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# Glutamate and GABA Imbalance Following Traumatic Brain Injury

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**Abstract** Traumatic brain injury (TBI) leads to multiple short- and long-term changes in neuronal circuits that ultimately conclude with an imbalance of cortical excitation and inhibition. Changes in neurotransmitter concentrations, receptor populations, and specific cell survival are important contributing factors. Many of these changes occur gradually, which may explain the vulnerability of the brain to multiple mild impacts, alterations in neuroplasticity, and delays in the presentation of posttraumatic epilepsy. In this review, we provide an overview of normal glutamate and GABA homeostasis and describe acute, subacute, and chronic changes that follow injury. We conclude by highlighting opportunities for therapeutic interventions in this paradigm.

**Keywords** Posttraumatic epilepsy · Parvalbumin interneuron · Glutamate transporter · NMDA receptor

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## Introduction

Advances in translational neuroscience research, coupled with increased attention from the medical community, have provided valuable insight into the mechanisms by which traumatic brain injury (TBI) leads to posttraumatic neurologic symptoms such as motor and cognitive deficits and posttraumatic epilepsy. While posttraumatic pathophysiology remains incompletely understood, much of the morbidity, particularly after moderate to severe TBI, may be referable to a pathologic shift toward excess excitability of the normal cortical excitation:inhibition ratio. Accordingly, the present review focuses on an overview of the major cortical excitation and inhibition mechanisms and relevant pathologic changes that follow TBI in humans and in experimental models.

Glutamate is the primary excitatory neurotransmitter in the brain, while $\gamma$-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter. The balance of glutamatergic and GABAergic tone is crucial to normal neurologic function. The role of glutamate signaling in TBI pathophysiology is twofold. On one hand, acute posttraumatic glutamate release is responsible for excitotoxicity following brain injury that leads to neuronal injury, cell death, and dysfunction of surviving neurons; on the other hand, delayed disruption of excitatory glutamate circuits leads to deficits in cognitive and motor function, and in experience-dependent plasticity.

Pyramidal neurons, located in the cortex and hippocampus of mammals, as well as neurons of the midbrain,

hypothalamus, and cerebellum produce glutamate that is central to excitatory signaling pathways [1]. Alternatively, GABA is produced in interneurons that modulate cortical and thalamocortical circuits that relay sensory information and play a role in coordinating motor functions, attention, and memory [2, 3].

GABA modulates excitatory pathways in the brain and, following injury, loss of GABA-producing cells disrupts the balance of excitation and inhibition leading to further cell injury and apoptosis. The results of glutamate excitotoxicity share common elements from mild [4] to severe TBI, as well as status epilepticus, ischemia, and neurodegenerative diseases [5]. In addition to this imbalance in excitation and inhibition, traumatic neuronal injuries may lead to cell damage via mitochondrial dysfunction, axonal shearing, oxidative stress, and abnormalities in cerebral vasculature that are beyond the scope of this review.

In this review, we discuss the effect of TBI on cortical glutamate and GABA balance, with particular focus on posttraumatic epilepsy (PTE) as it provides a clear, objective endpoint following TBI and has a number of well-characterized animal models. Severe TBI leads to PTE in approximately 20 % of the civilian population after closed head injury and 50 % in war veterans with penetrating head wounds [6–8]. It is the most frequent cause of acquired epilepsy in young adults [9]. Notably, PTE does not immediately follow TBI. Rather, PTE follows a latent period of epileptogenesis [10] that follows acute posttraumatic glutamate release [11, 12, 13••, 14] and leads to immediate and delayed neuronal death and dysfunction [15, 16]. This latent period provides the ideal setting to investigate the cellular dysfunction and plasticity that follow TBI and is a unique opportunity to test neuroprotective or antiepileptogenic agents.

We begin with a review of normal glutamate and GABA signaling and homeostasis. We follow with the pathophysiology of TBI beginning acutely and then injury progression over time, including subacute changes in receptors and chronic changes in cellular networks. We conclude with opportunities for neuroprotective intervention.

## Normal Glutamate and GABA Balance

### Glutamate Homeostasis

Glutamate has been appreciated as important in metabolic brain processes for over 60 years [17]. Glutamate is synthesized from glutamine in presynaptic glutamatergic neurons. It is then stored in presynaptic vesicles. A depolarizing current prompts entry of calcium into the presynaptic cell via voltage-gated channels, triggering an intracellular calcium sensor that leads to vesicular release into the synaptic cleft [18]. Following release of glutamate and its action mainly on postsynaptic receptors, it is taken up by astrocytes, largely by excitatory amino acid transporter (EAAT2) in humans or glutamate transporter-1 (GLT-1) in rodents [19, 20]. In astrocytes, glutamate is converted to glutamine by glutamine synthase and shuttled back to the presynaptic neuron [21] (see Fig. 1a).

