Review Article Altered GABA Signaling in Early Life Epilepsies

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The incidence of seizures is particularly high in the early ages of life. The immaturity of inhibitory systems, such as GABA, during normal brain development and its further dysregulation under pathological conditions that predispose to seizures have been speculated to play a major role in facilitating seizures. Seizures can further impair or disrupt GABA_A signaling by reshuffling the subunit composition of its receptors or causing aberrant reappearance of depolarizing or hyperpolarizing GABA_A receptor currents. Such effects may not result in epileptogenesis as frequently as they do in adults. Given the central role of GABA_A signaling in brain function and development, perturbation of its physiological role may interfere with neuronal morphology, differentiation, and connectivity, manifesting as cognitive or neurodevelopmental deficits. The current GABAergic antiepileptic drugs, while often effective for adults, are not always capable of stopping seizures and preventing their sequelae in neonates. Recent studies have explored the therapeutic potential of chloride cotransporter inhibitors, such as bumetanide, as adjunctive therapies of neonatal seizures. However, more needs to be known so as to develop therapies capable of stopping seizures while preserving the age- and sex-appropriate development of the brain.

1. Introduction

Epilepsy is a disease of recurrent seizures: that is, unprovoked episodes of aberrant synchronous excitation of brain regions that disrupt normal functioning [1, 2]. Epileptic seizures are thought to reflect a failure in the ability to maintain the balance between excitation and inhibition. The mechanisms underlying seizures are complex and not uniform across the numerous seizure types that exist [1]. Furthermore, our ability to study these mechanisms is often limited by the tools we can use: we can only see as far and as much as those tools allow. Consequently, many of the hypotheses describing the pathogenesis of seizures are biased by the dominant ictal phenomena, unbalanced excitation-inhibition and aberrant neuronal synchronization, which may not necessarily be the actual ictogenic mechanisms. Neurotransmitters involved in neuronal inhibition, such as GABA, have attracted the major focus of research aiming to decipher mechanisms involved in ictogenesis. Under certain conditions, and definitely not in the majority of cases, seizures may lead to epilepsy or neurodevelopmental deficits. The early periods of life,

when brain development is still incomplete, susceptibility to seizures is increased [3, 4]. However, a combination of biological factors (genetic, age-related processes, epigenetic or environmental factors) protect neurons from seizureinduced injury, epileptogenesis, or mortality to a greater extent than the adult brain is protected [5]. It is increasingly recognized that seizures may leave their imprint on the developing brain by altering the way that neurons differentiate, connect, and communicate to each other, even if, in many cases, such changes may be ultimately compensated for. As extensively outlined in the reviews included within this special issue, GABA plays a central role in controlling neuronal development and communications. A major focus of research has therefore been thrown into efforts to elucidate its role not only in ictogenesis but also in the pathogenesis of the sequelae of early life seizures, whether this may be epilepsy, cognitive, or behavioral deficits [6].

There are three types of GABA receptors reported in the literature: GABA_A, GABA_B, and GABA_C, the latter classified more recently along with GABA_A receptors, due to their functional similarities. Both GABA_A and GABA_C receptors

are ligand-gated ionotropic channels that allow primarily chloride but also bicarbonate to cross their pore in response to GABA binding. GABA_B is a metabotropic receptor that signals through cascades that modify potassium and calcium current (reviewed in [7]), direct migration [8], and control gene transcription [9, 10]. In this review, we will focus primarily on GABA_A receptors.

GABA_A receptors are pentameric channels usually comprised of 2 α and 2 β subunits, whereas the fifth is either a γ or a δ subunit. Less frequently, ε , θ , or π subunits are present [11–13]. There are 16 known mammalian GABA_A receptor subunits ($\alpha 1 - \alpha 6, \beta 1 - \beta 3, \gamma 1 - \gamma 3, \delta, \varepsilon, \theta, \pi$), which contribute towards the different pharmacokinetic, subcellular localization or affinity properties of each GABA_A receptor complex. The presence of a ρ subunit defines the GABA_C receptors. Unlike GABA_A receptors, GABA_C are insensitive to bicuculline. The expression of GABA_A receptor subunits changes with development and as a result the responsiveness of immature and adult neurons to GABA_A ergic modulators are significantly different.

