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GABA actions and ionic plasticity in epilepsy

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Concepts of epilepsy, based on a simple change in neuronal excitation/inhibition balance, have subsided in face of recent insights into the large diversity and context-dependence of signaling mechanisms at the molecular, cellular and neuronal network level. GABAergic transmission exerts both seizure-suppressing and seizure-promoting actions. These two roles are prone to short-term and long-term alterations, evident both during epileptogenesis and during individual epileptiform events. The driving force of GABAergic currents is controlled by ion-regulatory molecules such as the neuronal K-Cl cotransporter KCC2 and cytosolic carbonic anhydrases. Accumulating evidence suggests that neuronal ion regulation is highly plastic, thereby contributing to the multiple roles ascribed to GABAergic signaling during epileptogenesis and epilepsy.

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Much of the neurobiological research on epilepsies has focused on the role of GABAergic transmission in various phases of disease progression. Alterations in GABAergic signaling, which include changes in the properties of interneurons and in their quantitative as well as qualitative postsynaptic effects, are intimately involved in the development and chronic manifestations of epileptiform activity. In this review, we focus on $GABA_A$ receptor ($GABA_AR$) functions and the ion transporters which affect the reversal potential of $GABA_AR$-mediated currents (E_{GABA}). 'Ionic plasticity' [1] (Figure 1) refers to changes in neuronal signaling related to the operation and functional modulation of plasmalemmal ion transporters (Figure 2a) which set E_{GABA} either directly (Cl^- and/or HCO_3^- transporters) or indirectly (the Na-K ATPase).

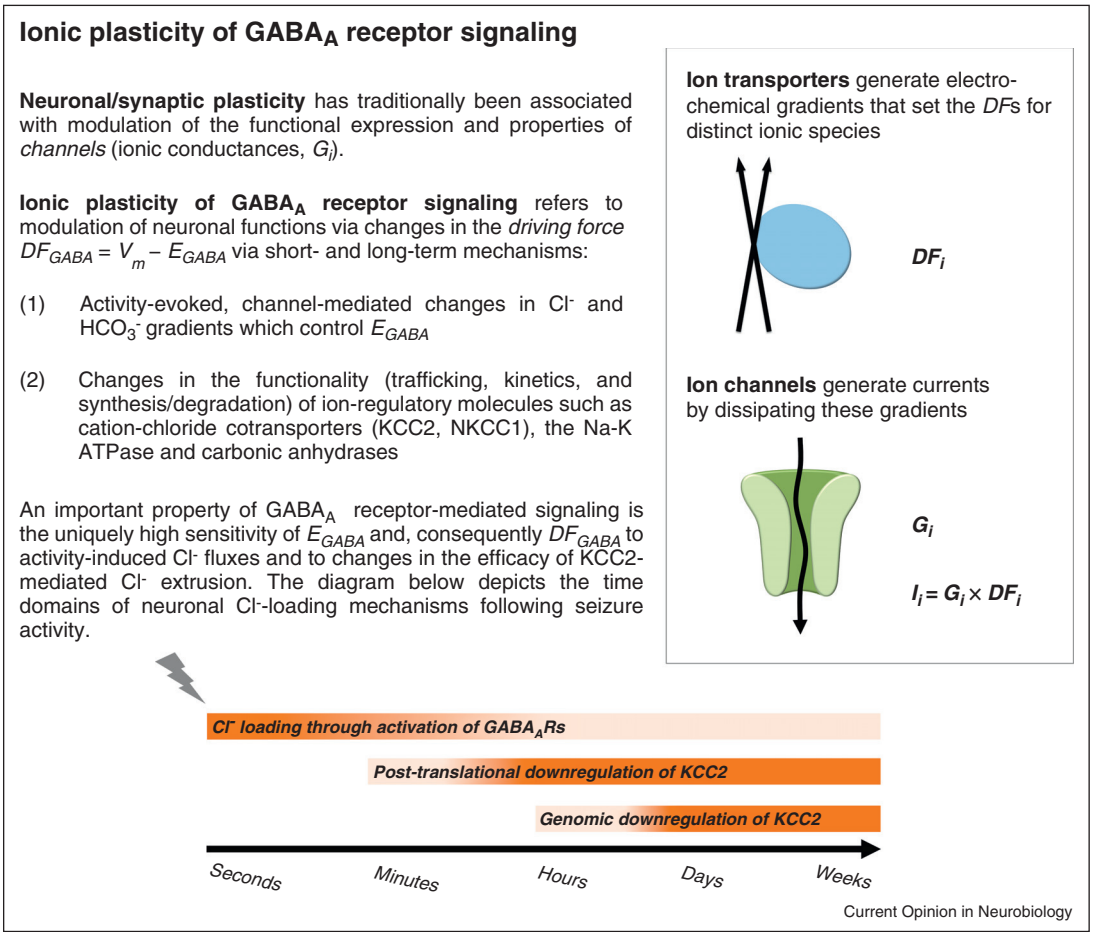
Epilepsies have turned out to be a spectrum disorder with a range of etiologies and comorbidities. Concepts of epilepsy and epileptogenesis seem likely to undergo revisions [2,3•]. Here we will mainly focus on mesial temporal lobe epilepsy (TLE) which is the most common type of refractory epilepsy. The primary cause leading to TLE is typically an insult to the brain (traumatic brain injury, inflammation, status epilepticus), but in patients the nature of the initial insult remains often unknown because of long delays between the insult and appearance of the recurrent seizures characteristic of TLE [4].

