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CRITICAL REVIEW

Excitatory/inhibitory balance in epilepsies and neurodevelopmental disorders: Depolarizing γ-aminobutyric acid as a common mechanism

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Abstract

Epilepsy is one of the most common neurological disorders. Although many factors contribute to epileptogenesis, seizure generation is mostly linked to hyperexcitability due to alterations in excitatory/inhibitory (E/I) balance. The common hypothesis is that reduced inhibition, increased excitation, or both contribute to the etiology of epilepsy. Increasing evidence shows that this view is oversimplistic, and that increased inhibition through depolarizing γ-aminobutyric acid (GABA) similarly contributes to epileptogenisis. In early development, GABA signaling is depolarizing, inducing outward Cl^- currents due to high intracellular Cl^- concentrations. During maturation, the mechanisms of GABA action shift from depolarizing to hyperpolarizing, a critical event during brain development. Altered timing of this shift is associated with both neurodevelopmental disorders and epilepsy. Here, we consider the different ways that depolarizing GABA contributes to altered E/I balance and epileptogenesis, and discuss that alterations in depolarizing GABA could be a common denominator underlying seizure generation in neurodevelopmental disorders and epilepsies.

KEYWORDS

epilepsy, GABA, inhibition, neurodevelopment

1 | INTRODUCTION

Epilepsy is one of the most common neurological disorders, and is associated with a very heterogeneous etiology, including brain injury and environmental or genetic factors. Increasing evidence supports that epileptic seizures and neurodevelopmental disorders (NDDs) go hand in hand. Although both epilepsies and NDDs are classified into different disease categories, they share phenotypic overlap and genetic risk factors.[1] Epilepsies and NDDs include rare monogenic disorders, caused by single gene variants, or more common causes predisposed by polygenic risk factors. Epilepsies with a monogenic origin, like developmental and epileptic encephalopathies (DEEs), are often

accompanied by comorbid neurodevelopmental problems, such as developmental delay, autism, neuropsychiatric disorders, or intellectual disability (ID).[2] Alterations in cognitive functioning in these DEEs are thought to be influenced by both seizure and interictal epileptiform activity, and the neurobiological process behind epilepsy.[3] The prevalence of epilepsy is higher in ID patients than in the general population, and epileptic seizures are an important comorbidity of monogenic causes of NDDs such as Rett syndrome, tuberous sclerosis, and fragile X syndrome.[4-6] Furthermore, evidence suggests fundamental etiological overlap between genetic generalized epilepsy, schizophrenia, and autism spectrum disorders (ASDs), as common genetic variants predispose to many of these diagnostic categories,[1] and protein products from NDD and epilepsy risk genes converge on similar molecular pathways.[7]

Especially defects in γ-aminobutyric acidergic (GABA) -ergic inhibition seem to underlie the strong bidirectional relationship between NDDs and epilepsy.[7] In the healthy adult brain, balance between excitation and inhibition (E/I) is tightly regulated and essential to maintain network dynamics, with GABA as the main inhibitory and glutamate the main excitatory neurotransmitter. E/I disbalance in the brain is a common mechanism underlying epileptogenesis and neurodevelopmental and psychiatric disorders.[8,9] It is generally thought that reduced inhibition, increased excitation, or both lead to E/I disbalance, for example, through changes in pathogenic variants encoding for essential players in the GABAergic signaling or ion channels.

Apart from these alterations, accumulating evidence suggests that increased depolarizing GABAergic signaling during development can be a contributor to epileptogenesis in both NDDs and epilepsy, and that this relationship is bidirectional.[10] We here review the influence of depolarizing GABA on altered E/I balance and epileptogenesis in the context of both NDD and epilepsy. We first explain the importance of depolarizing GABA during brain development, and discuss evidence that depolarizing GABA can underlie seizure generation, but that sustained seizure activity can in turn also cause a depolarizing GABA shift. Finally, we substantiate the hypothesis that alterations in depolarizing GABA could be an additional common pathway that contributes to seizure generation and altered neurodevelopment in NDDs and epilepsies.