The classical inhibitory GABA_A signaling, as occurs in most adult neurons, is due to chloride influx through the channel pore, which hyperpolarizes the cells. This is achieved because the intracellular chloride concentration is maintained at a low level, allowing chloride to flow in along its electrochemical gradient, when GABAA receptors open (Figure 1). Multiple studies over the last few decades have confirmed that this electrochemical chloride gradient is developmentally regulated by changes in the expression of cation-chloride cotransporters (CCCs). CCCs are the electroneutral ion symporters that establish the chloride gradient between cells and their extracellular environment. There are 3 CCC classes. The chloride importing CCCs are either the sodium/potassium/chloride cotransporters (NKCCs), with known representatives the NKCC1 and NKCC2, or the sodium chloride cotransporters (NCCs). Chloride exporters are the potassium/chloride cotransporters (KCCs), with 4 known isoforms: KCC1-4 (reviewed in [11, 12, 14, 15]) (Figure 1). Immature neurons express predominantly chloride-importers, such as NKCC1 [16], which generate high intracellular Cl⁻ levels. This forces the open GABA_A receptors to permit Cl- efflux through their channel pore, giving rise to depolarizing GABAA responses [16-18]. During developmental maturation, the expression of chlorideextruding CCCs, like the potassium/chloride cotransporter 2 (KCC2), dominates over NKCCs [19-22], decreasing the intracellular chloride concentration [23]. As a result, when GABA opens GABA_A receptors the ensuing influx of chloride results in hyperpolarizing currents [19] (Figure 1). However, cell type, sex, and species/strain differences occur in the timing of this developmental shift. KCC1, KCC3 and KCC4 are widely expressed, but KCC2 is specific to neurons. This makes KCC2 particularly interesting for the pathogenesis and therapy of neural diseases. NKCC2 expression is specific to the kidney, leaving NKCC1 as the most relevant chloride-importing cotransporter for the brain, though it is expressed ubiquitously. Bicarbonate, generated by carbonic anhydrase, is another negatively charged ion that can permeate the GABAA receptor, generating a depolarizing

response [12, 24, 25]. The cytosolic carbonic anhydrase VII (CAVII) increases around postnatal day 12 (PN12) in the rat hippocampus [26], rendering bicarbonate-mediated GABA_A depolarizations more prominent [25].

There is considerable evidence that alterations in GABA signaling can cause seizures, as well as that seizures can change GABAergic signaling. In this review, we will discuss the bidirectional relationship of seizures to GABA_A signaling at the level of the neurons, GABA_A receptors, and the ionic symporters that control chloride homeostasis and the efficiency of GABA_A receptor mediated inhibition.

2. Correspondence of Developmental Stages between Rodents and Humans

To facilitate the translation of the experimental data into humans, it is worth reminding that the accepted correspondence of developmental stages between rodents and humans considers that the first week of life in rodents is equivalent to a premature newborn human, whereas the time of birth in rodents is considered to correspond to PN8-10. The rodent infantile stage is thought to extend till PN21, the onset of puberty is at PN32-35 in rodents, whereas PN60 rodents are considered young adults. However, it is important to emphasize that this is a very oversimplified translation, based mostly on correspondence of protein and DNA content in the brain. Each developmental process occurs at different tempos and is not always in synchrony with the above sequence of events. For example, by the end of the first postnatal week, rats are able to walk away from the nest, quite unlike the human newborns who cannot yet ambulate [27]. Direct demonstration of the time of shift of GABA_A receptor responses to hyperpolarizing has not been demonstrated in humans, though it has been suggested to occur before or soon after birth, based on the developmental patterns of the relative expression of NKCC1 and KCC2 [21, 28].

3. The Immaturity of GABA_Aergic Systems as an Age and Sex-Specific Risk Factor for Early Life Seizures

Seizures are more common in the early periods of life and especially in males [3, 4]. The immaturity of GABAergic inhibitory systems has been implicated in the heightened susceptibility of neonates to seizures and may also underlie the increased vulnerability of males, in whom the maturation of these systems is delayed compared to females. GABA is depolarizing in the neonatal life and it stays depolarizing for longer developmental periods in the male brain than in females [17, 29–33]. Paradoxical exacerbation of seizures by GABA-acting drugs has been reported in newborns, especially in low weight premature babies [34]. GABA-acting drugs, such as benzodiazepines and barbiturates, however, still remain the mainstay of treatments for neonatal seizures, even if they may not always be as effective in newborn human babies as in older patients [21, 35-39]. This is thought to be due to shunting inhibition or inhibition via excitatory effects upon inhibitory interneurons [40]. The composition



FIGURE 1: CCCs control GABA_A receptor-mediated inhibition. Panels (a) and (b) show the effects of NKCC1 activity in the absence (panel (a)) or presence (panel (b)) of GABA. NKCC1 mediates the electroneutral cotransport of Na⁺, K⁺, and 2 Cl⁻, increasing the intracellular Cl⁻ concentration. As a result, upon binding of GABA upon the GABA_A receptor, the channel pore opens and Cl leaves the neuron, causing a depolarization. Panels c and d show the effects of NKCC1 activity on GABA_A receptor function in the absence (panel c) or presence (panel d) of GABA. KCC2 in contrast exports K⁺ and Cl⁻ reducing intracellular Cl⁻. Activation of GABA_A receptors therefore results into influx of Cl and hyperpolarizing current. Their function is dependent upon the gradients of Na⁺ and K⁺, which are controlled by various factors, including background conductances, membrane voltage, and by the Na⁺/K⁺ ATPase.