Seizures in the non-epileptic brain

Seizures can take place in disease states other than epilepsy. Refractory status epilepticus (SE), a life-threatening epileptic crisis characterized by prolonged recurrent seizures which do not respond to diazepines [5], is caused by factors such as inflammation and stroke, and it is seen in a minority of patients with established epilepsy. Much of our knowledge on the mechanisms and consequences of seizures come from *in vivo* and *in vitro* work on animals with no previous history of epilepsy or epileptogenesis. Induction of experimental SE has been shown to produce fast and robust changes in neuronal plasticity and a fast development of pharmacoresistance to conventional antiepileptic drugs which enhance GABAergic transmission. A straightforward explanation (see [6,7]) is that recurrent seizures lead to a progressive internalization of postsynaptic $GABA_AR$s and to a consequent erosion of inhibition. Work on brain slices has shown that the efficacy of feedforward inhibition declines rapidly after recurrent seizure-like activity, leading to a loss of the powerful inhibitory surround that is initially associated with these paroxysmal events [8,9•,10]. Seizures evoked in healthy adult brain tissue induce a fast decrease in the expression of KCC2 [11–13], the main neuronal Cl^- extruder. This molecule underlies classical, 'Eccles-type' hyperpolarizing inhibitory postsynaptic potentials (IPSPs) in central neurons [14]. GABAergic inhibition also has a shunting effect on electrical signals in the postsynaptic membrane (Box 1). The downregulation of $GABA_AR$s and KCC2 in response to trauma and/or intense seizure activity (Figure 2b) leads to a long-lasting decrease in the efficacy of both shunting and voltage inhibition, respectively.

The extrusion capacity of KCC2 can saturate even in the absence of functional downregulation [15•] of the transporter. Therefore, *the loss of voltage inhibition in response to*