### Glutamate Receptors and Physiology

Glutamate acts on either ionotropic receptors enabling ion passage into the cell or metabotropic G-protein coupled receptors, both of which prompt an intracellular signaling cascade. The ionotropic receptors include *N*-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainate. The NMDA receptor (NMDAR) has been the most extensively studied due to its role in use-dependent synaptic plasticity, particularly long-term potentiation [22] and its potential contribution to diseases such as schizophrenia and Alzheimer's disease [2, 23, 24]. Of note, this review highlights observed receptor and subunit changes following TBI (see Table 1), and is not meant to be a comprehensive discussion of subunits and receptor physiology.

The NMDAR is a tetramer of combinations of its NR1, NR2, and NR3 subunits [25]. NR1, NR2A, and NR2B subunits have received the most attention in TBI research. The NR1 subunit binds glycine, an inhibitory neurotransmitter and is responsible for receptor deactivation [25]. NR2A-containing NMDARs are often colocalized with NR1, predominantly at the synapse, and activation of these receptors strengthens synapses and induces proplasticity signals, such as pErk, pCREB, and BDNF [26, 27]. Conversely, NR2B-containing NMDARs are localized extrasynaptically, and their activation results in signals more detrimental to the cell. Activation of NR2B results in prolonged calcium influx that appears to be more specifically taken up by mitochondria, leading to subsequent mitochondrial dysfunction and increased activity of caspases, all of which promote cell damage and demise [28–30]. Finally, NR2B-containing neurons are more susceptible to mechanical injury versus NR2A neurons and a greater proportion of NR2B neurons leads to impaired neuronal connectivity and plasticity [31••, 32]. Shifts in these NMDAR subunits have important ontogenic and functional consequences. During normal circuit development, whether it be in the brain in vivo, hippocampal slices or neuronal cell culture, immature NMDARs are predominantly NR2B-containing and, with maturation, there is increasing expression of NR2A-containing NMDARs [33]. As we will see later, TBI also influences the expression of these subunits.

Typically ionotropic receptors require binding of the neurotransmitter alone to open the ion channel. The NMDA channel, however, has the unique property of requiring binding of glutamate, as well as postsynaptic membrane depolarization, often by neighboring AMPA channels, to prompt ion influx. It

