (Attwell and Laughlin, 2001; Harris et al., 2012; Sibson et al., 1998; Yu et al., 2018). During synaptic transmission, glutamate is mainly released via exocytosis where the release of a single vesicle induces a prompt rise of glutamate in the synaptic cleft. The released glutamate can activate postsynaptic glutamate receptors, thereby mediating excitatory transmission. Restoring
Several enzymatic reactions connect glutamate to cellular metabolism and neurotransmitter recycling at the glutamatergic synapse. The carbon skeleton of glutamate can be converted to the TCA cycle intermediate α-ketoglutarate, an important part of the malate-aspartate shuttle (MAS, see Fig. 3). Glutamate aminotransferase (BCAT). The most active aminotransferase in brain is AAT, which is also closely linked to the synthesis and metabolism of the inhibitory neurotransmitter GABA in the GABAergic synapse (Andersen et al., 2020; Graf et al., 2018). As the specific cellular mechanisms underlying glutamate transport into these vesicles. In particular, we review the known and recently discovered role of astrocytic and neuronal glutamate transporters in synaptic glutamate homeostasis. Finally, we summarize and discuss current advances in cellular glutamate metabolism in the context of two major neurodegenerative diseases: Alzheimer’s disease (AD) and Huntington’s disease (HD).

2. Synaptic glutamate – storage, uptake and recycling

It is estimated that 90% of all synapses in the brain utilize glutamate, making this neurotransmitter the predominant mediator of excitatory signals in the mammalian central nervous system (CNS) (Braitenberg and Schütz, 1998). Excitatory glutamatergic signaling is related to significant energetic costs estimated to account for 80% of the entire brain energy expenditure (Attwell and Laughlin, 2001; Harris et al., 2012; Yu et al., 2018). As the specific cellular mechanisms underlying glutamate metabolism and bioenergetics are still not fully understood, the study of glutamate-dependent metabolic dynamics at the synapse remains an open and fascinating field of investigation.

2.1. Energy dependence of glutamate storage in synaptic vesicles

In the glutamatergic presynaptic terminal, a fraction of the cellular glutamate pool accumulates in synaptic vesicles destined for release. This glutamate accumulation is accomplished by vesicular glutamate transporters (VGLUTs) present in the membrane of the synaptic vesicles. Notably, glutamate transport into these vesicles is considered a critical step in diverting glutamate from its metabolic role to its neurotransmitter function. It has been estimated that glutamate can reach...
intravesicular concentrations of 60 mM in vivo (Burger et al., 1989) while the cytosolic glutamate concentration is approximately 5–10 mM (Danbolt, 2001; Featherstone and Shippy, 2008). The mechanism for glutamate storage into synaptic vesicles involves an energy-dependent system coupling glutamate accumulation with ATP hydrolysis (Naito and Ueda, 1985; Ozkan and Ueda, 1998). The vacuolar H+-ATPase (V-ATPase) uses cytosolic ATP to generate an electrochemical proton gradient that drives inward glutamate transport into the vesicles (Juge et al., 2010). The VGLUTs display a complex biphasic dependency on extravesicular chloride ions (Cl−) (Wolosker et al., 1996). For instance, in isolated synaptic vesicles, glutamate transport was found to be low both in the absence of extravesicular Cl− or at high Cl− concentrations, whereas low extravesicular Cl− concentrations (4 mM) strongly activated glutamate transport. The Cl− biphasic effect was proposed to be associated with the allosteric modulation of VGLUT and reduction in the proton gradient caused by intravesicular Cl− loading (Wolosker et al., 1996). Interestingly, it has been demonstrated that metabolic intermediates such as ketone bodies have an allosteric role on VGLUT activity by competing for the Cl− binding site and thereby inhibiting VGLUT activity, which results in reduced glutamatergic neurotransmission in vivo (Juge et al., 2010). This finding further supports a strong association between the metabolic state, glutamate homeostasis and brain function.