Key Points

- Depolarizing GABA is an essential developmental mechanism
- Depolarizing GABA can be both the cause and the consequence of epileptic seizures
- Alterations in the timing of the depolarizing to hyperpolarizing GABA switch can underly E/I disbalance in both NDDs and epilepsies

2 | DEPOLARIZING GABA: AN IMPORTANT DEVELOPMENTAL MECHANISM

In adult neurons, classical inhibitory signaling typically occurs due to Cl^- influx through the $GABA_A$ receptor channel, hyperpolarizing the cell. This is achieved through the maintenance of low intracellular Cl^- concentrations, facilitated by secondary active extrusion through cation Cl^- cotransporters (CCC).[11] The CCCs are encoded by the *SLC12* family genes, and the two main CCCs in the central nervous system are KCC2 and NKCC1.[12] KCC2 specifically localizes to neurons and maintains a low intracellular Cl^- concentration through extrusion of Cl^- by harnessing K^+ gradients.[13] Proper KCC2 functioning depends on stimulatory phosphorylation of serine 940 (Ser940), important for KCC2 activity and membrane localization.[14] KCC2's counterpart NKCC1 utilizes the inward Na^+ and K^+ gradient to drive Cl^- influx.[15] In adult neurons, the internal Cl^- concentration is low due to high expression of KCC2, transporting Cl^- outward. $GABA_A$ receptor activation therefore causes Cl^- influx, and hyperpolarization of the cell, leading to inhibition.

Overall, passive Cl^- in- or outflow from the $GABA_A$ receptor is mediated by the difference between the Cl^- equilibrium potential (E_{Cl}^-) and the membrane potential (V_m).[16] In mature neurons, the internal Cl^- concentration is low and E_{Cl}^- is typically more negative relative to the neuronal resting membrane potential (V_{rmp}). The activation of the $GABA_A$ receptor will therefore result in Cl^- influx and hyperpolarization of V_m, reducing the likelihood of action potential initiation. When the E_{Cl}^- is more positive in relation to the V_{rmp}, $GABA_A$ receptor activation results in Cl^- efflux, and as a consequence neuronal depolarization. Therefore, low intracellular Cl^- facilitates inhibition, and high intracellular Cl^- facilitates GABA-mediated excitation.[16] Besides inhibition through hyperpolarization, GABA can in addition exert inhibitory action through shunting inhibition when E_{Cl}^- is equal to V_{rmp}. In this case, the membrane resistance is decreased through activation of $GABA_A$ receptors, increasing background conductance, and therefore reduced efficacy of excitatory signals.[17]

Inhibitory input is of crucial importance to tightly regulate E/I balance in the adult brain. Blocking GABAergic synapses leads to seizures, whereas enhancing inhibition results in sedative, anticonvulsive, and anxiolytic effects. This delicate balance poses a problem specifically in the

developing brain. A mismatch between the strength of GABA and glutamate could either result in the prevention of synapse formation and development or cause toxicity.[18]

The switch in GABA action from depolarizing to hyperpolarizing during brain development, also called GABA switch or shift, offers an interesting hypothesis to address this mismatch.[18] During early development, GABA action is depolarizing, due to high intracellular Cl^- and low expression of the Cl^- extruder KCC2 (Figure 1). Depolarizing GABA controls early network activity by contributing to synchronous giant depolarizing potentials (GDPs), which facilitate circuit and synapse formation by allowing large Ca^{2+} oscillations in immature cells, even in neurons without, or with only a few, synapses.[18,19] This Ca^{2+} influx is an important instruction signal to regulate activity-dependent plasticity and neurite growth.[19,20] Depolarizing GABA moreover mediates cell proliferation and cell migration, and by doing so, regulates important developmental switching points (for a detailed review see Peerboom and Wierenga[19]). During maturation, the lower intracellular concentration of Cl^- is facilitated by an increase in the expression of the chloride extruder KCC2, leading to the conventional hyperpolarizing and inhibitory effects of GABA. This switch is thought to be driven by activity and sensory input, transforming the primitive network activity pattern, driven by excitatory GABA, into a more sophisticated and diverse network activity.[18] It is hypothesized that when these delicate developmental processes go awry, the brain can become susceptible to epileptogenesis.

3 | ROLE OF DEPOLARIZING GABA IN ACQUIRED EPILEPSIES WITHOUT A GENETIC ORIGIN

Throughout life, the neonatal period has the highest seizure probability, especially in males.[21] There is indirect evidence that immature GABAergic signaling plays a role in epileptogenesis in young infants. For example, the increased vulnerability of male neonates could be partially explained by the observation that the maturation of the GABAergic system is slightly delayed in the substantia nigra of male rats.[22] Treating epileptic seizures with GABA agonists in young infants, especially in preterm neonates, can have adverse effects, suggestive of a role of depolarizing GABA in seizure activity early in life.[23,24] Moreover, barbiturates and benzodiazepines often suppress only the motor aspect of neonatal seizures, probably by suppressing seizure activity in the spinal cord and brainstem, but do not suppress the seizure activity observed