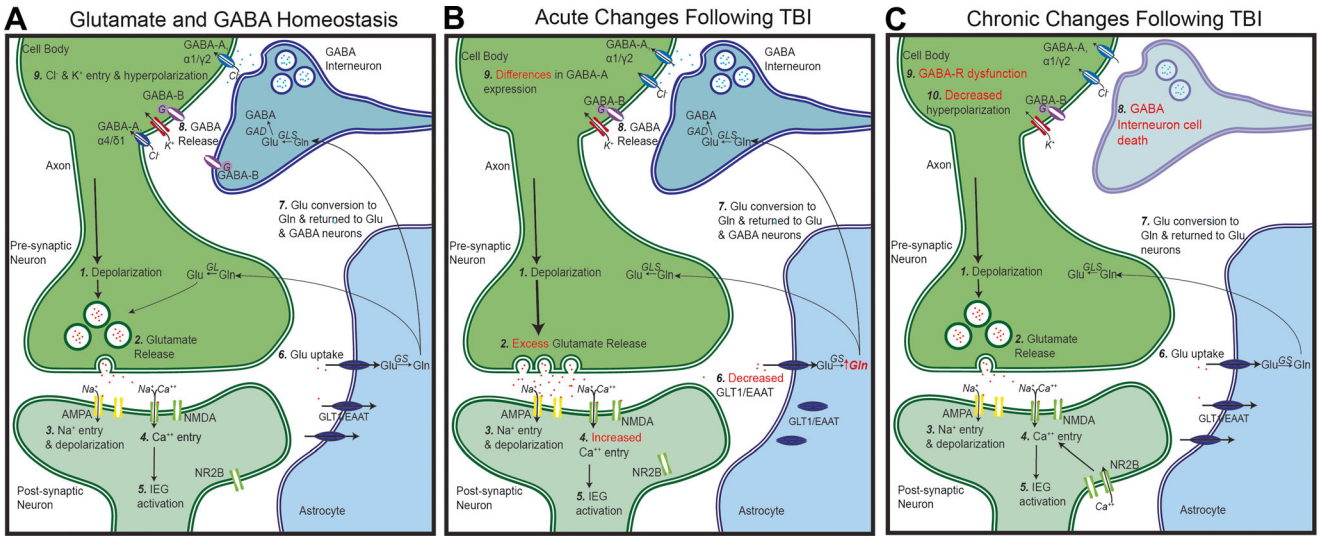


**Fig. 1** Summary of glutamate and GABA homeostasis and changes following traumatic brain injury. The figure illustrates a schematic relationship of a glutamatergic synapse between pyramidal neurons (*green neurons*), an astrocyte (*bottom right*), and an inhibitory GABAergic synapse between an interneuron (*blue neuron in the top right*) and, in this case, the cell body of the pyramidal cell. **a**. Baseline homeostatic relationship of glutamate and GABA begins with (*1*) a depolarizing current traveling down a pyramidal cell. (*2*) This is followed by $Ca^{++}$-mediated release of glutamate from the presynaptic neuron and action on local AMPA and NMDA receptors. (*3*) $Na^{+}$ enters the cell triggering depolarization, (*4*) followed by $Ca^{++}$ via NMDA receptors. (*5*) There is subsequently immediate early gene (*IEG*) activation. (*6*) Glutamate is taken up by the GLT-1/EAAT transporter on nearby astrocytes. (*7*) Glutamate is converted to glutamine by glutamine synthase (*GS*) and shuttled back to the presynaptic cell and nearby interneurons for conversion to GABA via glutaminase (*GLS*) and then glutamate decarboxylase (*GAD*). (*8*) GABA is released from local interneurons and acts on GABA-A and GABA-B receptors and is taken back up by GAT-1. (*9*) $Cl^{-}$ and $K^{+}$ enter the presynaptic pyramidal cell restoring the cell membrane to its resting state. **b**. Acutely following TBI, there is (*1*) rapid depolarization of the pyramidal cell and increased $Ca^{++}$ entry into the presynaptic cell prompting (*2*) increased glutamate release into the synaptic cleft. Glutamate then acts on AMPA and NMDA receptors, as there are local changes that attempt to compensate for the increased glutamate, e.g., downregulation of NMDA subunits. (*3*) $Na^{+}$ enters the cell triggering depolarization, (*4*) followed by increased $Ca^{++}$ via NMDA receptors and (*5*) increased IEG activation. (*6*) Less glutamate is removed from the synapse given decreased expression of GLT-1/EAAT transporter on astrocytes. (*7*) Glutamate that is taken up is rapidly converted to glutamine and recycled. (*8*) GABA is released from local interneurons; however, (*9*) changes in GABA-A subunit expression lead to changes in the phasic inhibition of the presynaptic pyramidal cell and deficits in membrane repolarization. **c**. Chronically following TBI, there is (*1*) depolarization of the pyramidal cell and (*2*) glutamate release into the synaptic cleft, which acts on AMPA and NMDA receptors, now with different expression of receptor subunits, e.g., NR2A shifts to NR2B. (*3*) $Na^{+}$ enters the cell triggering depolarization, (*4*) followed by $Ca^{++}$ via NMDA receptors and (*5*) IEG activation. (*6*) Glutamate is taken up by the GLT-1/EAAT transporter on nearby astrocytes and (*7*) converted to glutamine. (*8*) There is GABA interneuron cell death and (*9*) persistent GABA-A receptor dysfunction that leads to (*10*) less hyperpolarization and a hyperexcitable state of the presynaptic cell. *Glu* glutamate; *gln* glycine; *$Cl^{-}$* chloride; *$K^{+}$* potassium; *$Na^{+}$* sodium; *$Ca^{++}$* calcium; *IEG* immediate early gene; *NR2B* NMDA receptor subunit 2B; *GLT/EAAT* glutamate transporter, excitatory amino acid transporter; *GS* glutamine synthase; *GLS* glutaminase; *GAD* glutamate decarboxylase; *GAT-1* GABA transporter

functions, in effect, as a coincidence detector, linking presynaptic and postsynaptic activation. At rest, the NMDAR's ion channel pore is blocked by a $Mg^{++}$ ion, which is removed by postsynaptic depolarization, rendering it permeable to both $Na^{+}$ and $Ca^{++}$ [2]. This is essential for normal neurotransmission, but has implications for excitoxicity following TBI. If excess glutamate is released, then the cell membrane remains depolarized leading to increased $Ca^{++}$ entry into the postsynaptic cell.

The AMPA receptor (AMPAR) is also formed by a tetramer of subunits, including GluR1-4. The GluR1 and GluR2 subunits will be focused on here. The GluR1 subunit, located synaptically, makes the AMPAR permeable to $Na^{+}$ and $Ca^{++}$ and has been implicated in synaptic plasticity and learning and memory [34, 35]. GluR1 is regulated by phosphorylation that may be driven by NMDAR binding, including activation of calcium/calmodulin-dependent protein kinases (CaMKs), specifically CaMKII. The selective activation of specific CaMKs is important for long term-potentiation and normal memory function; however, indiscriminate activation can lead to impairments [2, 34, 36]. The GluR2 subunit is also synaptic; however, it makes the AMPAR impermeable to calcium [37] and, thus, is protective against the pro-apoptotic effects of increased $Ca^{++}$. Phosphorylation of GluR2 by protein kinase C (PKC) leads to endocytosis of the GluR2 subunit and impaired synaptic plasticity [38] (see Table 1).