2.2. Glutamate uptake from the synapse

Glutamate and aspartate were identified as excitatory transmitters of the CNS in classic studies by Curtis and Watkins (Curtis et al., 1960; Curtis and Watkins, 1966; Watkins and Evans, 1981). Decades later the three primary glutamate transporters expressed in the mammalian forebrain were cloned: GLT-1 (SLC1A2 (Pines et al., 1992)), GLAST (SLC1A3 (Stockck et al., 1992)), and EAAT3 (SLC1A7 (Ariza et al., 1997)). EAAT3, also known as excitatory amino acid transporter 3 (EAAT3), is expressed in astrocytic processes (Lehre et al., 1995), whereas EAAT3 is expressed in neurons facilitated by expression of distinct isoforms of glutamine synthetase (GS) which is exclusively expressed in neurons (Watkins and Evans, 1981). Neuronal GLT-1 is estimated to represent 10% of the total GLT-1 expression in the brain, based on studies in the hippocampus (Furness et al., 2008). It may therefore seem unlikely that neuronal GLT-1 contributes significantly to glutamate clearance, but this question has not been directly addressed. It is possible that the location of presynaptic GLT-1 in neurons places it in a prime location to modulate extracellular glutamate concentrations near synaptic glutamate receptors, especially those located on the presynaptic terminal (Rimmele and Rosenberg, 2016).

2.3. Glutamate recycling – the glutamate-glutamine cycle

Synaptic glutamate participates in a highly active recycling process between presynaptic glutamatergic neurons and astrocytes, known as the glutamate-glutamine cycle (Fig. 2) (Bak et al., 2006; Hertz, 2013; Hertz and Rothman, 2016). Astrocytes are closely associated with the glutamatergic synapse and are responsible for the majority of extracellular glutamate uptake, whereas a minor fraction is recovered by neuronal uptake (Hertz and Schousboe, 1987; Lehre and Danbolt, 1998; Zhou and Danbolt, 2013). Although microglia and oligodendrocytes have the capability to take up glutamate, the spill over from the synapse is low and the role of these cells in overall synaptic glutamate homeostasis is currently considered negligible. It is estimated that around 80% of all glutamate taken up by astrocytes is turned into glutamine (Hertz and Rothman, 2016; Rothman et al., 2011), a process catalyzed by the enzyme glutamine synthetase (GS) which is exclusively expressed in astrocytes (Norenberg and Martinez-Hernandez, 1979). The synthesized glutamine is subsequently released from the astrocytes and taken up by neurons facilitated by expression of distinct isoforms of glutamine transporters (primarily by sodium-coupled neutral amino acid modulates long-term potentiation (Scimemi et al., 2009) and is important for GABA synthesis (Sepkuty et al., 2002). Furthermore, EAAT3 is capable of transporting cysteine (Zerangue and Kavanaugh, 1996b), which is important for the synthesis of neuronal glutathione (Aoyama et al., 2006) as neurons lack the cystine-glutamate antiporter xCT (Ottestad-Hansen et al., 2018).
transporters (SNATs) of the SLC38 family) in neurons and astrocytes (Leke and Schousboe, 2016; Rodríguez and Ortega, 2017). The ability of glutamine efflux by the SNATs expressed in astrocytes has been proposed to be linked to the additional exchange of H⁺ (Chaudhry et al., 1999), which is not found in the primary neuronal SNATs (Chaudhry et al., 2002). Note that multiple SNAT isoforms and glutamine transporter systems are present in the brain and that the specific cellular localization and functional role of these have not been fully elucidated (Leke and Schousboe, 2016). Once taken up by the neurons, glutamine is converted back to glutamate by the enzyme phosphate-activated glutaminase (PAG) (Hogstad et al., 1988; Kvamme et al., 2001), thereby completing the cycle. The glutamate-glutamine cycle is highly active and astrocyte-derived glutamine is quantitatively the most important substrate for the replenishment of the neuronal glutamate pool (Hertz and Rothman, 2016; Rothman et al., 2011; Shen et al., 1999; Ward et al., 1983). Although the glutamate-glutamine cycle has been challenged (Kam and Nicoll, 2007), astrocyte-derived glutamine seems particularly important during sustained glutamatergic signaling (Tani et al., 2010, 2014). Indeed, experimental inhibition of astrocytic glutamine synthesis causes disruptions of both neurotransmitter and energy homeostasis (Andersen et al., 2017a) and leads to seizures (Eid et al., 2008). The glutamate-glutamine cycle does not operate on a stoichiometric basis, as glutamate is also utilized as a substrate to support cellular energy metabolism as discussed in the following sections. The oxidative loss of glutamate has to be correspondingly matched by a de novo synthesis of glutamate, which primarily takes place in astrocytes. De novo glutamate synthesis is dependent on sufficient anaplerosis, i.e. reactions providing a net increase in TCA cycle intermediates (Sonnewald, 2014). The primary anaplerotic enzyme in the brain, pyruvate carboxylase (PC), is exclusively expressed in astrocytes and serves as the main pathway of de novo glutamate synthesis (Fig. 2) (Schousboe et al., 2019; Yu et al., 1983; Oz et al., 2004). The selective expression of PC in astrocytes, in combination with the essential role of astrocyte-derived glutamine, underlines the fact that neuronal glutamate homeostasis is under tight astrocytic control (Schousboe et al., 2013).

