Minireview

Influence of glutamate and GABA transport on brain excitatory/inhibitory balance

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Impact statement

Excitatory and inhibitory (E/I) balance broadly refers to a stable global neuronal activity that is predominately achieved in brain by a coordinated balance between excitatory and inhibitory inputs. E/I imbalance contributes to the pathobiology of neurodevelopmental disorders, neurodegenerative/neurological disease, as well as, acute neurological disorders. Hence, a deeper understanding of the cellular and molecular mechanisms regulating physiological E/I balance is needed to improve current clinical strategies for managing disorders/diseases associated with E/I perturbations. Herein, we review the synthesis, release, and signaling of the principle CNS excitatory and inhibitory neurotransmitters (namely glutamate and GABA, respectively) and discuss the capacity of glutamate and GABA transporters to modulate release and uptake of neurotransmitter, as well as neural network activity.

Abstract

An optimally functional brain requires both excitatory and inhibitory inputs that are regulated and balanced. A perturbation in the excitatory/inhibitory balance—as is the case in some neurological disorders/diseases (e.g. traumatic brain injury Alzheimer's disease, stroke, epilepsy and substance abuse) and disorders of development (e.g. schizophrenia, Rhett syndrome and autism spectrum disorder)—leads to dysfunctional signaling, which can result in impaired cognitive and motor function, if not frank neuronal injury. At the cellular level, transmission of glutamate and GABA, the principle excitatory and inhibitory neurotransmitters in the central nervous system control excitatory/inhibitory balance. Herein, we review the synthesis, release, and signaling of GABA and glutamate followed by a focused discussion on the importance of their transport systems to the maintenance of excitatory/inhibitory balance.

Keywords: Excitatory/inhibitory balance, glutamate transport, GABA transport, EAATs, GATs, system x_c^-

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Introduction

An optimally functional brain requires both excitatory and inhibitory inputs that are regulated and balanced. Optimal balance is necessary for efficient information processing at both the cellular and network level, each of which ultimately subserves cognition and behavior.[1,2] At the cellular level, modulation of the intrinsic excitability and synaptic strength maintain balance, thereby regulating the overall firing probability of a neuron.[3–6] At the network level, optimal balance maintains stable circuitry (reviewed in Gao and Penzes[7] and Nelson and Valakh[8]). A perturbation in E/I balance has been implicated in the etiology and expression of autism spectrum disorders (ASD), schizophrenia and anxiety, cerebral ischemia, traumatic brain injury, epilepsy and substance abuse.[9–13] As such, a deeper understanding of the cellular and molecular mechanisms regulating physiological E/I balance would allow for improvement of current clinical strategies for managing such disorders. In this review, we will focus solely on the neurotransmitters glutamate and GABA, including how they are made, released, and signal, as well as, whether and how specific transport processes influence their activity.

Gabaergic neural transmission

Gamma-aminobutyric acid (GABA) is present in high concentrations in the CNS. Studies in cortex showing that application by ionotophoresis inhibits cell firing paved the way for its classification as an inhibitory neurotransmitter in mature, adult mammalian brain.[14–17] Nonetheless, when the potassium/chloride cotransporter KCC2 levels are low, as occurs early in development, GABA signaling

is excitatory, exerting trophic effects that contribute to normal neuronal growth and expansion.[18,19]

GABA synthesis and packaging. GABA is formed predominately by the enzymes glutamate decarboxylase 65 (GAD65) or GAD67, both of which use pyroxidine as a co-factor to convert glutamate to GABA in the CNS. GAD65, located in nerve terminals, produces GABA for classic tonic neurotransmission.[20,21] GABA produced via GAD 67, expressed principally in the neuronal somata, functions in a non-neurotransmitter, metabolic, capacity contributing to synaptogenesis as well as to oxidation-reduction (redox) regulation.[20–22] Glutamine, serving as a precursor for glutamate synthesis via phosphate activated glutaminase, is also a substrate of GABA.[23,24] Once synthesized, GABA is packaged for release into synaptic vesicles by vesicular transporters specific for GABA (VGATs) in a manner dependent on both the electrochemical and pH gradient.[25,26]

GABA signaling. GABA signals via membrane bound receptor proteins that either open chloride channels ($GABA_AR$ and $GABA_CR$) or activate a G protein ($GABA_BR$) (for review see Bormann[27]). Both lead to hyperpolarization of the cell membrane albeit via different mechanisms.[28] $GABA_ARs$ are composed of an obligatory α and β subunit (α1-6, β1-4) and at least one other subunit (γ1-4, δ, ε, π, and θ) and are ubiquitously expressed throughout the vertebrate CNS. $GABA_CR$ are composed exclusively of ρ subunits (ρ1–3) (see Hedblom and Kirkness[29] Bonnert *et al*.[30] and reviewed in Zhang *et al*. and Macdonald and Olsen)[31,32] and are near exclusively found in the retina.[27,33] Sedative-hypnotics of the barbiturate and benzodiazepine family increase channel opening frequency,[32,34,35] while bicuculline competitively antagonizes $GABA_ARs$,[36] but these drugs have no effect on $GABA_CRs$.[33] The $GABA_BR$ is a G-protein coupled receptor formed by the dimerization of $GABA_{B1}$ and $GABA_{B2}$ subunits (reviewed in Heaney and Kinney[37]).