## GABA Homeostasis

GABA is the primary inhibitory neurotransmitter of the central nervous system and its release from interneurons acts to modulate excitatory neurotransmission. Glutamine in

**Table 1** NMDA, AMPA, and GABA receptors and subunits relevant in TBI

| Receptor | Subtype | Localization | Function | Expression and/or function after TBI |
|---|---|---|---|---|
| NMDA [25–30, 31••, 32, 33] | NR1 | Synaptic | Enables receptor deactivation via glycine binding and particular localization with NR2 may lead to glycine-dependent desensitization | Acute: decreased<br>Chronic: increased |
| | NR2A | Synaptic | Enhances excitatory synaptic strength through colocalization with NR1 and protects NR2B-mediated $Ca^{++}$ influx; NR2A-containing NMDARs respond to high-frequency stimulation to initiate LTP | Acute: decreased<br>Chronic: decreased |
| | NR2B | Predominantly extrasynaptic | Enables prolonged $Ca^{++}$ influx; NR2B-containing NMDARs respond to low-frequency stimulation to initiate LTD | Acute: decreased<br>Chronic: increased |
| AMPA [2, 34–38, 69] | GluR1 | Synaptic | Enables $Na^{+}$ and $Ca^{++}$ permeability; phosphorylated and upregulated in LTP | Acute: increased<br>Chronic: decreased |
| | GluR2 | Synaptic | Limits $Ca^{++}$ permeability | Acute: decreased<br>Chronic: unknown |
| GABA-A [2, 29, 30, 31••, 32–43] | α1/γ2 | Synaptic | Enables phasic inhibition; responds to higher and quicker GABA concentration changes | Acute and chronic: decreased |
| | α4/δ1 | Extrasynaptic | Enables tonic inhibition; responds to lower and slower GABA concentration change | Acute and chronic: increased |
| GABA-B [2, 21, 39, 82•] | | Extrasynaptic and presynaptic | Hyperpolarizes membrane via opening of $K^{+}$ channels | Acute and chronic: increased |

Representation of receptor subtypes, commonly accepted localization and function, and general consequences following TBI based on the available literature. $Ca^{++}$ influx into the postsynaptic cell leads to mitochondrial dysfunction and increased caspases and CaMK activation. The extent of these receptor and subunit changes likely differs by injury severity and brain region, which is not represented here

*LTD* long-term depression, *LTP* long-term potentiation

astrocytes is transported to GABAergic neurons to be converted to glutamate and then immediately to GABA by glutamate decarboxylase (GAD). Glutamate, glutamine, and GABA rely on intermediaries from the tricarboxylic acid (TCA) cycle; therefore, deficits and inefficiencies in cellular energy metabolism, such as those that follow compromised tissue perfusion and increased neuronal metabolic demand after TBI, also lead to deficits in transmitter production [21]. GABA is stored in presynaptic vesicles and is released onto postsynaptic terminals that may be located on the dendritic projections, the cell body, axon, or another axon terminal [2]. Following the release and its postsynaptic actions, the majority of GABA is taken back up by its transporter, GAT-1 into the presynaptic neuron. It is then recycled back into presynaptic vesicles for subsequent release (see Fig. 1a).

## GABA Receptors and Physiology

Following release, GABA acts on GABA-A and GABA-B receptors. GABA-A receptors are postsynaptic ionotropic receptors that cause the opening of $Cl^{-}$ channels and lead to hyperpolarization of the postsynaptic cell. GABA-A receptors may be either synaptic or extrasynaptic. GABA-B receptors are metabotropic, G-protein-coupled receptors that act via a second messenger cascade. GABA-B receptors may be postsynaptic or presynaptic and lead to the opening of $K^{+}$ channels, which in the presynaptic terminal limits GABA release. Postsynaptically, $K^{+}$ leads to even more pronounced hyperpolarization than $Cl^{-}$, lasting longer than the action of GABA-A receptors [2, 21, 39].

The GABA-A receptor contains at least 16 subunits. Different subtype combinations lead to different physiologic characteristics and pharmacologic profiles. The combination of these subtypes relies on colocalization on the neuronal membrane [40]. The GABA-A subunits that have received the most attention in TBI research are α1, γ2, α4, and δ1. These subunits contribute to GABA interneurons' ability to modulate neuronal signals via phasic and tonic inhibition. These two types of inhibition have unique functions. Phasic inhibition decreases the hyperexcitability of the postsynaptic cell and plays an important role in the creation and modulation of theta and gamma oscillations. Alternatively, tonic inhibition provides more constant maintenance of the amount and duration of the postsynaptic depolarization [41]. This inhibition may contribute to rhythmic oscillations that act to time and synchronize the excitatory impulses [2, 42]. Phasic and tonic inhibitions are dependent on the amount and speed of GABA release as well as the differential action of postsynaptic receptors. The GABA-A receptor subunits involved in phasic inhibition are α1 and γ2, while tonic inhibition involves α4 and δ1 subunits [43]. When GABA is released rapidly from presynaptic vesicles, it diffuses quickly across the synaptic space and acts on α1 and γ2 containing GABA-A receptors leading to phasic inhibition. Alternatively, lower concentrations of

ambient or released GABA act on extrasynaptic receptors containing α4 and δ1 subunits. These lower, more constant concentrations lead to tonic inhibition [40, 43] (see Table 1).

The phasic action of GABA inhibitory signals creates a network of competing rhythmic oscillations that aid in the timing and synchronization of neuronal signals. The paravalbumin positive, fast-spiking GABAergic interneurons, which approximate 40 % of the total cortical GABAergic interneuron population, are key to this process [44]. This GABAergic interneuron subtype creates gamma frequency oscillations that are felt to enhance information processing through an improved signal to noise ratio [45]. Therefore, damage to specific receptor subtypes or cell populations may affect the modulation of cell signaling, leading to abnormal synchronization and decreased inhibitory tone that render the cortex vulnerable to seizures.