3. Cellular glutamate synthesis and metabolism

Glutamate is linked to cellular energy metabolism via the TCA cycle intermediate α-ketoglutarate catalyzed by several enzymatic reactions.
The two primary enzymes mediating glutamate synthesis and metabolism in the brain are aspartate aminotransferase (AAT) and glutamate dehydrogenase (GDH) (Schousboe et al., 2014). AAT is the most active aminotransferase in the brain and the reaction proceeds easily in both directions. AAT is present in both a cytosolic and mitochondrial form (Fonnum, 1968), whereas GDH is strictly a mitochondrial enzyme (Mastorodemos et al., 2005; Mathioudakis et al., 2019; Salganicoff and De Robertis, 1965). AAT activity, and thus glutamate, is closely linked to cellular energy metabolism, as both the cytosolic and mitochondrial isoforms of AAT participate in the malate-aspartate shuttle (MAS, Fig. 3). The MAS mediates the transfer of reducing equivalents of NADH from the cytosol into the mitochondria needed for sustained glycolytic activity (Dienel, 2019; McKenna et al., 2006). While the rate of the AAT catalyzed reaction is primarily determined by the availability of substrates, GDH has a more intricate regulation. Both leucine and ADP serve as positive allosteric modulators, whereas GTP is a potent negative modulator of GDH (Li et al., 2011). In the cell, although the thermodynamic equilibrium constant for GDH is very low (6 × 10^{-15} M (Engel and Dalziel, 1967)), the low intracellular levels of ammonia and NADH/NADPH drives the oxidative deamination of glutamate to α-ketoglutarate by GDH, i.e. net catabolism of glutamate (Fig. 1). In contrast, during pathological conditions of elevated cerebral levels of ammonia such as hepatic encephalopathy, GDH can mediate ammonia fixation via glutamate synthesis (Drews et al., 2020; Voss et al., 2021). Although AAT and GDH are regarded as the two main enzymes for glutamate synthesis and metabolism, the role of the branched-chain amino acids aminotransferase (BCAT) and alanine aminotransferase (ALAT) should not be underestimated (Fig. 1). The branched-chain amino acids (BCAAs) leucine, isoleucine and valine are readily transported across the blood brain barrier and are important nitrogen donors for glutamate synthesis via BCAT activity (Sperringer et al., 2017; Yudkoff, 1997) which is crucial for de novo synthesis of glutamate (Conway and Hutson, 2016). Since there is a net flow of glutamate from neurons to astrocytes (Fig. 2), the cell-specific metabolism of glutamate is an area of great interest as discussed in detail below.