Figure 1



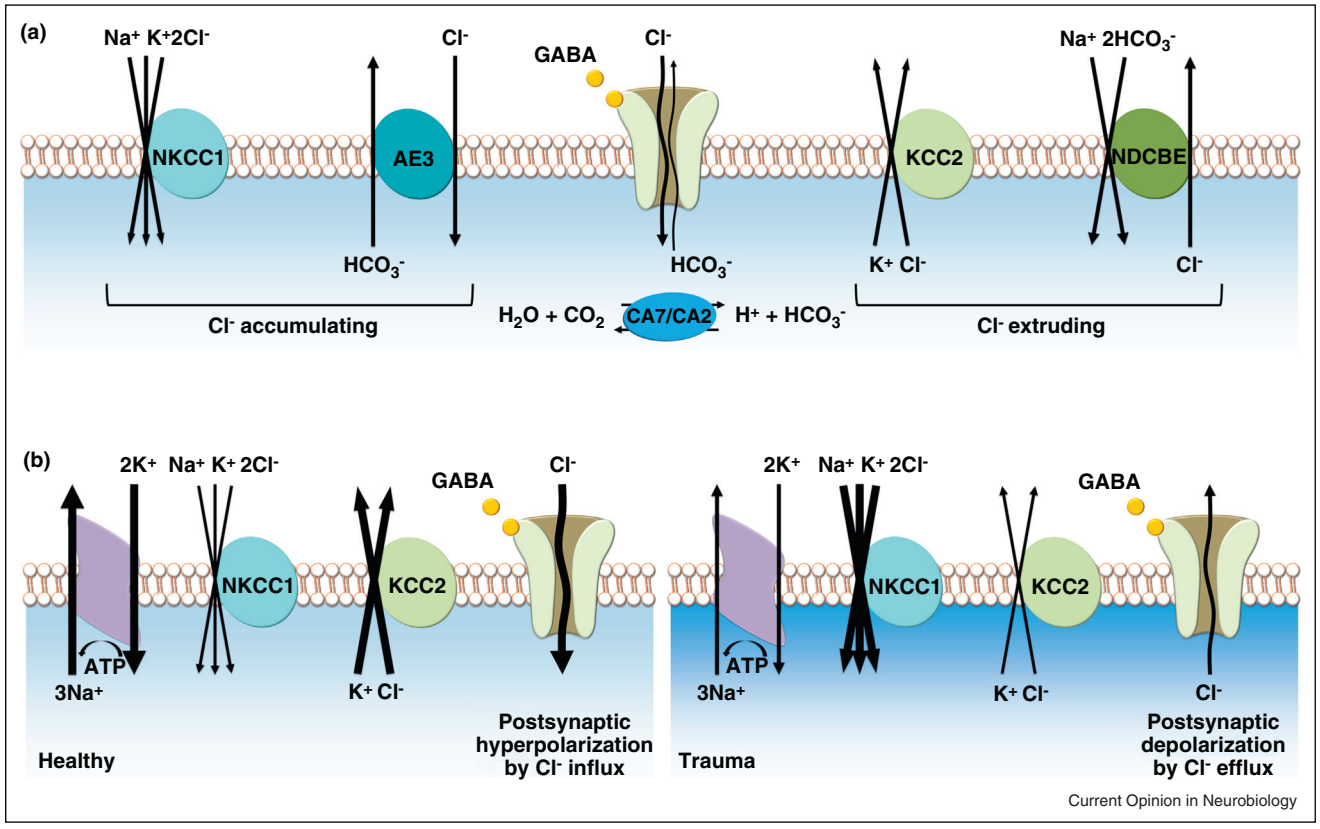
Ionic plasticity of GABA$_A$ receptor signaling.

seizure activity is likely to be much faster than the loss of shunting. This is because the intense activation of interneurons will lead to a massive Cl^- influx which is aided by the depolarizing currents mediated by HCO_3^- across $GABA_A$Rs and by coactivation of glutamatergic ionotropic receptors [16], as shown by continuous monitoring of E_{GABA} [17]. In fact, the depolarization mediated by the HCO_3^- current can drive a Cl^- influx that is large enough to induce a qualitative change in $GABA_A$R-mediated signaling from inhibitory to excitatory [17,18•]. During intense $GABA_A$R activation, the driving force of the inward HCO_3^- current is much more stable than that of the outward Cl^- current because the intracellular HCO_3^- is effectively replenished by neuronal cytosolic carbonic anhydrase [19,20•]. Indeed, under experimental conditions with enhanced synaptic release of GABA, pharmacologically isolated $GABA_A$R-mediated transmission alone is able to produce spontaneous paroxysmal activity in the brain slice [21]. The effect of the HCO_3^--dependent anion shift is augmented by KCC2-mediated net accumulation of K^+ in the interstitial space [18•], leading to further, non-synaptic depolarization and excitation of neurons. This probably includes (the at its time enigmatic observation of) GABA-driven antidromic spiking [22]. The membrane potential of glial cells is highly sensitive to extracellular K^+, and the above positive feedback loop may act in synergy with glutamate release [23] from depolarized glia [17] to sustain a seizure. $GABA_A$Rs and the Na-K-2Cl cotransporter NKCC1 (but not KCC2) are expressed in presynaptic terminals [14], and thus the antidromic spiking might also involve a direct presynaptic $GABA_A$R depolarization following an activity-dependent anion shift within the terminal. The role of extracellular K^+ is a classical focus in epilepsy research [24], and the time is ripe to readdress this topic with the novel insight pointing to GABAergic K^+ transients as a major pro-convulsant mechanism [25,26].