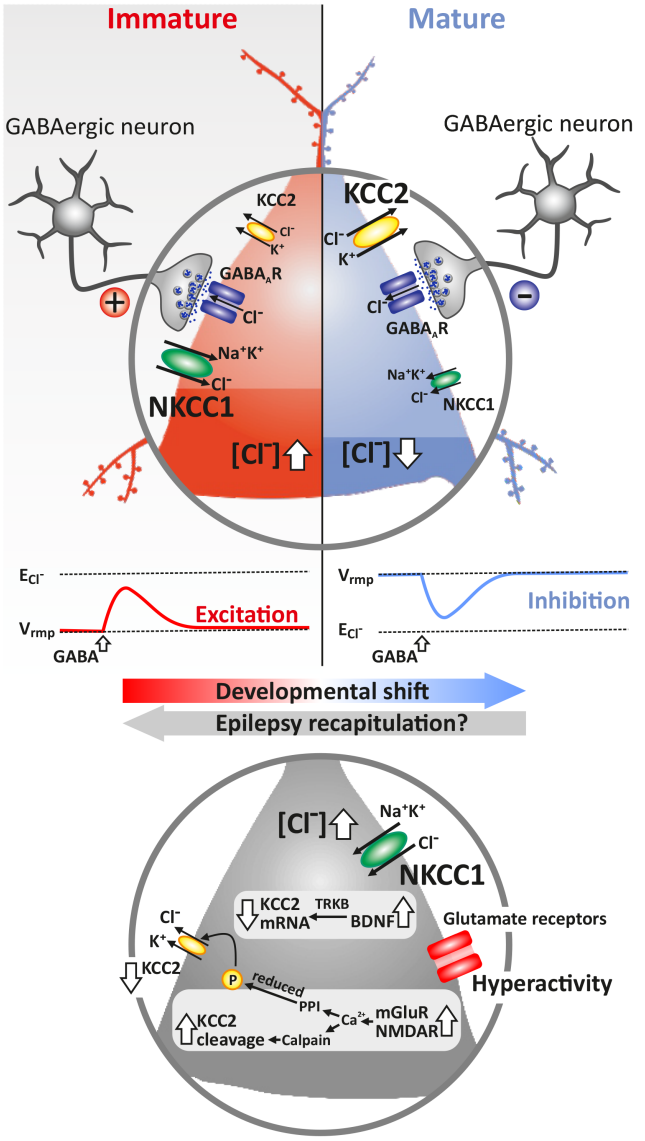

FIGURE 1 Schematic overview of γ-aminobutyric acid (GABA) action on both immature (left) and mature (right) neurons. Cl^- intruder NKCC1 maintains high intracellular Cl^- in immature neurons, leading to Cl^- efflux and neuronal depolarization upon $GABA_A$ receptor activation. During development upregulation of Cl^- extruder KCC2 leads to low intracellular Cl^- in mature neurons, leading to Cl^- influx and neuronal hyperpolarization upon $GABA_A$ receptor activation. The activity and expression of NKCC1 and KCC2 determine the value of the Cl^- equilibrium potential (E_{Cl}^-), relative to the resting membrane potential (V_{rmp}), and consequential excitation or inhibition of GABA action. Conditions associated with reduced expression or function of KCC2 can lead to reversal of this developmental shift, leading to reduced inhibitory actions of GABA. Hyperactivity in turn causes reduced KCC2 expression and functioning through glutamate receptor-mediated molecular pathways. mGluR, metabotropic glutamatergic receptor; NMDAR, N-methyl-D-aspartate receptor; P, phosporylation; PPI, protein phosphatase I. Figure adapted from Liu et al.[49]

on electroencephalogram (EEG).[25] This could be due to the observation that hyperpolarizing GABA signaling in neurons matures in a caudal to rostral fashion,[26] and that more rostral structures are therefore still under the influence of depolarizing GABA.[27,28] Finally, Cl^- intrusion by NKCC1 could be an additional contributing factor promoting hyperexcitability.[23,27] Evidence in hippocampal slices of status epilepticus rats showed that an increase in NKCC1 expression after neonatal seizures resulted in further hyperexcitability.[27,29] In conclusion, an underdeveloped GABAergic system could be an important contributor to epileptogenesis in early life seizures without an underlying genetic or structural malformation. However, additional evidence is needed, as a direct link is not yet established. Especially the exact timing of the GABA switch is difficult to determine in humans, because it is proposed to differ between cell types or brain regions.[27,30] Due to the nature of material collection, only a few studies have looked into KCC2 expression in the human brain. Jansen et al. examined postmortem and surgically removed cortical brain tissue samples from healthy children and adolescents, and found that the NKCC1/KCC2 ratio was higher up until 2 years, and subsequently remained low into adulthood.[31] This further suggests that in the first years of life, GABAergic signaling might still be immature in some cortical regions, supporting the hypothesis that neonatal seizures are less responsive to GABAergic anticonvulsants.