Bicuculline-resistant, $GABA_BRs$ are activated by baclofen and inhibited by phaclofen.[38] Located both presynaptically and postsynaptically, the primary effects of activation of $GABA_BRs$ are the inhibition of adenylate cyclase, inhibition of voltage-gated Ca^{2+} channels, and activation of inwardly rectifying K^+ channels, all of which contribute to a gradual and protracted synaptic inhibition.[39–41]

GABA uptake. The length and size of GABA R mediated responses are controlled by four different sodium symporters belonging to the solute carrier 6 (Slc6) family. The high affinity GABA transporters—GABA transporter (GAT) 1–3—and low affinity betaine-GABA transporter 1 (BGT1) are all coupled to Na^+ and Cl^- gradients.[42–47] In the CNS, GAT1 is expressed on inhibitory interneuron axon terminals, on the somato-dendritic compartment of developing interneurons, in pyramidal neurons, and in astrocytic processes and has a Km value of 8 μM.[44,45,48] Selectively expressed on astrocytes,[42,49–54] the Km of GAT3 is 0.8 μM. GAT2 (Km = 18 μM) is found in high abundance in kidney and liver and is only weakly expressed in brain, most auspiciously in cells forming the pia and arachnoid barrier and in a subset of blood vessels.[55,56] Transcript for BGT-1 has been found in both mouse and human brain [45,57] with a demonstrated Km of 80 μM.

Following its synaptic release, GABA uptake by neurons can be recycled/reloaded into synaptic vesicles to sustain subsequent rounds of release.[58,59] Alternatively, it can be metabolized in both neurons and astrocytes by GABA-transaminase and succinic semialdehyde dehydrogenase, a process known as the GABA shunt, to replenish the TCA cycle,[60,61] thereby constituting an alternative energy substrate.

Contribution of GABA transport to the maintenance of E/I balance. Because GABA released into the extracellular space is not enzymatically broken down, GAT activity is positioned to control the basal extracellular GABA in the extracellular space and E/I balance by acting as the primary mechanism to terminate synaptic inhibitory neurotransmission.

GAT1. In the CNS, GAT1 signaling accounts for ~75–80% of GABA uptake,[62] with high expression in GABAergic neurons of the neocortex, hippocampus, basal ganglia, brain stem, cerebellum, olfactory bulbs, and retina.[63] Indeed, $GABA_AR$-mediated currents derived from hippocampal slice recordings from $GAT1^{-/-}$ mice are increased compared to control slices.[62] These results were recapitulated by using GAT1 selective inhibitors at wild-type synapses.[64,65] Interestingly, a reduction in inhibitory tone at hippocampal presynaptic $GABA_BR$ has been reported in $GAT1^{-/-}$ mice,[62] which is potentially due to receptor desensitization following prolonged agonist exposure, as has been reported with other G-protein coupled receptors.[66] This occurs alongside decreased phasic inhibition—manifest as a reduction in miniature inhibitory postsynaptic current (mIPSC) frequency as compared to wild-type.[62] Given that presynaptic $GABA_BR$ activation typically inhibits neurotransmitter release, this, and the finding that spontaneous IPSCs were unaffected by presynaptic $GABA_B$ tone,[62] suggests that diminished phasic inhibition in $GAT1^{-/-}$ mice likely occurs via a $GABA_B$ receptor-independent mechanism.

Loss of GAT1 signaling also has a profound effect on behavior. Mice null for $GAT1^{-/-}$ display decreased depression and anxiety-like behavior,[67,68] are less aggressive,[69] and display signs of hypoalgesia, when compared to wild-type control mice.[70] They also demonstrate impairment in hippocampus-dependent learning and memory.[67,71] Moreover, selective GAT1 inhibitors—including tiagabine, NO-711, and DDPM-2571 [72,73]—have been demonstrated to recapitulate $GAT1^{-/-}$ behavioral phenotypes in wild-type rodents.[73–76] These findings have paved the way for human clinical trials of GAT1 inhibitors for treatment of behavioral complications of psychiatric disorders including aggression,[77,78] anxiety,[79,80] cocaine addiction [81] and for the improvement of pain symptoms in individuals suffering from sensory neuropathy.[82]

Inhibition of GAT1 also reduces the hyperexcitation of GABAergic neurons produced by opiate withdrawal.[83,84]