3.1. Astrocyte glutamate metabolism

Glutamate is an excellent oxidative substrate in astrocytes and is preferred over most other substrates, including lactate, ketone bodies and even glucose (McKenna, 2012; Sonnewald et al., 1993). However, the relative contributions of AAT and GDH in glutamate synthesis and metabolism in astrocytes is still a topic of debate (McKenna, 2007; McKenna et al., 2016). AAT inhibition in cultured astrocytes has been shown to significantly reduce glutamate synthesis, but produced little effect on glutamate entry into the TCA cycle (Sonnewald et al., 1996; Westergaard et al., 1996; Yu et al., 1982), suggesting that GDH is the primary enzyme mediating oxidative glutamate metabolism in astrocytes. However, other studies also in cultured astrocytes, reported a significant reduction in glutamate oxidation after AAT inhibition (Farinelli and Nicklas, 1992; McKenna et al., 1993, 1996b). These discrepancies are likely explained by differences in the applied glutamate concentrations of the studies. This would indicate that GDH is primarily recruited for sustained oxidative metabolism of glutamate at elevated concentrations, whereas AAT may be sufficient at lower concentrations (McKenna et al., 2016). This notion is in accordance with the observation that astrocytes increase the capacity of oxidative metabolism of glutamate in the TCA cycle, with a parallel reduction in glutamine synthesis, as the glutamate concentration increases (McKenna et al., 1996a). An interesting study by Pardo et al. showed that neuron-derived aspartate is a prominent nitrogen donor for astrocytic glutamate and glutamine synthesis (Pardo et al., 2011). However, since neuronal aspartate is an excitatory amino acid, as discussed above, Hertz has argued that it is not suited as a transcellular nitrogen donor (Hertz, 2011). This has led to a proposed model in which both astrocyte glutamate synthesis and metabolism is primarily driven by AAT (Hertz and Rothman, 2016). Instead of aspartate being transferred from neurons to astrocytes, astrocytic aspartate is generated from oxaloacetate during the oxidative metabolism of glutamate by AAT (Hertz, 2011; Hertz and Rothman, 2016). This model proposes a more prominent role.
of glutamate and the subsequent δ13C enrichment in TCA cycle intermediates and amino acids was determined by mass spectrometry (Andersen et al., 2017b; Hohnholt et al., 2018) (Fig. 4). We found that the δ13C enrichment in glutamate was unchanged between control and GDH KO mice, suggesting that lack of GDH does not affect neuronal glutamate uptake capacity. However, large reductions were observed in the δ13C enrichments of the TCA cycle intermediates fumarate, malate and citrate, clearly demonstrating a reduced capacity of glutamate oxidation in the GDH KO synaptosomes. Furthermore, the δ13C enrichment of aspartate was highly reduced, suggesting that AAT was not able to compensate for the lack of functional GDH in synaptosomes. Collectively, these results provide genetic evidence that functional GDH activity is important for neuronal glutamate metabolism. Interestingly, acute pharmacological inhibition of GDH has been shown to reduce glutamate uptake in both cultured astrocytes (Bauer et al., 2012) and synaptosomes (Whitelaw and Robinson, 2013), which is in contrast to the unaffected glutamate uptake in the GDH KO synaptosomes. This discrepancy may be caused by off-target effects of the applied GDH inhibitors or by compensatory actions in glutamate uptake in the GDH KO synaptosomes.

We have recently shown that deletion of GLT-1 in neurons has functional consequences for neuronal metabolism (McNair et al., 2019, 2020). Apart from reduced capacity of neuronal glutamate metabolism, a key finding of these studies was that neuronal GLT-1 deletion led to a robust decrease in aspartate concentrations in several brain regions (McNair et al., 2019, 2020). This decrease was caused by a hampered aspartate synthesis demonstrating that neuronal uptake of glutamate is essential for maintaining the neuronal aspartate pool (McNair et al., 2019). In the light that neuronal aspartate may be important for astrocyte glutamine synthesis (Pardo et al., 2011), it could be expected that the decreased neuronal aspartate synthesis would affect astrocyte glutamine homeostasis. However, this was not the case as both glutamine synthesis activity and glutamine amounts were maintained in the neuronal GLT-1 KO mice (McNair et al., 2019, 2020). One study, using a different neuronal GLT-1 KO mouse, even reported elevated glutamate synthesis in the hippocampus, which may suggest complex astrocytic compensatory mechanisms (Zhou et al., 2019). This notion is in line with the observation that both adjacent astrocytic processes and excitatory synapses displayed an elevated density of mitochondria in the absence of neuronal GLT-1 (McNair et al., 2019, 2020). Collectively, these studies demonstrate that neuronal uptake of glutamate supports synaptic oxidative TCA cycle metabolism and is important for neuronal aspartate synthesis and mitochondrial function.