In light of this, it is important to evaluate the most suitable treatment for neonatal seizures, especially as evidence suggests that severe and prolonged seizures could lead to KCC2 cleavage and inhibition, and therefore further rises in intracellular Cl^- concentrations, providing a negative feedback loop.[32] Studies in rats have shown that the NKCC1 blocker bumetanide could be used to treat neonatal seizures, in addition to the antiepileptic drug phenobarbital.[27,33,34] A pilot study in patients with temporal lobe epilepsy (TLE) and a phase 2 randomized controlled clinical trial in neonatal seizures provided promising preliminary observations.[35,36] However, other studies provide conflicting results, and have reported that bumetanide did not increase the efficacy of phenobarbital treatment in a rat model of birth asphyxia.[37] Furthermore, an earlier clinical trial was halted after adverse reactions (ototoxicity) with no evidence of seizure reduction.[38] The effectiveness of bumetanide as a possible additional treatment is currently therefore heavily debated, especially given that bumetanide has poor blood–brain barrier permeability. It is proposed that bumetanide can halt epileptogenesis only shortly after a few seizures, and the efficacy might depend on the severity and frequency of seizures.[39] Efforts investigating treatment options should therefore also look into other possibilities. For example, the diuretic torasemide, blocking NKCC1, potentiated antiseizure effects of midazolam in a rat model of birth asphyxia, and prevented cognitive impairment postasphyxia.[40] Enhancing KCC2 could be another potential target to investigate in addition to blocking NKCC1,[41-43] although the protein is short lived and rapidly internalized following seizures.[44] Future studies should investigate the precise molecular mechanisms and timing of the developing GABAergic system in humans, considering different brain regions or cellular subtypes. This might help explain the conflicting results regarding bumetanide as treatment for epilepsies and provide avenues for new therapeutical interventions.

In addition to primary seizurogenic effects of delayed GABA maturation, secondary effects from prolonged seizurogenic activity might further implicate depolarizing GABA.[30] Evidence that prolonged seizure activity can potentially result in altered GABAergic signaling comes from postmortem or surgically removed tissue of patients with idiopathic generalized epilepsy. For example, surgically removed tissue from patients with chronic TLE exhibited depolarizing, and sometimes even excitatory GABAergic transmission, and altered activity of Cl^- transporters, especially in tissues with severe sclerosis as a result from seizures.[45,46] Moreover, a decreased KCC2 and increased NKCC1 mRNA and protein expression was observed in surgically removed tissue from drug-resistant TLE patients.[47] Depolarizing GABA is also implicated in patients with brain tumors or gliomas, which are often highly epileptogenic in nature. In cortical tissue of patients with gliomas, from both postmortem and surgical origin, peritumoral neurons exhibited decreased KCC2 and increased NKCC1 expression, accompanied by reduced hyperpolarized responses to application of GABA.[48] The loss of GABAergic inhibition in these peritumoral regions leads to further hyperexcitability and vulnerability to seizures.[48,49] Moreover, GABA depolarizes 65% of pyramidal neurons in surgically removed tissue from glioma patients, likely due to a 144% increase of NKCC2.[50]

Due to the nature of tissue collection for these studies, from patients eligible for brain surgery or from postmortem tissue, it is challenging to extrapolate these findings to earlier phases of epileptogenesis.[51] Scientists use seizurogenic compounds to induce epileptogenesis in animal models of acquired epilepsies, which are a more suitable candidate to study seizurogenic mechanisms. The induced epileptic insults are followed by a latent period, after which the animal develops chronic spontaneous seizures.[52] These animal models provide further evidence that induction of spontaneous seizures leads to a depolarizing shift in the GABA reversal potential (E_{GABA}).[53] In wild-type mice, kainic acid-induced seizures reduced the expression of KCC2, already 30 min after seizure induction.[54] Pilocarpine, a