Apart from its role in mediating GABA uptake, multiple lines of *in vitro* evidence demonstrate GAT1 reversal represents a route of non-vesicular GABA release in the brain. Wu *et al.* found that the reversal potential of GAT1 is close to equilibrium to the cell's resting membrane potential, such that action potentials and high-frequency firing are sufficient to induce GAT1 reversal.[85] GAT1 reversal is observed in response to membrane depolarization in hippocampal cultures.[86] Interestingly, an increase in GAT1 immunoreactivity has been observed in the hippocampus of rats following 4-AP and kainic acid-induced epileptiform activity [87,88] and recent studies show that the anti-seizure medications gabapentin and vigabatrin enhance GAT1-mediated GABA release,[86,89] with vigabatrin potently increasing ambient $[GABA]_e$ and inducing tonic inhibition of neurons.[90] Additionally, tiagabine, a selective GAT1 inhibitor—commonly prescribed as an add-on therapeutic option for epileptics with complex partial seizures [91]—has been demonstrated to elevate the pentylenetetrazole (PTZ)-evoked seizure threshold and reduce generalized seizures in amygdala kindled rats.[92,93] Thus, GAT1 inhibition or reversal—the latter occurring either naturally or in response to drug treatment—may represent a potent neuromodulatory mechanism to terminate ongoing seizure activity (E/I imbalance) by directly increasing GABAergic transmission.

Taken altogether, the bi-directionality of GAT1-mediated GABA transport, controlled by the dynamic driving force equilibrium, underscores its ability to modify brain excitability and behavior by modulating the level of both tonic and/or phasic inhibition.

GAT2. In the adult rodent brain, GAT2-mRNA is found in leptomeningeal cells, in ependymal cells that line the ventricles, and in cells that constitute the pia and arachnoidea.[94,95] As might be expected based on these localization studies, GAT2 does not appear to influence network function, at least under physiological conditions.[55,56,96] However, GAT2 knockout mice do have a slight elevation in brain taurine levels,[55] in agreement with evidence that GAT2 expressed on blood vessels permits taurine efflux from brain to blood.[55,56,96]

BGT-1. BGT-1 levels are nearly one thousand times lower than those of GAT1.[97] This, as well as its low affinity for GABA, suggests it may lack a role in the reuptake of extracellular GABA under physiological conditions. In keeping with this contention, seizure severity of BGT1 deficient mice (both male and female) elicited by acute administration of PTZ did not differ from wild-type littermates.[97] Yet, pharmacological inhibition of BGT-1 reduced spontaneous interictal-like bursting activity recorded from brain slices taken from rats who experienced prolonged kainic acid-induced seizures.[98] Also of interest, is data demonstrating that BGT-1 is up-regulated in astrocytes of cortical and hippocampal tissue taken from human Alzheimer disease (AD) patients, thus begging the question as to whether BGT-1 might regulate neuronal excitability imbalances demonstrated to occur in AD.[99–101]

GAT3. Evidence indicates that concurrent block of both GAT1 and GAT3 in the hippocampus *in vivo* results in a synergistic enhancement of extracellular GABA levels and increased $GABA_A$ receptor tonic inhibition—suggesting that GAT3 represents an important GABA reuptake mechanism in brain.[102] In support of this assertion, rats subjected to juvenile stress have decreased GAT3 mRNA expression in hippocampus, which was experimentally demonstrated to underlie increased inhibition and reduced paired-pulse facilitation, which persisted into adulthood.[103] Interestingly, GAT3 expressed in Xenopus oocytes is inhibited by physiological levels of zinc, and immunohistochemistry studies in rat hippocampus indicate GAT3 is expressed at zinc-containing glutamatergic synapses in regions CA1 and CA3.[104] These results suggest that zinc co-released with glutamate [105,106] could serve to enhance GABAergic transmission via GAT3 inhibition. However, in at least one study, selective antagonism of GAT3 using SNAP-5114 [93] increased the excitability of neocortical interneurons, suggesting that (as discussed for GAT1) a reduction in transporter-mediated GABA release may be responsible for the reduction in GABA levels.[107] Interestingly, an increase in GAT3 mRNA is observed in the amygdala and cortex of rats following amygdala-kindling,[108] whereas GAT3 mRNA levels are decreased in the amygdala of alcohol-preferring rats as compared to controls, an effect also observed in the amygdala of alcohol-dependent humans.[109] Whether these changes reflect dynamic regulation of GABA transport (uptake or release) in efforts to restore E/I balance requires confirmation. Given that GAT3 is predominately localized to astrocyte processes surrounding symmetric (typically inhibitory) and asymmetric (typically excitatory) synapses,[50] future studies should address the cell-type specificity of all of these effects using astrocyte conditional knockout mice.

Glutamatergic neural transmission

Glutamate, the most abundant amino acid in the vertebrate nervous system, is found at concentrations three- to four-fold higher than the next three most abundant amino acids, aspartate, glutamine, and taurine.[110,111] As mentioned previously, glutamate is a substrate for GABA synthesis as well as a precursor for other intermediates of the TCA cycle. It participates in both osmotic balance and ammonia homeostasis [112–114] and is also incorporated into peptides, fatty acids, lipids, and proteins.[115] The pivotal discovery that application of glutamate to brains of rats resulted in seizure activity provided evidence for its role as an excitatory neurotransmitter.[116–118] Later work determined that glutamate fulfills the five criteria for classification as a neurotransmitter: (1) localization to nerve terminals; (2) release upon neuronal stimulation; (3) activation of cognate receptors; (4) a rapid termination mechanism; and (5) application of glutamate mimics neuronal stimulation.[110,119]