of GABA_A receptors is also different in newborns, with less α 1 and more α 2/3 subunits, rendering them less responsive to benzodiazepines [41, 42]. Furthermore, the subcortical GABAergic networks that control seizures, like the substantia nigra pars reticulata (SNR), have not fully developed [31, 42–46]. The excessive GABAergic stimulation of the SNR, as is thought to occur due to GABA release during seizures, has proconvulsant effects early in life and anticonvulsant in older animals and this switch occurs earlier in females [44, 45]. It is therefore important to investigate and clarify the exact molecular determinants that control GABA_A inhibition in the young brain so as to optimize the treatment of seizures.

4. Aberrant GABA_A Signaling Predisposes to Seizures

Clinical and experimental evidences indicate that an initial perturbation of GABA_A signaling may facilitate seizures. A loss of inhibition could result in runaway excitatory circuits. Too much inhibition could also cause a seizure, either by disinhibiting epileptogenic networks or via promoting

neuronal synchronization ([67] reviewed by [68]). Excessive inhibition has been implicated in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) ([69] reviewed in [70]) or absence seizures [71]. Moreover, as GABA_A signaling is critical for brain development and early synaptogenesis [72–74], a disorder of GABA_A signaling early in life may cause miswiring or malformations that predispose to seizures (Figure 2).

Many GABA-related mutations are known to cause early life epilepsy. These include loss of function mutations or deletions of GABA_A receptor subunit genes that reduce their expression, or the duration, amplitude or agonist sensitivity of GABA_A currents. GABA_A receptor subunit mutations have been implicated in childhood absence epilepsy (CAE) [50, 51, 75], autosomal dominant epilepsy with febrile seizures plus (ADEFS⁺) [76], and other epileptic syndromes (reviewed in Table 1 and [77, 78]). Conditional mutants indicate that the developmental period of exposure to insults that disrupt GABA_A signaling may be critical in ictogenesis and epileptogenesis. Chiu et al. proposed that loss of function mutations of the GABA_A receptor subunits may have

GABA-related mutations	Species	Epilepsy type	Age at first observation	Ref.
	G	ABA _A receptor mutations		
GABRA1	Human	ADJME, CAE	Childhood, Juvenile	[47, 48]
GABRA6	Human	CAE	Childhood	[49]
GABRB3	Human	CAE	Childhood	[50– 52]
GABRD	Human	ADJME	Juvenile	[53]
GABRE	Human	Febrile, ADEFS ⁺ IGE	Infantile, childhood	[49]
GABRG2	Human, mouse	CAE ⁺ Febrile, ADEFS ⁺ , SMEI ADEFS ⁺ , SMEI, Febrile	Infantile, childhood	[54– 59]
GABRP	Human	IGE, ADEFS ⁺ , Febrile	?	[49]
		Other mutations		
GAD65 knockout	Mouse	Stress-induced, Limbic seizures	12 weeks	[60, 61]
ARX mutations	Human, mice	Early life epileptic encephalopathies (infantile spasms, Ohtahara)	Neonatal, Infantile	[62– 66]

TABLE 1: GABA-related mutations linked with seizures.

developmental effects in addition to their direct electrophysiological consequences [79]. Using a conditionally expressed loss of function mutation of the γ 2 GABA_A receptor subunit in mice, the investigators expressed the mutant allele for different periods of time. Mice that were induced to express the mutant allele for longer developmental periods displayed higher seizure susceptibility to pentylenetetrazole (PTZ), a drug that acts as a GABA_A receptor antagonist, compared to mice with late disruption of the γ 2 subunit expression.

Glutamic acid decarboxylase (GAD) isoforms GAD65 and GAD67 synthesize GABA in the brain. Knockout mice for the pyridoxal-5'-phosphate inducible GAD65 isoform, that generates the GABA reserve pools, have lower seizure threshold to picrotoxin, a GABAA receptor antagonist [61], or spontaneous seizures that can be precipitated by stress [60]. Although total GABA content in the brain may be normal or decreased in GAD65 knockout mice, depending upon the genetic substrate, it has been proposed that GAD65 loss of function may preferentially decrease the presynaptic reserve pool of GABA and decrease the tonic GABA inhibition, leading to increased seizure susceptibility [80-82]. Although no human GAD mutations have been found to consistently cause epilepsy [83], mutations in co-factors that are necessary for GAD65 function have been linked with early life seizures, as occurs in pyridoxine-de-pendency disorders [84, 85]. GAD65 or GAD67 loss suf-ficiently compensates for each other and does not appear to affect early brain development; albeit, cleft palate has been reported with GAD67 knockout mice [86]. Dual GAD65/67 knockout mice are not viable [87]. A small subset of patients manifests epilepsy secondary to an autoimmune response against

GAD65/67, although these appear mostly in adults [88–91].