The notion that glutamate and GABA dysregulation leads to excitotoxicity is not a new concept [46]. Excitotoxicity initiates a metabolic cascade following both mild and severe TBI that may ultimately contribute to the neurologic sequelae that follow injury [4, 47]. Depending on the severity of injury, a patient may recover fully or be left with impairments ranging from subtle neurocognitive difficulties to posttraumatic epilepsy. Glutamate and GABA are central to this pathophysiology and the complicated dynamics of transmitter release, receptors, and neurophysiology that follow TBI are addressed here.

## Acute Consequences of TBI

### Glutamate and GABA Changes

Following focal traumatic brain injury, such as a focal contusion or penetrating injury there is direct tissue damage at the site of injury that may cause local swelling, ischemia and, or hemorrhage. The area of focal injury may be irreversibly damaged and the lesion expands as excitotoxic injury spreads through the surrounding tissue, analogous to the penumbra following ischemia. Following many mechanisms of TBI, including concussion, there is a more diffuse injury that impacts widespread areas of the brain, including axonal injury. At a molecular level, there is membrane swelling and potentially rupture, neurotransmitter and ion release into the extracellular space, and $Na^+/K^+$ pump failure. With indiscriminate glutamate release, there is rising intracellular $Ca^{++}$ that perpetuates neuronal injury [48, 49].

Microdialysis studies in both humans [12, 48, 50] and rodents [14] demonstrate an immediate rise in extracellular glutamate following severe TBI. In humans, Chamoun and colleagues demonstrated that this rise in extracellular glutamate, recorded from 24 h after injury, lasts for as long as 4 days and is directly proportional to posttraumatic mortality [12]. In a rodent model, using controlled cortical impact (CCI), microdialysis studies have demonstrated increased extracellular glutamate at 1 h after injury [14], although the initial peak with fluid percussion injury (FPI) is much more acute [51].

In contrast to these microdialysis studies, magnetic resonance spectroscopy (MRS) studies demonstrate a decrease in glutamate at 2 and 4 h after a CCI model with an open skull injury [52]. Additionally, over the same time period, there is an increase in "pericontusional" glutamine. Similarly, in a moderate–severe TBI model using CCI that leads to a brain contusion, there was a decrease in glutamate as measured by MRS from days 0–14. There was a corresponding transient increase in glutamine in lesional cortex on days 0 and 1. In deeper, perilesional tissue of the hippocampus, the greatest increase in glutamine was on day 3, while glutamate remained decreased to a lesser extent over the course of the 2-week observation [53]. Although, there are likely differences in pathophysiology between mild TBI in humans and these severe TBI animal models, there is a similar pattern of MRS changes. Following concussion in humans, there was a decrease in glutamate 1–6 days in motor cortex, but not in the dorsal lateral prefrontal cortex (DLPFC) or hippocampi. Glutamate returned to baseline in the chronic phase of injury at 6 months [54]. The authors conclude that this change was related to the motor cortex's vulnerability to injury, although it remains unclear why this should occur in the motor cortex but not in the DLPFC.

To summarize, microdialysis studies demonstrate an increase in synaptic glutamate following TBI, while MRS exhibits a decrease in total glutamate and increase in glutamine. The differences between microdialysis and MRS findings may be related to injury severity [55] and models, but also highlights two points of distinction: 1. Microdialysis is capable of testing solely extracellular glutamate, while MRS tests both intra- and extracellular glutamate. 2. There is a difference in the pathophysiology of lesional and perilesional cortex.

Microdialysis studies confirm the rapid release of glutamate into the extracellular space, while MRS detects glutamate in vesicles in the presynaptic cell plus glutamate in the synaptic space. Thus, the MRS-microdialysis discrepancy may be explained by glutamate depletion from presynaptic vesicles, the uptake of glutamate from the synaptic cleft by neighboring astrocytes, and rapid conversion into glutamine leading to a total decrease in glutamate as measured by MRS. This contributes to a net increase in synaptic glutamate, as demonstrated by microdialysis studies, leading to further postsynaptic excitotoxic injury. These findings may be related to the severity of injury or due to damaged compensatory mechanisms, such as the glutamate transporter, both resulting in a net increase in synaptic glutamate.

Synaptic glutamate is taken up by astrocytes expressing EAAT2/GLT-1 [19, 20]. These transporters are downregulated in a number of pathologic processes, including hypoxic-

ischemic injury [56], stroke [57], and Huntington's disease [58]. Specifically in severe TBI, our lab has demonstrated that GLT-1 is downregulated 7 days after TBI in a rat lateral fluid percussion model. These rats reliably develop posttraumatic seizures at 12 weeks after injury. Seven days of treatment with ceftriaxone, a common beta-lactam antibiotic, restores GLT-1 expression to normal levels and reduces posttraumatic epilepsy [13••]. The mechanism by which ceftriaxone restores GLT-1 protein levels is unclear; however, ceftriaxone has demonstrated neuroprotective capacity in a range of CNS models [59]. The decrease in GLT-1 does not appear to persist when measured 2–4 weeks after injury [60••].