A large proportion of neuronal synapses in the CNS (≈80–90%) release glutamate [111,120] contributing to a

myriad of sensory, cognitive, and behavioral processes (for review see Hassel and Dingledine[121]). Maintenance of low basal extracellular glutamate concentrations as well as efficient release and uptake of the neurotransmitter are necessary to maintain proper balance of synaptic excitation and inhibition with imbalance leading to neurological disorders and disease states. For example, cognitive deficits associated with schizophrenia may result from glutamatergic hypofunction,[12] while disproportionate release of glutamate and/or prolonged glutamate receptor activation can lead to over-excitation and excitotoxic neuronal cell death.[122–124] Hence, it is imperative that the release of glutamate be exquisitely controlled by specific and efficient uptake, as will be discussed below.

Glutamate synthesis and packaging. Because of its inability to cross the blood–brain barrier, glutamate is synthesized primarily from glutamine (glutamate-glutamine cycle) in both neurons and astrocytes by the action of phosphate-dependent mitochondrial glutaminase (for review see McKenna[125]). An additional source of glutamate results from the transamidation of α-ketoglutarate, a key intermediate in the TCA cycle, by the enzyme glutamate dehydrogenase.[126] Once formed, 70–210 mM glutamate can be packaged into synaptic vesicles via one of three vesicular glutamate transporters ((VGLUT1, Slc17a6); (VGLUT2, Slc17a7); and (VGLUT3, Slc17a8)),[127–129] all of which rely on the vacuolar type H+-ATPase for function.[127,130]

Vesicular and non-vesicular release of glutamate. The majority of fast synaptic excitatory neurotransmission is facilitated by action potential driven, Ca^{2+}-dependent vesicular release of glutamate from neurons. However, glutamate can be released by both astrocytes and neurons by other cellular mechanisms. For instance, when the gradients of Na^+, K^+ and H^+ are disrupted across the plasma membrane, as occurs during cerebral ischemia, the Na^+-dependent excitatory amino acid transporters (EAATS) reverse, dumping glutamate into the extracellular space.[131–133] Volume-regulated anion channels (VRACs) are glutamate permeable when physiological and/or pathological swelling occurs.[134,135] Functional hemichannels in astrocytes can efflux amino acids, including glutamate.[136] This mechanism of glutamate release may occur under physiological [137–140] as well as pathophysiological [141,142] conditions. Additionally, purinergic $P2X_7$ receptors are responsible for ATP-induced glutamate release.[143] Finally, astrocytes express the protein machinery (see literature[144–147]) that would support fusion-related release of neurotransmitter [148–150] and, indeed, vesicular glutamate release has been described from astrocytes in response to neuronal activity.[151,152] There is a suggestion that this may be a significant source of extracellular glutamate during development, but not in adulthood.[153] Overall, the existence and importance of vesicular glutamate release from astrocytes *in vitro* appear incontrovertible; however, whether this occurs *in vivo* remains contested (for dual perspective reviews see Savtchouk and Volterra[154] and Fiacco and McCarthy[155]) Finally, the ambient, basal levels of extracellular glutamate that bathe the CNS are maintained by the activity of a heteromeric amino acid transporter known as system x_c, found near exclusively on astrocytes, that facilitates entry of cystine in exchange for glutamate in a one-to-one fashion.[156]

Glutamate signaling. Fast and slow excitatory synaptic transmission in the CNS occurs via ligand-gated ion channels (i.e. ionotropic (iGluRs)) and G-protein coupled (i.e. metabotropic (mGluR)) receptor subtypes, respectively (see reviews Traynelis *et al*. [157]Niswender and Conn [158]).

Cognate iGluRs, composed of four subunits that assemble as dimer pairs, were classified over 40 years ago according to the exogenous ligands that activate them, namely α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs), kainic acid receptors (kainate, KARs), and *N*-methyl-D-aspartate receptors (NMDARs) (for review see Traynelis *et al*.[157]Lodge[159]). Of interest, delta receptors have also been classified as iGluR subtypes, but this is based solely on sequence homology as neither δ1 and δ2 are gated by glutamate.[160,161] They do, however, respond to D-serine and glycine.[162]

AMPAR tetramers arise from a combination of GluA1-4 subunits.[163] Glutamate binding to AMPAR facilitates the fast opening of an ion channel pore, fluxing Na^+ in and K^+ out, which then rapidly desensitizes.[164] The editing of the GluA2 subunit (Q/R) is responsible for the impermeability of AMPARs to calcium..[165] However, AMPARs can flux calcium when the tetramer contains unedited GluA2 subunits and/or lacks GluA2 altogether.[166,167] Enriched at glutamatergic synapses, AMPARs mediate fast synaptic transmission and are a key determinate of the morphology of the dendritic spine.[168,169] Membrane trafficking of AMPARs into and out of the synapse also regulates synaptic strength and plasticity.[170]