5. Disrupting CCC Function May Predispose to Seizures

Decreased expression or function of chloride extruders may change seizure susceptibility by not only diminishing the efficacy of GABAA inhibition and promoting cellular swelling and degeneration under hypotonic conditions, but also by exerting broader developmental effects. Human linkage studies or transgenic knockout animal studies document that, at least in certain cases, seizures and epilepsy may ensue. There is currently no known human mutation of KCC2 associated with epilepsy. This may rather reflect the indispensability of KCC2, as complete KCC2 knockout mice die postnatally from respiratory failure, due to the immaturity of the respiratory system [93]. KCC2 has two known isoforms, KCC2a and KCC2b, of which KCC2b is thought to contribute to the developmental shift to hyperpolarizing GABA_A receptor currents [106]. KCC2bknockout mice demonstrate hyperexcitability at PN10 to PN16 (equivalent to human infantile age) [94] (Table 2). Although the expected intracellular accumulation of chloride and depolarizing shift of GABAA responses could easily explain the hyperexcitability, application of the GABA_A receptor antagonist picrotoxin paradoxically retains its excitatory responses [94]. Similarly, a different hypomorphic mutation in KCC2 causes a lower PTZ threshold for induction of clonic seizures in mice, despite the absence of gross morphological changes [95]. Such observations are

Neural Plasticity

CCC	Location	Mutation	Species	Neurological effect	Ref.
KCC1	Ubiquitous	Knockout	Mouse	None seen	[92]
KCC2	Brain	KCC2a and KCC2b knockout	Mouse	Death at birth	[93]
	Brain	KCC2b knockout	Mouse	Seizures, low weight, early mortality	[94]
	Brain	Hypomorph	Mouse	Increased seizure susceptibility and anxiety	[95]
	Brain	Heterozygote	Mouse	Hyperexcitability	[96]
KCC3	Ubiquitous	KCC3a-c knockout	Human, mouse	Peripheral neuropathy; seizures have been reported	[97– 100]
KCC4	Kidney, heart, lungs, liver	Knockout	Mouse	Deafness	[101]
NKCC1	Ubiquitous	NKCC1a knockout	Mouse	Deafness, circling behavior	[102]
	Ubiquitous	NKCC1a and NKCC1b knockout	Mouse	Deafness, circling behavior, growth retardation, defective spermatogenesis, increased threshold to thermal stimulation	[103, 104]
NKCC2	Kidney	Knockout	Human	Bartter's syndrome	[105]

TABLE 2: Phenotype of CCC mutations.

indicative of a residual inhibitory capacity of KCC2, either in the form of less potent hyperpolarizing GABA_A receptor currents or shunting inhibition [107]. However, the function of KCC2 is more complex, due to interactions with dendritic cytoskeletal proteins [108] or with other modulators of neuronal activity (i.e., increasing extracellular potassium) [109] which need to be further analyzed as to their ability to influence the phenotype of these mice.

Loss of function mutations in KCC3, which is expressed in many tissues, have been reported in patients with hereditary motor sensory neuropathy, some of whom have seizures as well as developmental deficits, like agenesis of the corpus callosum [100].

Altered CCCs may also affect brain development in a more subtle fashion, which could predispose a brain to epilepsy even if it does not directly cause seizures. From various fronts evidence emerges that shifts in the timing of emergence of hyperpolarizing signaling may have significant impact on neuronal and brain development and connectivity. Precocious appearance of hyperpolarizing GABA_A receptor signaling, either by KCC2 overexpression [72] or via loss of NKCC1 activity [110], disrupts cortical morphogenesis. Pharmacological inhibition of NKCC1 with bumetanide from embryonic day E15 to PN7 in otherwise normal mice disrupts cortical dendritic formation [74]. Abnormal cortical development and synaptic connectivity may predispose to seizures or cognitive impairment, which is both a predisposing factor and a common comorbidity of young patients with epilepsy [111].

6. Secondary Disruption of GABAergic Signaling in Risk Factors for Early Life Epilepsy

Conditions that predispose to epilepsy, genetic or acquired, may also create an imbalance in excitation/inhibition. Although their effects are not restricted to GABA_A signaling, in certain cases they may show a predilection to preferentially impair GABAergic inhibition.