Harris and colleagues highlight a difference in the temporal course of glutamate release and reuptake between lesional and perilesional tissue. In "pericontusional" hippocampus, glutamine did not peak until day 3 implying a slower, ongoing glutamate release that may indicate that tissue remains viable beyond the initial injury. They also demonstrated a decrease in GABA on day 1 in lesional, but not deeper hippocampal tissue, which they propose is due to decreased conversion of glutamate to GABA [53]. Therefore, ongoing excitatory and inhibitory imbalance may not be related to abnormalities in the amount of glutamate in the synaptic space or its reuptake. Instead, changes in receptors or cell populations may be responsible (see Fig. 1b).

### Acute Receptor Changes

Following CCI, NMDAR show significant acute downregulation subunits NR1, NR2A, and NR2B that return to baseline levels after 24 h [61]. In rodents following FPI, total NR2B protein and phosphorylation are downregulated leading to destabilization of the NMDA receptor [62•]. In addition to cumulative receptor downregulation, lateral FPI leads to a decrease in NR2A and increase in NR2B subunits in cortical NMDAR. In the postnatal day 19 rat pup, lateral FPI elicits a similar NR2A to NR2B shift in both the cortex and particularly in the hippocampus [63]. The timing of this subunit shift in hippocampal NMDARs occurs predominantly within the first post-injury week, and corresponds to the time window of diminished experience-dependent plasticity normally induced by rearing in an enriched environment (EE). Rat pups reared in EE during the first post-injury week show impaired cortical thickening [64], altered dendritic arborization [65], and a failure of EE-induced enhancements in spatial learning and memory [66]. Additionally, following lateral FPI in rats, NMDA receptor blockade blocks immediate early gene (IEG) activation in the hippocampus 24 h after injury. Specifically, elevations in brain-derived neurotrophic factor (BDNF) and *Bax*, a marker of apoptosis, are decreased [67•].

AMPAR expression and function is also impacted by TBI and may lead to excitotoxic injury. In the acute phase, GluR1 receptors are upregulated following TBI, leading to increased calcium entry and subsequent nondiscriminate activation of all calcium/calmodulin-dependent protein kinases (CaMKs). The selective activation of these CaMKs is important for normal memory function; therefore, the lack of specific activation patterns leads to impaired function even in the absence of cell death [68]. Following an in vitro and in vivo TBI model, there is decreased GluR2 subunit expression, which is NMDAR mediated [69]. Reduced GluR2 allows for increased intracellular $Ca^{++}$ flux [37]. Increased intracellular $Ca^{++}$ flux through AMPARs, and via the relatively increased numbers of NR2B-containing NMDARs, leads to greater post-TBI vulnerability and likely increased neuronal death.

Blocking GABA-A receptors acutely induces seizures in rats following lateral FPI and leads to more pronounced structural damage, which underscores the critical contribution of GABA signaling to neuronal health in acute injury [70]. There are also differences in GABA-A subunit expression that occur acutely that vary by animal and TBI model; however, subunits responsible for the phasic inhibition ($\alpha1/\gamma2$) are generally downregulated following TBI, while those responsible for the tonic inhibition ($\alpha4/\delta1$) are upregulated. Raible and colleagues found a decrease in the $\alpha1$ subunit at 24 and 48 h that persisted for at least 1 week in rats injured by FPI, while there was an increase in the $\alpha4$ subunit at 24 h, but not 1 week. The authors point to previous evidence of a similar pattern of subunit expression that plays a role in the hyperexcitability of the hippocampus in models of status epilepticus [71•].

These changes in GABA-A subunit expression appear closely related to the glutamate-induced excitatory signal. GABA $\alpha1$ and $\gamma2$ subunit expression are increased in the hours after diffuse FPI in rats, but decreased by 24 h. Changes in expression can be blocked by MK-801, an NMDA receptor blocker that prevents $Ca^{++}$ influx into the postsynaptic cell following TBI and glutamate release. The authors suggest that $Ca^{++}$ blockade may prevent the $\alpha1$ subunit mediated role in post-TBI apoptosis [72] (see Table 1).

## Subacute Consequences of TBI

### Glutamate and GABA Changes

The window of posttraumatic epileptogenesis, as well as the post-TBI window of vulnerability to a second injury, extends beyond the acute period. The pathophysiology that follows in the days, weeks, and months after injury involve compensatory processes of receptor up- and downregulation, alterations in subunit composition, and a growing imbalance of glutamate-driven excitation and GABA-mediated inhibition. A recent study by Cantu and colleagues highlights the early phases of this imbalance in glutamate and GABA and points to mechanisms that may lead to posttraumatic epilepsy. In slice preparations, using a glutamate biosensor 2–4 weeks

following controlled cortical impact, they demonstrated extracellular glutamate signaling was increased in cortical networks. The highest glutamate signal occurs in perilesional tissue adjacent to the direct injury. Additionally, at the onset of a seizure, the glutamate biosensor signal spreads from medial to lateral and proximal to distal away from the site of direct injury [60••]. The mechanism of these changes may be related to changes in cell populations, particularly loss of parvalbumin positive GABA interneurons [45, 60••, 73, 74] and/or differences in receptor populations for glutamate and GABA.