Tetraheteromers of GluK1-5 form KARs that show prominent localization to both pre- and post-synaptic sites in the cerebellum (comprised of GluK1, 2, and 5) and hippocampus (comprised of GluK2, 3, 4, and 5).[171] Interestingly, KARs are allosterically modulated by monovalent anions and cations, which serve to stabilize the ligand binding core domain.[172,173] Similar to AMPARs, Q/R RNA editing, in this case of GluK5 and GluK6 subunits, renders the receptor impermeable to calcium.[174,175] Unlike AMPARs, KAR-mediated excitatory postsynaptic currents (EPSCs) are small, with both slow rise and decay times.[176,177] Depending on the concentration of agonist, activation of presynaptic KARs results in either synaptic facilitation or depression at excitatory CA3-CA1 or mossy fiber-CA3 synapses.[178–180] Presynaptic KAR activation can also depress GABA release in the hippocampus, presumably through a novel second messenger metabotropic signaling mechanism.[181]

Functional NMDARs are composed of a combination of two GluN1 subunits (termed the obligate receptor subunit; glycine/serine binding) and two GluN2 (GluN2A-D, glutamate binding) and/or GluN3 (GluN3A-B, glycine binding) subunits, the unique composition of which renders distinct physiological properties to each receptor

combination along with regional specificity (reviewed in Sanz-Clemente *et al*. [182]). For instance, assembly of GluN1 with GluN3, in the absence of GluN2, creates an excitatory glycine receptor located at sites distant from synaptic terminals.[183] In contrast, a significant proportion of forebrain NMDARs are triheteromers found post-synaptically and feature two GluN1 subunits together with two different GluN2 subunits (GluN2A-D), the activation of which creates the slow component of EPSCs. These NMDARs have several features that differentiate them from other iGluRs (for more detail see Glasgow *et al*.[184]) First, they function as coincidence detectors, requiring both ligand and voltage for channel opening. Second, their deactivation kinetics are slow, creating an opportunity for temporal integration of synaptic activity.[185,186] Third, they show remarkable calcium permeability, thus making them essential players in both Hebbian and homeostatic types of plasticity.[187,188]

Metabotropic glutamate receptors (mGluRs), eight in total, do not flux ions but instead are coupled to G-proteins that possess seven transmembrane spanning regions, the activation of which initiates distinct intracellular signaling cascades that result in diverse cellular and electrophysiological effects (for reviews see literature[158,189,190]). mGluRs are divided into three groups (Group I, II, and III) based on amino acid sequence homology and the intracellular second messenger cascade that they initiate. Coupled to phospholipase C, Group I receptors (mGluR1 and 5) hydrolyze phosphoinositol, mobilize calcium, and facilitate protein phosphorylation. Negatively coupled to adenylate cyclase, Group II (mGluR 2 and 3) and Group III (mGluR4 and mGluR6-8) receptors decrease cyclic AMP production and ultimately protein phosphorylation. Activation of mGluRs in the CNS has diverse functional outcomes ranging from activation or inhibition of K^+ and Ca^{2+} channels, potentiation and inhibition of AMPA and NMDA receptor-mediated responses, and/or presynaptic facilitation or inhibition of neurotransmitter release.[189,190]

Glutamate uptake. Glutamate is not broken down in the extracellular space, and as such excitatory amino acid transporters (EAATs) are responsible for termination of glutamate signaling by its removal from the synapse following release.[191] EAAT1-5 are members of the Slc1 family of transporters.[192] Removal of glutamate by EAATs is said to be electrogenic as one K^+ ion is transported out of the cell each time a glutamate anion and three Na^+ ions are transported in.[131,193–197] Uptake via EAATs is also associated with an uncoupled Cl^- gradient,[198–200] which may also contribute to a reduction in excitability.

Found throughout the brain, EAAT1 (GLAST) [201,202] is localized exclusively on astrocytes.[203] EAAT2 (GLT-1a,b) [204] is also predominately an astrocyte protein although a minor proportion of GLT-1a can be found on certain axon terminals.[205–208] EAAT3 (EAAC1) [209,210] is localized to neuronal somata and dendrites.[211,212] EAAT4 localizes to cerebellar Purkinje cells,[213,214] but also astrocytes,[215] while EAAT5 expression appears to be limited to photoreceptors and bipolar cells of the retina.[216] In the CNS, the majority of extracellular glutamate clearance is performed by EAAT2[217–219.]

Role of glutamate transport in maintenance of E/I balance

Excitatory amino acid transporter 1. In humans, a heterozygous mutation in EAAT1 phenocopies with reductions in glutamate uptake that likely contributes to neuronal hyperexcitability resulting in episodic ataxia and, depending upon the extent of the reduction, seizures.[200,220,221] In mice, loss of EAAT1 does not result in spontaneous seizure generation, but the duration of seizures elicited by electrical stimulation of the amygdala is significantly prolonged, whereas the latency to seizure induced via systemic administration of PTZ is shortened and the seizures themselves more severe.[222] EAAT1 null mice also show locomotor hyperactivity when placed in a novel open field.[223] Additional studies with this mouse demonstrate abnormalities on behavioral symptoms (positive, negative, and attentional/cognitive symptoms) associated with the developmental disorder schizophrenia,[224] which arises from alterations in E/I balance.[7]