Seizures in the epileptic brain

It is likely that seizure mechanisms in chronically epileptic tissue differ dramatically from those evoked in brain tissue in healthy experimental animals or observed in patients with no previous history of epilepsy. Even if no cognitive defects are detectable during seizure-free periods of time, the cortex of chronically epileptic patients generates

Figure 2



Neuronal ion regulation sets the driving force for $GABA_A$ receptor-mediated currents. **(a)** The cation chloride cotransporters govern neuronal Cl^- regulation. In mature neurons the K-Cl cotransporter isoform 2, KCC2, mediates Cl^- extrusion driven by the K^+ gradient while the Na-K-2Cl cotransporter isoform 1, NKCC1, mediates Na^+ driven Cl^- uptake. In addition to this, the Na^+-independent and Na^+-dependent Cl-HCO_3 exchangers AE3 and NDCBE, respectively, may modulate intracellular Cl^- levels. The main function of these HCO_3^- transporters, together with the ubiquitous Na-H exchanger (not depicted), is to keep the intracellular pH level significantly more alkaline than what is predicted on the basis of passive distribution of H^+ and HCO_3^- ions. Hence, the equilibrium potential for HCO_3^- is much more positive than the resting membrane potential and HCO_3^- invariably mediates a depolarizing current across $GABA_A$Rs. Intracellular HCO_3^- concentration is rapidly replenished even during prolonged $GABA_A$R activation via the activity of carbonic anhydrase isoforms 2 and 7 (CA2 and CA7) which catalyze the formation of HCO_3^- from CO_2 [20•]. **(b)** The cation-chloride cotransporters shown in panel (a) are fueled by the Na^+ and K^+ gradients generated by the Na-K ATPase. The conventional, $GABA_A$R-mediated hyperpolarizing IPSPs seen in mature neurons depend on the functional expression of KCC2 (left) that maintains a low intracellular Cl^- level that favors conductive Cl^- influx. Seizure-induced post-translational changes in CCC functional expression, e.g. via altered membrane expression, commence in tens of minutes. Prolonged changes at the levels of post-translational modification and transcription convert $GABA_A$R signaling back to its immature, depolarizing/excitatory mode of action during the course of epileptogenesis (right). The positive shift in E_{GABA} assists in reducing energy consumption during 'energy crisis' by reducing the driving forces of temporally overlapping and mutually counteracting excitatory and inhibitory postsynaptic ion fluxes (see [16]). For further details, see text.

abnormal interictal activities which are seen as brief (tens of milliseconds) spikes in the EEG. In hippocampal tissue from TLE patients, the *in vitro* counterpart of interictal activity is highly sensitive to bumetanide [27], a drug that selectively blocks Cl^- uptake mediated by NKCC1 in neurons *in vitro* [26]. In a manner similar to the depolarizing $GABA_A$R actions and associated NKCC1-dependent network events in the immature rodent hippocampus [14], interictal activity *in vitro* shows an obligatory dependence on excitatory GABAergic and glutamatergic excitatory synaptic drive [28,29••]. In hippocampal tissue resected from human TLE patients, intracellular recordings have revealed a subpopulation of pyramidal neurons where KCC2 levels are low and GABA has an excitatory action [27,28] (see also [13]). Thus, $GABA_A$R signaling appears to resume its immature, depolarizing/excitatory mode of action at least in some pyramidal neurons during the course of epileptogenesis. However, while the changes in cation-chloride cotransporter (CCC) expression levels (low KCC2, high NKCC1) in these cells provide an explanation for the generation of interictal activity, there are no data to suggest that ictogenesis is based on these mechanisms (for review see [26]).