4. Glutamate metabolism in neurodegeneration

Neurodegenerative diseases comprise a variety of disorders of the CNS leading to gradual loss of brain tissue and neuronal death. The mechanisms underlying the neuronal loss are complex and not completely understood, however, glutamatergic dysfunction and neuronal overstimulation, i.e. excitotoxicity, has been a driving hypothesis for many years (Beal, 1992; Choi, 1988; Greenamyre, 1985; Greene and Greenamyre, 1996; Lewerenz and Maher, 2015; Lipton and Rosenberg, 1994). Furthermore, alterations in brain energy metabolism are implicated in many neurodegenerative diseases (Camandola and Mattson, 2017; Cunnane et al., 2020). Since cellular glutamate uptake and metabolism are closely linked to brain energy metabolism, investigation of functional glutamate metabolism may provide new metabolic insights of these diseases. In the following sections we focus on two prominent neurodegenerative diseases, Alzheimer’s disease (AD) and Huntington’s disease (HD), in which dysfunctional glutamate uptake and metabolism are important for neuronal loss and mitochondrial dysfunction.

3.2. Neuronal glutamate metabolism

Astrocytes take up and process the majority of synaptic glutamate (Danbolt, 2001; Rose et al., 2018). However, as mentioned, neuronal uptake of glutamate is gaining attention (Rimmele and Rosenberg, 2016) and it is well-known that neurons are able to utilize glutamate as an energy substrate (Ostad et al., 2007; Sonnewald et al., 1996). In contrast to astrocytes, AAT activity has been suggested to play a more prominent role in neuronal oxidative glutamate metabolism (McKenna, 2007). This may be related to the high activity of the MAS in neurons (Fig. 3) (McKenna et al., 2006; Pardo et al., 2011). However, GDH has also been shown to be important for neuronal glutamate metabolism. Isolated nerve endings, known as synaptosomes, lacking GDH displayed a lower respiratory capacity during stimulation, indicating that GDH may be particularly important for neuronal metabolism during intense signaling events (Hohnholt et al., 2018). Furthermore, oxidative metabolism of glutamate, derived from hydrolysis of glutamine by PAG activity, seems to be dependent on GDH in neurons (Hohnholt et al., 2018; McKenna, 2007).
homeostasis has been extensively associated with the progressing pathology and neurodegeneration (Brymer et al., 2021).

4.1. Alzheimer’s disease

Alzheimer’s disease (AD) is an age-related unremitting neurodegenerative disease leading to dementia (Masters et al., 2015). The complex pathology of AD gradually develops over decades and is characterized by the formation of insoluble plaques of aggregated amyloid-β (Aβ) peptide and neurofibrillary tangles of hyperphosphorylated tau protein (Querfurth and LaFerla, 2010). AD is further characterized by imbalances of neuronal signaling, reduced cerebral blood flow, impaired brain energy metabolism and mitochondrial dysfunction (Busche and Konnerth, 2016; Cardoso et al., 2016; Gordon et al., 2018; Moreira et al., 2010; Nortley et al., 2019). Hyperactive glutamatergic signaling arise in the early phases of AD pathology (Busche et al., 2008, 2012), which has been suggested to be mediated by reduced glutamate uptake (Li et al., 2009; Selkoe, 2019; Zott et al., 2019). Reduced synaptic glutamate clearance and concurrent excitotoxicity has been widely proposed as a pathological mechanism in AD (Acosta et al., 2017; Greenamyre and Young, 1989; Mattson, 2004). Several studies have reported reduced expression of GLT-1 in both aging (Pereira et al., 2017; Potier et al., 2010) and AD brain tissue (Jacob et al., 2007; Scott et al., 2011). Furthermore, Aβ has been shown to directly impair astrocyte glutamate uptake (Matos et al., 2006) and to reduce the surface expression of GLT-1 (Scimemi et al., 2013). Studies have furthermore shown that reducing GLT-1 expression in the APP/PS1 mouse model of AD exacerbates cognitive dysfunction (Moskhovjee et al., 2011) and that deletion of either astrocytic or neuronal GLT-1 leads to cognitive impairments (Sharma et al., 2019). In particular, loss of astrocytic GLT-1 displayed a large transcriptomic overlap with human aging and AD (Sharma et al., 2019), suggesting a crucial role of astrocytic glutamate clearance in AD.