Excitatory amino acid transporter 2. Consistent with its outsized role in synaptic glutamate uptake, mice with a genetic deletion of astrocyte (but not neuronal) EAAT2 demonstrate excessive synaptic glutamate, which precipitates spontaneous seizures that are lethal by three–six weeks of age.[225,226] Of interest, Amara *et al.* determined that *Eaat2* (*Slc1a2)* is located on mouse chromosome 2 near quantitative trait loci shown to modulate seizure frequency in mouse models of epilepsy and alcohol withdrawal.[227] In humans, glutamate levels are increased in interictal epileptogenic foci,[228] leading to the speculation that clearance is impaired. In keeping with this idea, protein expression of EAAT2 (and EAAT1) in the CA1 hippocampus of patients with temporal lobe epilepsy was reduced by 25% (and 40%), respectively.[229] Reduction of EAAT2 protein expression at human neocortical epileptic foci has also been described.[230] These reductions could be caused by the production of alternative EAAT2 mRNA splice variants.[231] Finally, seizure control in both mouse and monkey models of epilepsy was achieved via strategies that upregulate EAAT2 expression.[232,233] Upregulation of EAAT2 expression was also shown to attenuate alcohol consumption in male alcohol preferring rats,[234] contributing to the idea that glutamate transport might be a target for treatment of alcohol dependence.[235]

Very recently, it was demonstrated that chemically elicited cortical spreading depression (CSD), a pathological neural depolarization that underlies migraine pathophysiology [236–238] as well as secondary neuronal damage and infarct expansion following cerebral ischemia,[239,240] occurs with increased frequency and velocity in EAAT2 astrocyte conditional knock-out mice.[241] In contrast, the germ-line EAAT1 and EAAT3 null mutants show no such effect.[241]

Excitatory amino acid transporter 3. Although approximately 100-fold less abundant than EAAT2 [246],

EAAT3/EAAC1, primarily found on dendrites and soma of hippocampal neurons,[212] is known to regulate the duration of glutamate in the synaptic space that immediately surrounds active terminals. This prevents glutamate spillover to extrasynaptic regions,[242,243] which are morphologically defined as receptors lying more than 100 nm from the postsynaptic density.[244] Accordingly, the slower component of CA1 pyramidal cell glutamatergic EPSCs is enhanced when EAAT3/EAAC1 [242] is absent and extrasynaptic NMDA receptors are activated in EAAT3 knockout mice.[245] This latter result could, at best, result in modulation of synaptic activity[246] and, at worse, contribute to neurodegeneration.[247,248] Interestingly, a reduction in both EAAT3 message and protein was found in human neocortical tissue taken from epileptic foci in comparison to non-epileptic regions of similar neuronal density using quantitative real-time PCR and immunoblotting, respectively.[230] Thus, it may not be surprising that chronic antisense oligonucleotide treatment against EAAT3/EAAC1 in rats resulted in behavioral episodes of staring/freezing that correlated with electroencephalogram changes manifest by short runs of rhythmic spikes.[249,250] The physiological basis of EAAT3/EAAC1 antisense oligonucleotide epileptogenesis was attributed, in part, to hippocampal GABA synthesis reduction.[250] However, these phenotypes were not recapitulated in an EAAT3/EAAC1 null mice.[251] Additionally, EAAT3 mRNA and protein expression are enhanced (not reduced) by approximately 3-fold in granule cells of the dentate gyrus of pilocarpine-treated rats that seize spontaneously as compared to control rats.[252] EAAT3 message is also higher in granule cells of the dentate gyrus taken from human patients with temporal lobe epilepsy.[252] Whether this increase represents a compensatory change to increase glutamate clearance or enhance GABA production/activity,[250,253] or is merely an epiphenomenon, remains to be definitively determined.

With respect to neuropsychiatric disorders, a functionally relevant deletion of *Slc1a1*, which encodes for EAAT3/EAAC1 has been shown to co-segregate with psychotic disorders (e.g. bipolar disorder and schizophrenia) in an extended 5-generation pedigree.[254] Furthermore, mice with EAAT3/EAAC1 haploinsufficiency show biochemical, behavioral, and histological changes that reflect an altered redox state, congruous with changes found in patients with schizophrenia.[255] Finally, genetic linkage and association studies performed in obsessive convulsive disorder (OCD), itself linked with cortical excitability abnormalities,[256] point to gene variants in *Slc1a1* (for review see Escobar *et al.* [257]). While mice null for EAAT3 do not show behaviors consistent with OCD, overexpression of EAAT3 in forebrain neurons alone does result in anxiety-like and repetitive behaviors, which are also often reported in persons diagnosed with OCD.[245]

Excitatory amino acid transporter 4. Soma and dendrites of cerebellar Purkinje neurons show prominent expression of EAAT4, although it is not restricted to these cells.[258–260] While EAAT4 shows high affinity for glutamate,[258,261] it takes up just 10% or less of released glutamate at climbing fiber (CF) synapses;[262] the balance is removed by Bergmann glial cells via EAAT1.[263,264] Consistent with this notion, no Purkinje cell death followed 5 min global ischemia in mice null for EAAT4, whereas significant loss occurred in mice lacking EAAT1.[265] Supporting the idea that EAAT4 transporters prevent glutamate spillover to adjacent synapses, a pronounced tail current (seconds in length) appears during the decay phase, the initial kinetics of which is not different, of both CF- and parallel fiber (PF)-EPSCs when evoked in slices derived from mice deficient in EAAT4.[266]