### Subacute Receptor Changes

Two to four weeks following CCI, in slice preparations, NMDA, but not AMPA, receptor blockade prevents epileptiform activity [60••]. Further NMDAR investigation in the subacute time-frame has found changes that may underlie a form of maladaptive neuroplasticity. Reger and colleagues, using a lateral FPI in rats demonstrated an increase in the NR1 subunit of the NMDAR in the ipsilateral basolateral amygdala 2 weeks after injury. In this setting, the animals had enhanced but perhaps maladaptive fear learning related to context and discrete cues. This may represent a potential molecular underpinning of the posttraumatic stress disorder associated with TBI and may be seen in patients with more mild injuries [75•, 76, 77].

There is also ongoing GABA-A receptor changes. Kharlamov and colleagues found a reduction in the $\gamma 2$ subunit (phasic inhibition) and an increase in the $\delta 1$ subunit (tonic inhibition) in rats at 7 days that developed seizures following CCI [43]. These changes in GABA-A subunit expression further tip the balance toward excitation over inhibition.

## Chronic Consequences of TBI

### Glutamate and GABA Changes

In the chronic stages of injury following TBI, there are accumulation of cellular injuries and compensatory changes that result in an imbalance of excitation and inhibition, leading to posttraumatic seizures or neurocognitive and behavioral changes. Recently, there is a growing number of studies in TBI that have employed transcranial magnetic stimulation (TMS) as a means to assess the balance of glutamate-mediated excitation and GABA-mediated inhibition [78, 79]. TMS is a method for focal cortical stimulation where small intracranial electrical currents are induced by a strong and fluctuating extracranial magnetic field [80, 81]. One method of testing couples motor cortex stimulation by TMS with surface EMG recordings in the contralateral limb that results in a motor evoked potential (MEP). From the MEP, the degree of corticospinal excitability mediated by glutamate, and intracortical inhibition mediated at least in part by GABA-B receptors, can be measured [82•]. In common TMS terminology, measures of short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), or long-interval intracortical inhibition (LICI) can be assessed based on timing, frequency, and amplitude of stimulation and EMG recordings. Abnormalities in these measures are indicative of imbalance between GABA-mediated inhibition and glutamate-mediated excitation or facilitation (excitatory:inhibitory, E:I ratio).

TMS studies in mild TBI have identified chronic changes in glutamate-GABA balance. Théoret's group has demonstrated an increase in GABA-B-mediated inhibition in asymptomatic athletes with at least two concussions at greater than 9 months after injury. The authors conclude that GABA upregulation may be a long-term compensatory mechanism against glutamate excitotoxicity [82•]. More recently, the same lab looked for abnormalities in the MRS spectra of GABA, glutamate, and glutamine, as well as TMS determined inhibition patterns of the motor cortex, occurring 3 years after 1–4 concussions. They found no differences in metabolism, cortical thickness, cortical connectivity, and contrary to previous studies no significant difference in GABA-B-mediated inhibition. This difference with their prior study may be attributed to the longer time elapsed since concussion and the fewer number of concussions in the latter group. They did, however, find alterations in the balance of excitation and inhibition in concussed athletes based on the lack of correlation between GABA and glutamate measured by MRS [83].

More severe TBI models that lead to posttraumatic epilepsy months to years following injury have received ongoing investigation in our labs and others. It has been appreciated for some time that neuronal circuits are modulated by inhibitory GABAergic neurons and that these neurons may be damaged by TBI [74, 84]. More recently, Pavlov and colleagues found a progressive loss of phasic inhibition with corresponding loss of parvalbumin-positive GABA interneurons (see Fig. 1c). They observed this in the ipsilateral hippocampus at 1 month following a lateral fluid percussion injury and by 6 months in the contralateral hemisphere. They did not see a significant change in subunit expression or deficits in tonic inhibition [85]. Loss of parvalbumin-positive GABA interneurons following TBI [60••, 86, 87] leads to deficits in gamma oscillations that are important for modulating excitatory signals [45]. In addition to loss of GABAergic interneurons and inhibitory synapses, there are also increased and abnormal excitatory synapses impacting neuronal connectivity [88].

Finally, deficits in the synthesis of neurotransmitters may play a role in tipping the balance of excitation and inhibition. GABA is synthesized by glutamic acid decarboxylase; therefore, a decrease in this enzyme could lead to decreased inhibition. Recently, genetic variability in humans in GAD gene expression was shown to increase the risk of PTE [89]. In an

animal study, GAD-67 has been shown to be decreased following lateral FPI [75•]. Conversely, GAD-67 was initially increased 1 month after a moderate to severe controlled cortical impact; however, this did not persist to 4 months despite ongoing deficits in working memory [90].