muscarinic acetylcholine receptor agonist, induces status epilepticus in mice, which in turn results in reduced cell surface stability of KCC2.[55] During the latent period of epileptogenesis, depolarizing GABA transmissions were observed in principal neurons in hippocampal slices of pilocarpine-induced status epilepticus rats.[29] Furthermore, several studies in rodent induced seizure models have shown that KCC2 remains downregulated during the latent period, up to 14 days after pilocarpine-induced seizures.[29,56,57,58,59] Similar deficits in KCC2 expression were observed in a mouse model for traumatic brain injury, where KCC2 protein and mRNA expression were reduced in the dentate gyrus, causing a depolarizing shift in E_{GABA} 7 days postinjury.[60] Finally, induced stroke in mice leads to overactivation of N-methyl-D-aspartate receptors through glutamate excitotoxicity, in turn resulting in reduced KCC2 and $GABA_A$ receptor expression.[61] This has resulted in decreased tonic inhibition post-stroke, which was associated with improved motor performance, but leads to seizures as an adverse side effect. Although KCC2 downregulation and depolarizing GABA are sustained during the latent periods of epileptogenesis, it remains unclear whether this mechanism is underlying the subsequent development of spontaneous seizures, or that it only contributes to seizure susceptibility, as numerous other mechanisms can contribute to altered E/I balance. Further studies examining the exact mechanism behind KCC2 downregulation and temporal modifications in KCC2 function are therefore needed.[51]

4 | ROLE OF DEPOLARIZING GABA IN EPILEPSIES WITH A GENETIC ORIGIN

Before the gene *SLC12A5*, encoding KCC2, was recognized as an epilepsy gene in patients, studies in animal models showed that reduced KCC2 functioning resulted in epilepsy phenotypes. Full homozygous *Slc12a5* knockout (KO) mice die right after birth, due to respiratory failure caused by reduced GABAergic inhibition in the spinal cord.[62,63] Heterozygous *Slc12a5* KO mice show increased seizure susceptibility and anticonvulsant resistance.[63] Comparable lethality and seizure phenotypes were reported in drosophila where the *kcc* gene was mutated.[64] Moreover, preventing S940 phosphorylation in KCC2, in S940A homozygous knockin mice, contributes to the severity and onset of status epilepticus, emphasizing the importance of this particular phosphorylation site for the proper functioning of KCC2.[54]

The first evidence that genetic variants in KCC2 play a role in the development of epilepsy in humans was observed in idiopathic generalized epilepsy, where an enrichment of two nonsynonymous *SLC12A5* risk variants was found in patients compared to controls.[65] One of these risk alleles was also identified in an Australian family with an inherited form of febrile seizures. Expressing this particular risk allele resulted in decreased Cl^- extrusion and depolarized E_{GABA} in mouse primary neurons.[66] Similar nonsynonymous variants were later described in human autism spectrum disorder and schizophrenia patients.[7] Although these studies provide evidence that *SLC12A5* variants enhance risk for epileptogenisis and neurodevelopmental disorders, causality was not proven.[51] Exome sequencing in two unrelated families with epilepsy of infancy with migrating focal seizures resulted in the identification of missense mutations in three different exons of *SLC12A5*, describing the first monogenic mutations in *SLC12A5*.[67] Whole exome sequencing uncovered another six additional compound heterozygous variants in *SLC12A5* causing severe infantile onset epilepsy and developmental delay in three other families.[68] These studies provided the most significant genetic association of KCC2 impairments with epilepsy to date, indicating that variants in KCC2 can cause a monogenic epilepsy disorder.[69]

KCC2 disease-causing variants are not very prevalent, and are only causal for a very small subset of genetic epilepsies. The most prevalent genetic cause of early childhood epilepsy is mutations in *SCN1A*, encoding the voltage-gated sodium channel $Na_V1.1$, which cause epilepsy in a broad phenotypic spectrum.[70] This spectrum ranges from febrile seizures to severe Dravet syndrome (DS), considered a DEE, as it is accompanied by neurodevelopmental comorbidities. The leading hypothesis explaining the pathological mechanisms behind mutations in *SCN1A* is that reduced Na^+ current in inhibitory neurons causes loss of inhibition and therefore hyperexcitability, but the exact cellular mechanisms remain unclear, as excitatory neurons also contribute to seizure generation.[71] In addition, few studies implicate depolarizing GABA in the E/I imbalance in *SCN1A*-related epilepsies. The first evidence came from observations in *Scn1b*-deficient mice, which exhibited GDPs, preceding hyperexcitability and epileptogenisis.[72] This observation was later confirmed in heterozygous $Scn1a^{+/-}$ mice, showing a depolarized $GABA_A$-reversal potential.[73] Moreover, bumetanide delayed the onset of sudden unexpected death in epilepsy in both $Scn1a^{+/-}$ and $Scn1b^{-/-}$ mice, supporting that depolarizing GABA contributes to one of the most debilitating symptoms in DS.[73] Transplanting postmortem membranes from DS patients into *Xenopus* oocytes revealed a depolarized shift in E_{GABA} and concordant reduction in KCC2 expression.[74] Furthermore, induced pluripotent stem cell (iPSC)-derived neurons from patients with a heterozygous missense mutation in *SCN1A* showed an increased