Astrocytes react strongly to Aβ aggregates, leading to changes in morphology and homeostatic properties (Bennett and Viana, 2021; Liddelow et al., 2017). It has been proposed that reactive astrocytes provide insufficient synaptic support and thereby potentially contribute to the neurodegeneration in AD (Steele and Robinson, 2012; Walton and Dodd, 2007). Since glutamate uptake is a highly energy requiring process, alterations in astrocyte energy metabolism in AD, which has been reported by multiple studies (Abramov et al., 2004; Allaman et al., 2010; Dematteis et al., 2020; Oksanen et al., 2017; Ryu et al., 2021; van Gijsel-Bonnello et al., 2017) may be linked to the insufficient glutamate clearance. This notion is in line with a recent extensive proteomics study of human AD brain samples, finding a strong correlation between the severity of AD pathology and changes in proteins related to astrocyte and microglia metabolism (Johnson et al., 2020). Several studies have also reported reduced glutamate amounts in the AD brain, again correlating with advancing neurodegeneration (Fayed et al., 2011; Guelli and Taibi, 2013; Rup Singh et al., 2011). The decreased brain glutamate levels could be due to the loss of glutamatergic neurons, but could also be a consequence of hampered astrocyte glutamine support. Both reduced activity and expression of GS have been reported in human patient tissue (Robinson, 2000; Smith et al., 1991) and mouse models of AD (Kulijewicz-Nawrot et al., 2013; Olabarria et al., 2011). We recently demonstrated that altered astrocyte glutamine synthesis has functional consequences for neurons in AD, as diminished astrocyte glutamine support directly impaired neuronal GABA synthesis in brain slices of the 5xFAD mouse model of AD (Andersen et al., 2021). This observation was further supported by an augmented capacity for neuronal metabolism of exogenously provided glutamine, suggesting a neuronal metabolic compensation for the reduced astrocyte glutamine supply (Andersen et al., 2021). Interestingly, we did not observe changes in the capacity of exogenous glutamate uptake and metabolism in the brain slices of the 5xFAD mice (Andersen et al., 2021). Both isolated mitochondria (Tao et al., 2009) and synaptosomes (Wang et al., 2016) from mouse models...
of AD have been reported to display reduced metabolic capacity with glutamate as a respiratory substrate. However, this likely reflects the general decline in mitochondrial function commonly observed in AD (Cardoso et al., 2016; Moreira et al., 2010). Several studies have also reported decreased amounts of cerebral aspartate in AD (Gueli and Taibi, 2015; Paglia et al., 2016; Xu et al., 2016), which may indicate dysfunctional AAT activity. In agreement with this, a reduced expression of both the cytosolic and mitochondrial isofoms of AAT has been reported in both human AD brain samples and animal models (Li et al., 2020; Mahajan et al., 2020; Puthiyedath et al., 2016; Savas et al., 2017). Lower activity or expression of AAT could disturb glutamate synthesis and metabolism, but could also have significant impact on MAS activity (Fig. 3) (McKenna et al., 2006), which in turn may contribute to the cerebral bioenergetic crisis of AD. Intriguingly, we recently found a selective reduction in astrocytic aspartate synthesis in hippocampal slices of 5xFAD mice (Andersen et al., 2021), which may indicate that astrocyte-specific metabolic deficiencies in AD disrupt aspartate and glutamate homeostasis.

An elegant study by Neuner et al. recently identified hippocampal GDH expression as a strong determinant of memory decline in both aging and AD (Neuner et al., 2017). The authors investigated the hippocampal proteome of 8 months old 5xFAD and control mice and found that reduced GDH expression strongly correlated with cognitive deficits in both aged control and 5xFAD mice. In line with this, reduced cerebral GDH expression has also been reported in the 3xTG mouse model of AD (Clavardelli et al., 2010). In contrast, increased expression of BCAT has been found in human AD brain samples (Hull et al., 2015), which may serve as an alternative pathway of glutamate synthesis in AD (Conway, 2020). Functional in vivo studies applying 13C enriched glucose and magnetic resonance spectroscopy have shown significant reduction in glutamate synthesis in both neurons and astrocytes in Aβ+ rodent models of AD (Nilsen et al., 2014; Tiwari and Patel, 2012). In contrast, rodent models of tau pathology have revealed elevated 13C enrichments in glutamate from metabolism of 13C glucose (Hebron et al., 2018; Nilsen et al., 2013), which may suggest complex differential mechanisms of Aβ and tau pathologies on glutamate synthesis and metabolism. Interestingly, a reduced activity of the anaplerotic enzyme PC, the activity of which is linked to glutamate metabolism via GDH (Nissen et al., 2015), has also been found in rodent models of AD (Nilsen et al., 2014; Tiwari and Patel, 2014).