Mutations of the aristaless-related and X-linked homeobox gene ARX have attracted a lot of interest due to their linkage with early life catastrophic epileptic syndromes, such as infantile spasms, Ohtahara syndrome, X-linked myoclonic seizures, spasticity and intellectual disability, idiopathic infantile epileptic dyskinetic encephalopathy, X-linked mental retardation [63–66, 112–116] (reviewed in [117]). ARX is a transcription factor that regulates the proliferation and



FIGURE 2: Schematic depiction of simple models through which dysregulation of GABAA receptor-mediated inhibition can increase the activity of neuronal networks, potentially generating seizures. GABA inhibition can fail when GABA or GABA_A receptor expression is low, when GABA depolarizes neurons, or when miswiring and mistargeting of synapses occur. Excessive GABA inhibition may trigger seizures by disinhibiting target cells, or via excessive synchronization of the neurons in the epileptogenic focus. Please note that the effects of dysregulated GABA signaling in more complex neuronal networks, especially in the presence of abnormal circuitry or with specific pathologies, may differ. In such cases a combination of the above models may be applicable at different sites of the epileptogenic network rendering the pharmacological effect of a GABAergic agonist not completely predictable by a single model. Furthermore, shunting inhibition may explain situations where GABAergic drugs silence excessive excitatory network activity, in neurons with depolarizing GABAergic signaling.

migration of GABA, calbindin, or neuropeptide Y positive interneurons but also of striatal cholinergic neurons [64, 66, 117]. Two recently published mouse models of ARX loss of function mutations, one of which specifically disrupted it in GABAergic interneurons destined to migrate to the neocortex, have recapitulated several phenotypes of infantile spasms and associated phenotype (cognitive, behavioral deficits and epileptogenesis) emphasizing the importance of deficient GABA inhibition for their pathogenesis [64, 66].

Angelman syndrome, a rare chromosomal deletion, involves the loss of ubiquitin-protein ligase 3A (UBE3A), but in certain patients there is a more extensive deletion of the 15q11-13 chromosomal locus that contains three GABA_A subunits, α 5, β 3, and γ 3 GABA_A receptor subunits [118]. Genotype-phenotype correlation suggested that deletion of the GABA_A receptor subunits is associated with more severe seizures, including infantile spasms, atypical absences, and myoclonus whereas patients with UBE3A mutations had a milder phenotype [118]. The β 3 subunit knockout mouse strain also develops a similar epilepsy phenotype [119].

Loss of function mutations of the voltage-sensitive sodium channel SCN1A gene is found in not only the severe myoclonic epilepsy of infancy (Dravet syndrome) but also in ADEFS⁺ syndrome [120–123]. SCN1A mutations have been proposed to preferentially impair the sodium channel activity of GABAergic interneurons, diminishing their activity [124]. Anti-NMDA autoantibodies detected in limbic encephalitis, a rare cause of refractory and frequent seizures [125], have been speculated to selectively target the NMDA receptors of presynaptic GABAergic terminals, reducing therefore GABA release [126].

Aberrant reappearance of depolarizing E_{GABA} and reduced GABA_Aergic responses have been proposed to underlie the pathogenesis of seizures from cortical malformations. Pathology and electrophysiological studies from human tissue specimens from patients with cortical dysplasias, that commonly predispose to early life seizures, have also suggested the presence of depolarizing GABA [20, 127, 128]. In the neonatal freeze lesion model, a shift to the immature pattern of high NKCC1/KCC2 ratio in the lesional site [129] as well as reduced $\gamma 2$ subunit expression and sensitivity to $\alpha 1$ subunit agonists in adulthood was described [130, 131]. In the rat model of cortical dysplasias induced by prenatal exposure to the 1-3-bis-chloroethyl-nitrosurea, reduced sensitivity to GABA was also seen in adulthood [132].

Traumatic brain injury in adults, such as in axotomized neurons, causes a reversal of GABAA signaling and CCC expression profile to the immature pattern (more depolarizing GABA and dominant NKCC1 over KCC2 activity) [133–135]. This appears to aid the survival and regeneration process, promoting the brain-derived neurotrophic factor-(BDNF-) dependent neuronal survival and may resolve with time, during recovery [135]. However, there is limited information as to the consequences of neuronal trauma upon the expression, physiology, and connectivity of GABAergic interneurons in developing animals. In the partially isolated undercut cortical model, reduced GABA_Aergic IPSCs and impaired chloride extrusion were found in juvenile rats, suggesting a possible correlation between impaired GABAergic inhibition and posttraumatic cortical excitability [136, 137]. Few studies have advocated against the use of GABA enhancing drugs and in favor of GABAA receptor inhibitors as interventions to improve cognitive outcomes [138]. More detailed studies are needed to determine the role of posttraumatic GABAA signaling changes for healing and regeneration in the developing brain as well as its impact on subsequent epileptogenesis and ensuing cognitive deficits.