Intriguingly, it seems to be more difficult to evoke seizures in human TLE tissue than in brain tissue from healthy animals [30•]. This is a difficult paradox, since surgically obtained human TLE tissue typically has a

Box 1 Synaptic and extrasynaptic $GABA_AR$ signaling

Synaptic $GABA_AR$-mediated inhibition is based on shunting and hyperpolarization of the postsynaptic membrane. *Shunting inhibition* has a duration set by the $GABA_AR$ channels' open time, and the associated increase in conductance acts to suppress the temporal and spatial summation of incoming excitatory synaptic signals, as well as intrinsic pro-excitatory currents generated in the dendritic tree. *Voltage inhibition*, which hyperpolarizes the postsynaptic membrane, is dependent on the inwardly-directed electrochemical gradient of Cl^-, maintained by KCC2, and it counteracts excitatory mechanisms for a longer time period, which is set by the time constant of the cell membrane. Unlike shunting, voltage inhibition does not take place in all types of mature CNS neurons because of cell-type specific lack of KCC2. The postsynaptic $GABA_ARs$ in the neocortex and hippocampus consist of α(1-3), β(x) and γ2 subunits [80] whereof the of $\alpha1\beta2\gamma2$ is the most common one.

Tonic $GABA_AR$-mediated signaling is based on the activation of high-affinity *extrasynaptic $GABA_ARs$* (with a subunit combination consisting of $\alpha5\beta\gamma2$, $\alpha4\beta\delta$ or $\alpha1\beta\delta$ in neocortical and hippocampal neurons) by ambient GABA. The subunit composition of $GABA_ARs$ undergoes marked changes during epileptogenesis with consequent changes in the abundance of postsynaptic and extrasynaptic receptors [81], often followed by an increase in tonic inhibition [82]. Tonic GABAergic signaling is highly sensitive to changes in the efficacy of GABA uptake transporters (GAT1-4) [83], and it produces a spatially extended shunting effect in the target neurons, with voltage changes set by DF_{GABA}. Excessive tonic inhibition is known to promote absence seizures by inducing slow-wave discharges in thalamo-cortical networks, while enhancing tonic inhibition has an anticonvulsant action in partial seizures and catamenial epilepsy [84]. Synaptic GABAergic signaling is often called 'phasic' (e.g. 'phasic inhibition') to underscore its distinct properties versus tonic signaling. Both phasic and tonic $GABA_AR$-mediated signaling can be functionally inhibitory or excitatory, depending on the ion-regulatory mechanisms which set E_{GABA} and DF_{GABA}; on the $GABA_AR$-mediated conductance; and on the intrinsic electrophysiological properties of the target neuron.

sclerotic CA1 region, and such macroscopic differences in the properties of the healthy versus chronically diseased circuitry will compromise evaluation of the effects of distinct CCCs or changes in their expression. In slices from human TLE tissue, seizure-like events are not preceded by interictal but rather by 'pre-ictal' events which are largely based on recurrent glutamatergic signaling [29••,31]. These bursts have a wide spatial extent and a high propagation speed which probably makes them particularly effective in activating interneurons [30•]. While dendritic GABAergic synapses are lost in animal models of chronic epilepsy [32], a wealth of data suggest that parvalbumin-positive, perisomatically targeting interneurons shape the rhythmicity and synchrony which makes it possible for the seizures to effectively spread across wide cortical territories [33].