4.2. Huntington’s disease

Huntington’s disease (HD) is a fatal autosomal dominant neurodegenerative disease characterized by loss of voluntary motor control, psychiatric disturbances and cognitive decline (Bates et al., 2015; Ross and Tabrizi, 2011). HD is caused by an expansion of the CAG repeat in the HTT gene leading to toxic gains of function for the translated mutant huntingtin protein. Excitotoxicity, caused by decreased synaptic glutamate clearance, has also been a central hypothesis in HD pathology research (Estrada Sánchez et al., 2008; Raymond et al., 2011; Tabrizi et al., 1999). Several reports have demonstrated significant reductions in both brain GLT-1 expression and synaptosome glutamate uptake capacity in multiple models of HD (Behrens et al., 2002; Estrada-Sánchez et al., 2009; Faideau et al., 2010; Huang et al., 2010; Liévès et al., 2001). Recent studies using fluorescent glutamate biosensors have conflictingly reported both reduced (Jiang et al., 2016) and increased (Parsons et al., 2016) real-time glutamate uptake capacities in the R6/2 mouse model of HD. Interestingly, a study has shown that treatment with the antibiotic ceftriaxone, which elevates the cerebral expression of GLT-1, reduces the pathological phenotype of R6/2 mice (Miller et al., 2008). In contrast, Petr et al. demonstrated that further reduction of brain GLT-1 expression did not worsen HD pathology in the R6/2 mouse (Petr et al., 2013). The authors generated a novel mouse model that, in addition to the pathological HTT gene, was also heterozygote for GLT-1 KO, i.e. only carrying the GLT-1 gene on one allele. The double mutant displayed the expected additional reduction in GLT-1 expression when compared to R6/2 mice. However, this did not affect the pathological phenotype, suggesting that the overall reduction in GLT-1 may in fact not directly contribute to HD pathology (Petr et al., 2013). The authors also reported a pronounced decrease in neuronal GLT-1 expression in the R6/2 mice (Petr et al., 2013). Transgenic mice lacking neuronal GLT-1 interestingly display a similar transcriptional dysregulation as those observed in HD (Laprairie et al., 2019). Furthermore, studies on neuronal cultures from induced pluripotent stem cells (iPSCs) of HD patients, revealed a downregulation of the genes SLC1A3 and SLC1A6, coding for the glutamate transporters GLAST and EAAT4, respectively (The HD iPSC Consortium, 2017). Collectively, these observations may suggest a critical role of glutamate uptake in HD pathology.

Brain energy metabolism is also impaired in HD (Browne and Beal, 2004; Mochel and Haller, 2011), which correlates with overall disease progression (Tang et al., 2013). The cerebral content of glutamate has generally been reported to be unchanged or slightly reduced in HD (Graham et al., 2016; Patassini et al., 2016; Sturrock et al., 2018; Zacharoff et al., 2012), whereas several studies have reported cerebral glutamine accumulation in HD models (Behrens et al., 2002; Heikkinen et al., 2012; Pépin et al., 2016; Tkac et al., 2007; Tsang et al., 2006; Zacharoff et al., 2012). The elevated levels of brain glutamine are surprising, as several studies have found reduced expression and activity of GS in HD (Boussicault et al., 2014; Carter, 1982; Hosp et al., 2017; Liévès et al., 2001; Tong et al., 2014). We have also recently reported reduced cerebral GS expression is the R6/2 mice (Skotte et al., 2018). Interestingly, we also found decreased expression of the glutamine transporter SNAT3 (Skotte et al., 2018), which is primarily expressed in astrocytes (Fig. 2). This could lead to a reduced capacity of astrocyte glutamine release, which may explain the reported accumulation of cerebral glutamine in HD. In the same study we also investigated functional metabolism in cerebral cortical and striatal slices of R6/2 mice. Surprisingly, we observed that glucose metabolism was largely maintained in the R6/2 slices, whereas astrocyte energy metabolism was highly impaired (Skotte et al., 2018). This perturbed astrocytic metabolism was also reflected as a significant reduction in oxidative glutamate metabolism, particularly in the striatal slices of the R6/2 mice. Finally, we found that functional astrocyte glutamine synthesis was reduced, which in combination with the diminished glutamine release, led to decreased neuronal synthesis of GABA (Skotte et al., 2018). All of these observations underline the importance of astrocyte metabolic function in HD (Khakh et al., 2017; Polyzos et al., 2019; Wilton and Stevens, 2020).