7. Seizures Alter GABA_A Signaling

Seizures can affect almost every neurotransmitter system in the brain. Seizures can have immediate effects on $GABA_A$ signaling, that is, during the ictal period, or delayed, appearing after the termination of seizures. In both scenarios, the observed changes are dynamic and evolving. Seizures may interfere with the expression, composition, and subcellular distribution of GABA_A receptors and their regulatory factors, such as CCCs or regulatory kinases. Defining the timing of these events is crucial, not only to better understand the pathophysiological mechanisms investigating these changes but also to best interpret their pathophysiological relevance for epileptogenesis and brain function. The temporal Neural Plasticity

Seizure model	Age	Region	Effects on GABA _A receptors	Ref.
		Ictal changes		
In vivo SE (Lithium-pilocarpine; continuous hippocampal stimulation)	PN30	Hippocampus	Reduced surface expression of $\beta 2/3$, $\gamma 2$ subunits but not of δ .	[139]
In vivo SE (lithium-pilocarpine)	4–7 week old	Hippocampus	Internalization of $\beta 2/3$, $\gamma 2$ subunits; reduced mIPSCs	[140]
		After seizures		
Recurrent flurothyl seizures	PN1-5	Hippocampus, somatosensory cortex	Decreased amplitude of GABAergic IPSCs	[141, 142]
Flurothyl seizures	PN6 or PN6-10	Hippocampus	Decreased numbers of α 1-ir neurons	[143]
Kainic acid SE	PN9	Hippocampus	At 3 weeks postictally: α 1, α 4, γ 2 decrease; α 2, α 3 increase; α 5 increase (CA3 only); β 3 increase compared to controls	[144]
Lithium-pilocarpine	PN10	Hippocampus (dentate gyrus)	In adulthood: increased α1 expression, larger GABA current, enhanced zolpidem sensitivity	[145]
Lithium-pilocarpine SE	PN20	Hippocampus	Decreased α 1 and increased α 4 expression in the hippocampus of epileptic versus non-epileptic rats	[146]

TABLE 3: Effects of early life seizures on GABA_A receptors and currents in rats.

evolution of these events is also particularly important in developing rats, given the maturational changes that are ongoing. In addition, the age at first seizure, the type and severity of seizures, sex, epigenetic factors, medications, but also the cellular diversity of specific operant signaling systems further modify the final outcomes.

7.1. Ictal Attenuation of GABA_A Receptor-Mediated Inhibition. The urgency in treating early SE has long been recognized in the clinical literature. GABA-acting drugs, like benzodiazepines or barbiturates, are more effective early at onset of seizures than later on, when SE has been established [147, 148]. The transience of the efficacy of GABAergic drugs has been attributed to either increase internalization of selective synaptic GABA_A receptor subunits, such as of $\beta 2/3$ and $\gamma 2$, which mediate the effects of benzodiazepines and barbiturates [139, 140]. On the other hand, extrasynaptically located subunits that mediate tonic GABA inhibition, like the δ subunit, are not affected [139]. Failure of GABA_A receptor-mediated inhibition during prolonged seizures may also occur due to a positive shift in E_{GABA} either because of buildup of intracellular Cl⁻ concentration, from intense $GABA_A$ receptor-mediated chloride inward pumping, or from impaired chloride extrusion mechanisms, due to increased NKCC1 activity or decreased KCC2-mediated Cl⁻ efflux [149–151].

7.2. Postictal Changes. Loss of GABAergic interneurons is a hallmark pathology of focal epilepsies, like mesial temporal sclerosis [152-157]. In experimental studies, prolonged seizures can lead to interneuronal loss but such effects are age-specific. In newborn rats, during the first week of life, even 3 episodes of status epilepticus (SE) do not injure GABAergic neurons [30]; yet cell death becomes a progressively more prominent feature as the age at exposure to SE increases [155, 158–160]. In contrast, early life seizures functionally disrupt the physiology of GABAA receptor system. Age at the time of seizures, etiology or model of seizures, biological factors such as sex, as well as cell type and region-specific features may determine the end effects upon GABAA receptor subunits or the direction of GABA_A receptor-mediated responses (Tables 3 and 4). These changes may be either compensatory attempts to repair or restore normal function or, on the contrary, may contribute

 TABLE 4: Effects of Seizures on CCCs.