What triggers seizures in TLE? The diversity of recent explanations shows that this fundamental problem has still not been satisfactorily solved. We note that the term 'trigger' is ambiguous in that it has been used to describe (i) extrinsic factors that increase the propensity of seizures (e.g. hyperventilation or fever in children) and (ii) the intrinsic neuronal and network mechanisms that act as immediate causes for seizure generation. Here, we will consider the latter. There is evidence that local desynchronization of neuronal activity is needed for the initiation of seizure activity [34]. Thus, a simple working hypothesis for the generation of TLE-related seizures and the role of KCC2 therein might be constructed as follows: In the seizure-triggering 'kernel' of diseased tissue, pre-ictal activity leads to a loss of phasic hyperpolarizing IPSPs (Box 1), which results (over short times) from the high Cl^- load [35,36] and is enhanced and consolidated largely by post-translational downregulation of membrane-associated KCC2 [37•,38•] and later (cf. [37•]) by block of KCC2 transcription (Figure 1). The lack of hyperpolarizing IPSPs will lead, in turn, to the local desynchronization and promotion of seizures [34]. This idea is consistent with and supported by the actions of hyperpolarizing inhibition on spike probability and timing in healthy tissue [39].

TrkB and calpain as coordinating factors in epileptogenesis and epilepsy

BDNF-TrkB signaling has been put forward as a coordinating factor in epileptogenesis [40–42]. Indeed, the parallel loss of postsynaptic $GABA_ARs$ and KCC2 after recurrent seizures may imply a shared mechanism, which most likely consists of signaling cascades down-stream of the tropomyosin-related kinase B (TrkB) receptor [40], the main target of brain-derived neurotrophic factor (BDNF). Seizures enhance BDNF secretion and the activation of TrkB [41] but, notably, BDNF itself is not always responsible for seizure-induced TrkB activation (cf. [43]). Conditional knockout of TrkB [44], transient inhibition of TrkB [45] or uncoupling of TrkB from the PLCγ1 cascade [46•] are all reported to suppress epileptogenesis. Enhanced TrkB activation in mature neurons rapidly decreases surface expression of $GABA_ARs$ [47,48] and downregulates KCC2 [12,49]. There exist close parallels between the role of BDNF-TrkB signaling in epilepsy and in chronic pain [50]. In both cases, inflammation may induce BDNF secretion from activated microglia, and cause a downregulation of KCC2 in adjacent neurons [50]. Notably, inflammation is also a major cause of SE and epileptogenesis [51].

Fast, seizure-induced downregulation of KCC2 activity (over tens of minutes to hours), depends on post-transcriptional mechanisms [15•,37•], including protein phosphatase 1-mediated dephosphorylation of KCC2 at serine 940 [38•,52] and cleavage by the protease calpain [37•,52], which is activated by Ca^{2+} and/or BDNF (for review, see [53]). A decrease in KCC2 mRNA occurs within hours of a seizure [12] and may contribute to consolidate KCC2 downregulation (Figure 1), but is not needed for chronic suppression of KCC2 protein expression or Cl^- extrusion [54]. Indeed, the increased level of calpain expression observed in TLE tissue [55,56] could account

for the chronic suppression of hippocampal KCC2 expression observed in patients with chronic epilepsy (see above). Interestingly, up-regulation of the gene encoding for the endogenous calpain inhibitor calpastatin is observed during the acute and latent phase of limbic epileptogenesis, whereas this enhancement appears to be lost in the chronic phase, characterized with spontaneous recurrent seizures [57]. Notably, calpain cleaves not only KCC2 [37•,52,54] but also other proteins involved in GABAergic transmission, including GAD65 [58], VGAT/VIIAT [59], GAT1 [60] and gephyrin [61]. Thus, mounting evidence suggests that activation of calpain is another coordinating factor in epileptogenesis with important effects on GABAergic signaling (see also [62]).