Excitatory amino acid transporter 5. EAAT5 is localized near exclusively to the retina (see Dalet *et al.*[260]) with expression in the synaptic terminals of photoreceptors and rod bipolar cells.[216,267] EAAT5 exhibits two distinct properties, acting both as a rather ineffective, low-affinity and low-capacity glutamate transporter and as a glutamate-gated inhibitory "receptor".[267–269] This EAAT5-mediated anion channel, optimized for conduction in the negative voltage range,[270] is postulated to reduce excitability of neurons by maintaining membrane potential at its optimum.

Electrogenecity and anions. Electrophysiological measurement of tonic NMDAR activity in acute brain slice has informed our understanding of the amount of ambient glutamate in the extracellular space, which has been reported to range from 25 to 90nM.[271–275] Studies using *in vivo* microdialysis report higher concentrations (0.2–35 μM),[276–279] which could be due to tissue damage inflicted by the sampling probe.[280] These low values are maintained despite the fact that intracellular concentration of glutamate ranges from high μM to mM concentrations in astrocytes and neurons.[128,129,281] This is because glutamate transport is electrogenic,[131,193–197] allowing for efficient uptake of glutamate against this concentration gradient. The ion-coupled substrate transport current generated by each EAAT subtype varies with the bioenergetics tightly controlling the rate and amount of glutamate removed (for detailed review see Divito and Underhill[282]). Glutamate uptake creates a chloride flux (anion channel) that is thermodynamically uncoupled to transport but is generated when Na^+ ions and/or glutamate bind to the transporter.[198,199,283] For a detailed review of the molecular transport mechanisms see Grewer *et al.*[284] EAAT1, EAAT2, and EAAT3 produce smaller anion currents as compared to EAAT4 and EAAT5, both of which show large chloride conductance.[216,283,285] It has been suggested that this chloride conductance shapes excitatory signaling by counterbalancing the entry of positive charges that occurs along with glutamate influx, thereby preventing depolarization of the cell.[286] Additionally, it could serve to clamp the membrane potential at negative values, further inhibiting glutamate release and/or supporting electrogenic glutamate uptake by favoring Na^+ entry.[285] Most interestingly, the anion current could effectively function as glutamate-dependent inhibitory receptor, thereby directly counteracting glutamate's excitatory effects.[284,287]

System x_c^-. System x_c^- (Sx_c^-)—described by Bannai and Kitamura in 1980—is a Na^+-independent, Cl^- -dependent, heteromeric amino acid transporter that functions physiologically to import L-cystine in exchange for L-glutamate in a 1:1 ratio.[156,288] Transport is electroneutral and limited to amino acids in their anionic forms. While Sx_c^- is expressed in cultured microglia,[289,290] neurons,[290–293] HT22 neuronal cell line,[294] rodent astrocytes, [290,295] and human glioma cell lines,[292,295–299] astrocytes appear to be the main cell type expressing Sx_c^- in the mature brain *in vivo*. To wit: Pow *et al.* demonstrated via immunocytochemical analyses that α-aminoadipate, a substrate inhibitor of Sx_c^-, was absent from neurons and oligodendrocytes but accumulated in astrocytes, radial, and Bergman glia.[299] Transcriptome analysis of parenchymal cells from mouse and human cortex, revealed enrichment of the transcript for the substrate specific light chain of Sx_c^- (xCT encoded by *Slc7a11*) in astrocytes when compared to neurons, microglia, endothelial cells, and other cell types.[144,300] Finally, immunohistochemical analysis for xCT in adult mouse brain showed that Sx_c^- is expressed in a subset of astrocytes but not in neurons, microglia, or oligodendrocytes.[301] Labeling of xCT was found in most brain regions including the molecular layer and the stratum lacumosum moleculare of the hippocampus, the striatum, the hypothalamus, the thalamus, and the cortex; it was also concentrated a the blood/brain/cerebral spinal fluid barriers.[301]