Interestingly, elevated GDH and PAG expression has been described in the brains of R6/2 mice, which may reflect early compensations to elevate the capacity of glutamate metabolism (Zabel et al., 2009). This elevation is in accordance with reports of increased GDH activity in brain samples of both HD patients and mouse models (Bird et al., 1977; Olah et al., 2008). Such increased GDH activity has also been found in samples from amyotrophic lateral sclerosis patients (Rothstein et al., 1992). This is intriguing as transgenic mice overexpressing neuronal GDH likewise present with age-associated neurodegeneration (Dao et al., 2009; Michaelis et al., 2011), which may suggest a link between over-activation of GDH and neuronal death. Finally, reduced levels of the BCAAs in plasma, cerebrospinal fluid and the brain have consistently been reported in HD (Graham et al., 2016; Mochel et al., 2007, 2011; Perry et al., 1969). The decreased BCAA levels correlate with HD progression and can be used as a biomarker of disease (Cheng et al., 2016; Mochel et al., 2011). We recently described elevated expression of BCAA metabolic enzymes along with an elevated capacity of leucine and isoleucine metabolism in brain slices of R6/2 mice (Andersen et al., 2019). Since the R6/2 mice also display decreased glutamate metabolism in astrocyte cultures of HD KO mice (Nissen et al., 2015), the results suggest that the reduced BCAA levels in HD may in fact be caused
by a compensatory increase in brain BCAA metabolism, potentially to sustain neuronal glutamate synthesis and counteract the reduced astrocyte glutamine support (Andersen et al., 2019).

5. Conclusions

Spatiotemporal glutamate homeostasis is dependent on a complex interplay between multiple cellular compartments and pathways. Glutamate metabolism at the excitatory synapse is the subject of an exquisitely fine-tuned regulation that when compromised, may lead to brain pathology as observed in several neurodegenerative diseases. Glutamate bioenergetics functionally involve astrocytes and neurons as glutamate is extensively metabolized in, and recycled between, these cell types. The development of more powerful methods to investigate glutamate dynamics will likely aid to reveal several yet unknown aspects of glutamate metabolism (Brymier et al., 2021). Moreover, modulation of brain energy metabolism is currently gaining momentum as a target in neurodegeneration (Cammane et al., 2020). An important open question is whether the alterations in glutamate uptake, recycling and metabolism in neurodegenerative diseases are a consequence of disease progression or if they could be causative mechanisms driving the neurodegeneration and hereby be potential targets for treatment (Kim and Baik, 2019).

Studying glutamate homeostasis in animal models of neurodegenerative diseases in the early pre-symptomatic phases may aid to address this issue. Although neurons and astrocytes are the predominant cell types, the brain consists of several other cell types that are crucial for overall brain homeostasis. Further studies are therefore needed on cell-specific glutamate synthesis and metabolism in both health and disease. Together, this will not only increase our understanding of the metabolic mechanisms underlying glutamate homeostasis, but may also provide new and important insights into the pathogenesis of neurodegenerative diseases.

Data availability

All data of this study is available from the corresponding authors upon request.

Author contributions

KHM performed the synaptosomal experiments. JVA, PR and BIAG drafted the manuscript with critical input from all authors.

Declaration of competing interest

The authors have no conflict of interest to declare.

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