Developmental stage and seizure mechanisms

Neuronal signaling mechanisms are radically different in developing and mature brain. One of the best examples is $GABA_AR$-mediated signaling, which undergoes a well-known 'developmental shift' from depolarizing/excitatory to hyperpolarizing (for review, see [14,63]). Initially neuronal Cl^- accumulation by NKCC1 is dominant, and KCC2 is expressed later during neuronal maturation [14]. The maturation of KCC2-dependent hyperpolarizing inhibition is accompanied by the expression of neuronal carbonic anhydrase isoform 7 (CA7), at around postnatal day (P) 12 in rodent hippocampus [64], followed by neuronal expression of the ubiquitous CA isoform 2 (CA2) at ~P20 [20•]. The *simultaneous presence* of KCC2 and CA activity is crucial both for the generation of GABA-dependent neuronal Cl^- loads in response to interneuronal activity, and for paroxysmal extracellular K^+ transients [18•]. The key role of NKCC1 in immature, depolarizing GABAergic transmission and GABA-driven network events has led to a number of studies in neonatal rodents on the possible therapeutic use of the NKCC1 blocker, bumetanide. This work has been largely disappointing, as described elsewhere [26]. Cortical development is much more advanced in the human newborn than in the rodent [65]. Unlike in neonate rodents, CA7 and KCC2 are both expressed at high levels in hippocampus and neocortex of full term human babies [15•,20•,66,67]. This major species difference has numerous implications for translational work on the mechanisms of GABAergic signaling and seizures.

Adaptive mechanisms

From an evolutionary point of view, it is easy to understand why neurons and neuronal networks are endowed with adaptive response patterns which promote their survival under various insults [68]. Adaptive mechanisms can be detected, for instance, in the slowing of disease progression induced by application of proconvulsant drugs such as atipamezole and rimonabant soon after an insult [69,70]. Moreover, whether disease stage-related expression patterns of the endogenous calpain inhibitor calpastatin [57] (see above) are causally involved here, is an interesting question for future work. Clearly, empirical information is required to judge whether a disease-related change at the molecular, cellular or network level is a 'dysfunction' (maladaptive, pro-epileptogenic) or an adaptive (anti-epileptogenic) process.

After trauma, neurons undergo processes of de-differentiation, seen as a shift in gene expression patterns to those of earlier developmental stages [1,71]. The adaptive value of such processes may be best explained from the factors involved in neuronal survival after trauma: (i) downregulation of energy metabolism under conditions of an 'energy crisis' (see [72]); as well as the presence of (ii) sufficient connectivity and (iii) trophic factor signaling, which promote neuronal survival. With regard to (i), the mammalian brain works close to theoretical limits on energy consumption, with most of it used to maintain the ionic driving forces which are needed for electrical signaling [16,73]. Changes in the functions and expression patterns of ion transporters and channels may thus have evolved as adaptive mechanisms to protect neurons during states of energy crisis. This idea fits well with the fast downregulation of both ion transporters and channels (e.g. $GABA_ARs$ and KCC2) in response to seizures (see above and Figure 2b). Furthermore, the Na-K ATPase, the major ion-regulatory and energy-consuming molecule of the brain, is functionally downregulated after trauma or seizure [26,74]. The Na-K ATPase and KCC2 are functionally tightly linked, and there is evidence that the two molecules form a structural ion-transport metabolon [75,76]. We note also that shutting down Cl^- permeable $GABA_ARs$ will reduce the energy-metabolic load imposed by cation-based glutamatergic signaling [16].

Conclusions

Changes in excitation–inhibition (E/I) balance are often used to explain epileptogenesis and seizure generation but, as should be obvious from the work reviewed above, the explanatory value of the E/I balance in the context of epilepsy is limited. Moreover, the postulated cause (E/I imbalance) is deduced from the outcome (seizures), which is an obvious circular argument. A *gross* change in the E/I imbalance is not, either, consistent with the fact that seizures in chronic epilepsy can occur infrequently and unpredictably, with intervening periods of intact cognitive and mnemonic cortico-hippocampal functions. The studies reviewed presently demonstrate that $GABA_AR$ signaling has multiple, context-specific and age-specific actions which can prevent or promote epileptogenesis and seizure generation. A context-specificity and age-specificity is true also for intracellular signaling cascades such as those down-stream of TrkB receptors [76–78], which exert a strong influence on neuronal plasticity. This context-dependent diversity in cellular

and molecular signaling is not only a major challenge for basic research on the etiology and mechanisms of epileptiform syndromes, but also for the design of novel, genuinely antiepileptic drugs [79] which, instead of having solely symptomatic anticonvulsant actions, would halt and even reverse the progression of epilepsy.

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