Apart from transporting cystine into cells, a process important for cellular redox balance, export of glutamate by Sx_c^-—estimated to be 0.6 μM/s [302]—contributes to/maintains basal extracellular glutamate concentrations,[276,279,303–309] which itself contributes importantly to brain E/I balance. [273,274,303,308,310–318] Numerous studies demonstrate that Sx_c^- -derived extracellular glutamate, specifically, is important for maintaining balanced transmission. For example, increased glutamate receptor clustering and excitatory junction potentials occurred in association with reductions in glutamate at the neuromuscular junction of the *Drosophila melanogaster* mutant for Sx_c^-, an effect phenocopied by bathing larvae in low glutamate concentrations.[308] Findings that Sx_c^- -derived glutamate is important for maintenance of synaptic strength were also reported in mouse CA1 hippocampus taken from male mice mutant for $xCT^{-/-}$. Specifically, AMPAR immunoreactivity was enhanced as was both spontaneous and evoked excitatory currents, effects phenocopied by maintaining slices in glutamate-free bathing solution and/or by incubation with a pharmacological inhibitor of Sx_c^-.[311] Given this, it is somewhat surprising that a higher dose of the chemoconvulsant pilocarpine or kainic acid, provided via intravenous infusion, is needed to precipitate behavioral seizures in transgenic $xCT^{-/-}$ mice when compared to wild-type control mice.[276] Also surprising is that following a single intraperitoneal (i.p.) dose of NMDA, latency to convulsive seizure is increased and incidence of mortality is reduced in a cohort of $xCT^{-/-}$ mice.[276] This contrasts with our own findings, which are in keeping with the electrophysiological results described above, that demonstrate a reduction in convulsive seizure threshold of *Slc7a11*$^{sut/sut}$, as compared to *Slc7a11*$^{+/+}$ littermates, in response to a single dose of the chemoconvulsant PTZ or kainic acid delivered i.p. (manuscript in review).

A deleterious role for aberrant Sx_c^- expression is demonstrated by the following few studies. A rapid increase in Sx_c^- activity, demonstrated by positron emission tomography, in rat brain followed a focal cerebral ischemic insult induced by transient occlusion of the middle cerebral artery.[319] In this same study, cell death and neuronal currents induced by oxygen–glucose deprivation in slice and slice culture—so called anoxic depolarizations—were both reduced by block of Sx_c^-.[319] Previously, we found that when astrocyte activity of Sx_c^- is enhanced, glutamate-mediated excitotoxic neuronal death during simulated ischemia is also increased.[290,320,321] Finally, both glutamate concentrations and Sx_c^- levels are enhanced in glioma tissue,[296,322] with evidence in humans showing that enhanced xCT expression in tumors positively correlates with degree of tumor invasion and with shortened survival.[323] Moreover, pharmacological inhibition of Sx_c^- reduces seizure frequency in glioma-bearing mice and peritumoral glutamate levels in human patients.[324,325] Increased xCT expression was found in post-mortem samples of dorsolateral prefrontal cortex of patients diagnosed with schizophrenia,[326] although the significance to disease pathogenesis or symptomology remains to be determined. Finally, reduced activity of Sx_c^- in the nucleus accumbens of rats followed repeated cocaine exposure was demonstrated convincingly to be associated with pathological changes in extracellular glutamate levels as well as their compulsive drug seeking behavior.[278,305,327–329] Likewise, xCT levels were reduced in nucleus accumbens and the ventral tegmental area of rats self-administering nicotine.[330] In this same study, human smokers treated with N-acetyl-cysteine, a cysteine prodrug that activates Sx_c^-, reported they smoked fewer cigarettes.[330]

With respect to more conventional behaviors, mice lacking Sx_c^- (both transgenic $xCT^{-/-}$ as well as *Slc7a11*$^{sut/sut}$ mice) show reduced alternations in the three arm spontaneous alternation task, indicating a deficit in spatial working memory.[276,331] Additionally, impaired functioning in both amygdala and hippocampal-dependent fear conditioning tasks, as well as in a hippocampal-dependent passive avoidance tasks, representing aberrant learning and/or memory, has been reported in male *Slc7a11*$^{sut/sut}$ mice.[332] Notably, CA1-Schaeffer collateral cellular long-term potentiation—a synaptic mechanism thought to underlie learning and memory–is reduced in these same mice.[332] Other studies demonstrate that physiological Sx_c^- signaling influences behavioral anxiety and despair. For instance, as compared to wild-type, male transgenic mice null for xCT occupy the illuminated portion of the light/dark box and spend an increased amount of time in the open during the open field test.[333] As compared to wild-type control mice, they also show reduced immobility and enhanced climbing behaviors in the tail suspension and forced swim tests.[333]

Overall, evidence across multiple species using different paradigms indicate that physiological Sx_c^- activity contributes importantly to the maintenance of E/I balance in brain.

Conclusions

Stable global neuronal activity is achieved in forebrain by a coordinated and dynamically regulated balance between excitatory (chiefly glutamatergic) and inhibitory (chiefly GABAergic) inputs. This coordination is essential for the normal functioning of most complex brain processes, with imbalances contributing to the pathobiology of neurodevelopmental disorders, neurodegenerative/neurological disease, as well as, acute neurological disorders. Most studies researching E/I imbalance focus on neurotransmitter levels and/or concentrate on receptor signaling. However, the capacity of glutamate and GABA transporters to both modulate release and uptake of neurotransmitter, as well as neural network activity, in a cell-type specific manner underscores their important contribution to maintenance of physiological balance. Hence, a comprehensive understanding of how these transporters work normally and how their physiological function may be altered under pathophysiological conditions are the first steps to identifying novel therapeutic avenues and targets to prevent or mitigate imbalance.

AUTHORS' CONTRIBUTIONS

SMSS and SJH both contributed equally to the writing of this review.

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