### Chronic Receptor Changes

An increase in the NR2B-containing NMDAR leads to persistent $Ca^{++}$ influx that stretches from the acute to chronic phases following injury [91]. In the chronic setting, rats that developed posttraumatic epilepsy up to 5 months following CCI had an increase in NR2B subunit compared with both rats which did not develop seizures after injury and controls [43]. Similarly, there is evidence of long-lasting AMPAR changes. In a more severe TBI rodent model leading to PTE, GluR1 was downregulated several months after CCI, related to possibly altered phosphorylation or a compensatory mechanism [43].

## Implications for Neuroprotection

TBI pathophysiology likely differs between lesional and perilesional tissue and severe and mild injury. Cell injury proceeds more slowly in the brain tissue that is immediately proximal to the most severe injury and may be reversible in this penumbra. Similarly, following concussion the majority of brain injury is reversible given the typical course of symptom resolution [92–95]. Therefore, the pathophysiology of mild TBI likely has similarities with more mildly affected perilesional tissue in severe TBI models. In these brain areas with reversible damage, the opportunity to decrease acute glutamate excitotoxicity holds promise for neuroprotection. For example, NMDA receptor blockade decreases epileptiform discharges [60••], excitatory synaptic strength [67•], and apoptosis [67•, 72]. Removing glutamate from the synapse using scavengers, such as pyruvate and oxaloacetate an hour after weight-drop TBI, leads to an increase in surviving hippocampal neurons 30 days after injury [96]. There is also evidence that by removing glutamate from the synapse more efficiently through upregulation of glutamate transporters, either by ceftriaxone [13••] or with the steroid dehydroepiandrosterone [97], there is a decrease in posttraumatic seizures.

Techniques in neuromodulation also hold promise for treatment following TBI. A recent study using anodal transcranial direct current stimulation (tDCS) combined with MRS demonstrated a reduction in GABA, but not glutamate or glutamine concentrations, in the motor cortex and improved motor memory [98•]. Although this was not a study on TBI patients, the ability to directly impact concentrations of neurotransmitters, as well as the ratios of excitation to inhibition with tDCS and TMS, holds promise for the treatment of TBI.

## Summary

Following traumatic brain injury immediate glutamate release sets off a cascade of metabolic changes, which includes both the expanding lesion itself and the brain's attempt to compensate (summarized in Fig. 1). Glutamate is released into the extracellular space and acts on AMPA and NMDA receptors prompting $Ca^{++}$ ions to flow into the postsynaptic neuron. Glutamate is then taken up into astrocytes by the glutamate transporter. TBI-induced decreases in this transporter allow excess glutamate to remain in the synapse and continue its excitotoxic actions. In astrocytes, glutamate is converted to glutamine and returned to its presynaptic cell or neighboring GABA interneuron for conversion back to glutamate and then to GABA, respectively. Acute decreases in GAD and/or a decrease in glutamine traveling to GABA interneurons may decrease GABA synthesis and impact local inhibition.

Early after TBI, in cortical and hippocampal regions, there is activation of extrasynaptic NR2B-containing NMDARs and downregulation of NR2A, resulting in persistent $Ca^{++}$ flux, impaired neuroplasticity, and an increased risk for neuronal cell death. Downregulation of the GluR2 AMPAR subunit renders these receptors calcium-permeable and may exacerbate injury. Later, NMDAR subunits may recover, with concomitant improvements in neural activation, increased expression of neurotrophins, and enhanced plasticity. This delayed re-activation of circuitry may be mediated not only by changes in NMDARs but also AMPARs. However, with the restoration of neural activation comes the risk of maladaptive excitatory neurotransmision, including enhanced fear/anxiety as well as the development of posttraumatic seizures.

In the subsequent phase of excitotoxic injury, there are changes in receptor composition that are likely a combination of direct injury, as well as compensatory changes that are meant to combat the excessive excitatory input. GABA interneurons act to synchronize and time neuronal signals across large networks, and dysfunction in particular GABA receptor subunits leads to abnormal patterns of phasic and tonic inhibition, thus, making the local environment hyperexcitable and at risk for seizures. Additionally, through normal patterns of inhibition, GABA-A receptors mediate gamma oscillations that play a role in higher cognitive functions. These gamma oscillations appear to rely on fast-spiking parvalbumin-positive GABA interneurons, which are uniquely susceptible to loss following TBI. Therefore, investigations into the mechanism of this cell loss are a key area to target to improve neurocognitive outcomes and posttraumatic epilepsy.

## Conclusion

Following experimental TBI, there are dynamic changes in excitatory-inhibitory balance that result in neuronal

dysfunction and may result in long-term sequelae. This imbalance is a result of increased glutamate release, faulty reuptake, and changes in the population of receptors and inhibitory interneurons. Given the complexity of the pathophysiology and the heterogeneity of injuries, it is crucial to continue building our understanding of this imbalance with the goal of finding therapeutic and neuroprotective targets.

#### Compliance with Ethics Guidelines

**Conflict of Interest** Réjean M. Guerriero declares no conflict of interest.

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- • Of importance
- •• Of major importance


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