Model	Species	Age at seizures	Region	Effects	Ref.
			Ictal changes		
Kainic acid	Rat	PN6-7	Hippocampus	Switch from hyperpolarizing to depolarizing E _{GABA}	[184]
Low Mg ²⁺ seizures	Mice	PN5	Hippocampus	Bumetanide sensitive increase in [Cl ⁻] _i	[150]
			After seizures		
Kainic acid	Rat (male)	PN4-6	Hippocampus (at least 4 days postictally)	Increased KCC2; decreased NKCC1 activity; more hyperpolarizing E _{GABA}	[185]
Kainic acid	Rat (female)	PN4-6	Hippocampus (at least 4 days postictally)	No change in KCC2; increased NKCC1 activity; more depolarizing E _{GABA}	[185]
Kainic acid	Rat (male)	PN5-7	Hippocampus (immediate postictal period)	Increased surface expression of KCC2; hyperpolarizing shift of E _{GABA}	[171]

to the postictal dysfunction, comorbidities, or sequelae of seizures, such as cognitive dysfunction or epileptogenesis. Unlike the adults, in which the physiology of GABA_A receptor-mediated signaling has reached a relative steady state, developmental research is further complicated by the evolving changes that normally occur during the period when brain matures[161]. There is no systematic research study taking us step-by-step through all the complexity of seizure-induced postictal alterations in GABA_A receptor physiology and any extrapolations should be cautiously done pending confirmation by actual experimentations.

Seizures selectively interfere with the expression of certain, but not all, GABA_A receptor subunits [141–146] (Table 3). Kainic acid SE at PN9 rats favors the preservation of the immature pattern of GABA_A receptor complex (less α 1, more α 2/ α 3 subunits) on the third postictal week [144] that typically attributes slower IPSC kinetics and less sensitivity to benzodiazepines. Similarly, recurrent flurothyl-induced seizures, in the first 10 days of life, decrease α 1 expression and the amplitude of GABA_A receptor-mediated IPSCs [141–143]. Looking at longer-term outcomes of early life seizures, during adulthood, Brooks-Kayal's group has demonstrated that age at onset of SE is key at defining the final composition of GABA_A receptors and that this, in turn, may contribute to epileptogenesis. Lithium-pilocarpine SE at PN10 increases α 1 subunit expression in the dentate granule

cells in adulthood; in contrast, if SE is induced at PN20, a decrease in α 1 subunit is noted, but only in the epileptic animals [145, 146]. Interestingly, reconstitution of α 1 subunit expression prevented the occurrence of spontaneous seizures [146, 162].

The reports of untimely appearance of depolarizing GABA_A receptor signaling in a subpopulation of subicular neurons from adult human epileptic resected temporal lobes have attracted a lot of interest as a possible mechanism of epileptogenicity and potential refractoriness to GABA-acting antiepileptics [163, 164]. Depolarizing GABA_A receptor signaling has been linked to a dominance of NKCC1 over KCC2 activity in certain neurons of the epileptic tissue. It may also occur because of effective replenishment of intracellular bicarbonate by carbonic anhydrase during intense GABA_A receptor activation, which leads to a depolarization and to a consequent influx of Cl-, that enhances KCC2mediated K^+/Cl^- efflux [109]. The sequential interaction between carbonic anhydrase/GABA_A receptors/KCC2 may therefore increase extracellular K⁺, a factor that promotes the generation of ictal events. In support, carbonic anhydrase inhibitors have been used in certain cases as anticonvulsant therapies [109, 165].

Seizures in adult animals tend to increase the ratio of NKCC1 over KCC2 activity, reverting to a more immature pattern of CCC balance that favors depolarizing E_{GABA} [151, 166]. This is believed to occur in humans as well [127, 167–170]. But what happens, then, after early life seizures, when neurons are already in an immature state and how does this impact epileptogenesis and functional outcomes? In the immediate postictal period, following brief recurrent kainic acid seizures or an hour of kainic acid SE, KCC2 is reshuffled towards the plasma membrane, increasing its capacity to export Cl⁻ [171]. As a result E_{GABA} becomes more negative, contributing perhaps to the ability of the neurons to stop seizures.

In the longer run, further changes in E_{GABA} function occur, which are attributed to altered CCC expression or activity [30]. In our lab, we were interested in determining whether the original E_{GABA} , at the time seizures occur, may control the effects of seizures on CCCs and the direction of GABA_A receptor-mediated signaling, in other words, whether seizures might have different effects upon GABAA receptor-mediated signaling in neurons with depolarizing or hyperpolarizing GABA_A receptor mediated responses at the time of seizures. Taking advantage from the earlier appearance of GABA_A receptor currents in females than in males, we compared the effects of 3 episodes of kainic acid SE elicited at PN4, 5, and 6 (3KA-SE) in CA1 pyramidal neurons with depolarizing E_{GABA} (i.e., male) or isoelectric/hyperpolarizing E_{GABA} (i.e., female) at the time of seizures [30]. We found that 3KA-SE caused only a transient appearance of depolarizing GABA_A receptor mediated responses in neurons that had already started to shift to mature and more hyperpolarizing EGABA, similar to what was previously described for the adult neurons. In contrast, in male neurons, with still depolarizing GABAergic responses, 3KA-SE caused a precocious emergence of mature, hyperpolarizing responses. These changes were attributed to

altered expression and/or activity of KCC2 and NKCC1. The precocious termination of depolarizing GABA_A signaling would be expected to deprive brain from its neurotrophic effects that are important for normal development [72, 74]. Indeed, 3KA-SE-exposed pups develop learning and memory problems when they grow up (unpublished data). However, the inability of the immature neurons to persistently exhibit depolarizing GABAA receptor-mediated responses after seizures could be a protective feature against the development of subsequent epilepsy [30]. Our results indicate that age-specific factors, including the depolarizing GABA, may be important for this protection. Another dual regulator of CCCs and EGABA through development is the brainderived neurotrophic factor (BDNF) pathway, which is also activated in certain seizure models. BDNF increases KCC2 in developing neurons but decreases it in mature neurons [172, 173]. The opposite patterns of KCC2 regulation by BDNF in certain systems has been proposed to be due to trkB-mediated activation of different intracellular signaling cascades that regulate KCC2 expression [151].

The maturation of GABA_A receptor system occurs asynchronously across different neuronal types and brain regions. As a result, since early life seizures change the direction and strength of GABA_A receptor-mediated inhibition, their effects will be region and cell type specific, further confusing the interneuronal communication protocols. They may also disrupt the basic neural processes of learning and cognitive processing that depend upon GABA neurotransmission, such as long-term potentiation (LTP) [174–176], or social interactions [177–182]. The result will be a state of postictal confusion or more sustained cognitive or behavioral deficits [6]. Of interest, bumetanide treatment has shown benefit in five infants with autism [183]. However the exact mechanisms underlying this therapeutic effect are not yet known.

8. Implications for Early Life Seizures and Their Treatment

Human and experimental evidence indicates that similar to adults, aberrant preservation of depolarizing GABA_A signaling may also be a feature of the medically refractory epileptogenic focus in early life epilepsies. At present we do not have any data to discuss the pathological features of the medically sensitive early life epilepsies. The idea of pharmacologically enhancing GABA inhibition to stop seizures by using NKCC1 inhibitors like bumetanide is under investigation as a rationally developed, smart intervention to overcome the barriers posed by the well-established molecular switch of GABA_A receptor function [21]. Beneficial effects have been shown in few animal models [21, 186-189] and a human case report [190]. However, model-specific differences, as well as the timing of administration, can influence its efficacy in suppressing seizures [96, 191]. Moreover, concerns have been raised about potential adverse developmental effects on innocent bystander normal brain tissues, as may occur in chronic use in patients with focal epilepsies [74]. Undoubtedly, more studies need to be done to determine which seizure types are more likely to respond,

when is the optimal time to administer, for how long, and how such interventions influence long-term outcomes in subjects who have already experienced seizures or have epilepsy. Similarly, by increasing our knowledge about the specific changes that occur in GABA_A receptor composition and pharmacology, it may be possible to design more selective and specific GABAA receptor agonists for the very young or epileptic brain that is refractory to the existing medications. At the anatomical and electrophysiological level, it might be feasible, one day, to design such specific, very targeted, and individualized therapies to enhance GABA inhibition and stop seizures. The biggest challenge will be however to predict the functional state of GABAA receptormediated inhibition at the target areas, so as to implement such rational therapies. Emerging evidence suggests that GABA-acting drugs, hormones, and different stressors are among the factors that can alter GABA_A receptor signaling, rendering it almost a moving target [11, 30, 31, 192–196]. The need for biomarkers of GABAA function is therefore a priority.

9. Conclusion

The study of GABA in seizure generation and consequences has become a very fruitful field not only by generating intriguing results but also by producing challenging new questions. We have learned a number of mechanisms that compromise GABA_A inhibition in the very young or epileptic brain, predisposing to seizures and the associated cognitive and neurodevelopmental deficits. We still need to better understand and, most importantly, predict which is the normal balance between excitation and inhibition with sufficient age, sex, cell type, and regional, context, and function-related specificity, so as to preserve normal brain function and development.

Abbreviations

ADEFS ⁺ :	Autosomal dominant epilepsy with
	febrile seizures plus
ADJME:	Autosomal dominant juvenile
	myoclonic epilepsy
ADNFLE:	Autosomal dominant nocturnal frontal
	lobe epilepsy
ARX:	Aristaless-related X-linked homeobox
	gene
BDNF:	Brain-derived neurotrophic factor
CAE:	Childhood absence epilepsy
GABA:	Gamma aminobutyric acid
GABR:	GABA _A receptor
GAD:	Glutamic acid decarboxylase
IGE:	Idiopathic generalized epilepsy
IPSC:	Inhibitory postsynaptic current
3KA-SE:	3 episodes of kainic acid SE at PN4,5,6
KCC:	Potassium chloride cotransporter
LTP:	Long-term potentiation
NKCC:	Sodium potassium chloride
	cotransporter

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