NKCC1/KCC2 ratio, suggesting an immature phenotype.[75] Finally, in a computational model of DS, depolarizing $GABA_A$ currents were sufficient to cause DS-related EEG activity to transition from background activity to interictal epileptic spikes and seizurelike activity.[76] Similar evidence comes from research investigating the DEE gene *SYNGAP1*. *Syngap1*$^{+/-}$ mice displayed decreased KCC2 expression in granule cells, including a decreased Cl^- reversal potential. Administration of a GSK3B inhibitor during the critical period rescued these deficits, which counteracted the increase in intracellular Cl^- concentrations, and restored cognitive deficits through an unknown mechanism.[77]

Together, these studies provide evidence that depolarizing GABA may play a role in hyperexcitability in epilepsies with a genetic origin, whether directly due to pathogenic variants in *SLC12A*, or due to indirect effects from mutations in *SCN1A* or *SYNGAP1*. Recently, research investigating *KIF4*, a newly identified DEE gene, uncovered that *Kif4*$^{-/y}$ mice have a lower seizure threshold and an overactivated TrkB pathway, leading to decreased KCC2 expression and a higher intracellular Cl^- concentration.[78] However, future research is needed to explore whether depolarizing GABA could be linked to other well-known DEE genes as well, such as *PCDH19*, *STXBP1*, or *SCN8A*.

5 | ROLE OF DEPOLARIZING GABA IN NEURODEVELOPMENTAL DISORDERS WITH EPILEPSY AS A COMORBIDITY

Patients with NDDs have an increased risk for epilepsy, and it is one of the most common comorbidities.[79] Although altered E/I balance and depolarizing GABA are implicated in NDDs that result from common genetic variants, such as ASDs,[80] we will focus here on the contribution of depolarizing GABA to epileptogenisis in NDDs with a monogenic origin.

Down syndrome results from chromosome 21 trisomy. Studies estimate the prevalence of epilepsy in Down syndrome between 1.4% and 17%.[81] Hippocampal neurons in the *Ts65Dn* mouse model for Down syndrome displayed depolarizing $GABA_A$ currents, which contributed to the learning and memory defects.[82] Moreover, postmortem brain tissue of Down syndrome patients showed enhanced NKCC1 expression.[82] Neither Ts65Dn mice nor Down syndrome patients exhibited downregulation of KCC2 expression, although KCC2 function through phosphorylation was not investigated.[51] More evidence is therefore needed to directly link depolarizing GABA to Down syndrome etiology.

The NDD Rett syndrome is the second major cause of ID in females, and is caused by mutations in *MECP2*. The majority of patients suffer from epileptic seizures.[83] Neurons differentiated from Rett patient-derived iPSCs showed deficits in KCC2 expression and a concordant delayed GABAergic switch, which could be rescued by overexpression of KCC2 or IGF1 treatment.[84] This study showed that *MECP2* could directly regulate KCC2 gene expression by binding to the KCC2 promotor region, suggesting that developmental upregulation of KCC2 fails in patients with Rett syndrome.[84] Similarly, *Mecp2*$^{-/y}$ KO mice exhibited E/I disbalance, caused by a reduced expression of KCC2 and a depolarized $E_{GABA.}$[85] Rett syndrome patients showed reduced KCC2 protein in cerebrospinal fluid[86] and reduced KCC2 expression in postmortem brain tissue.[87] Using high-throughput screening, Tang and colleagues developed a compound library with KCC2-enhancing molecules, and tested these molecules in vitro in *MECP2*-deficient human iPSC-derived neurons, and in vivo in *Mecp2*$^{-/y}$ KO mice. Treatment with hit compounds restored KCC2 levels, and rescued electrophysiological and morphological phenotypes, suggesting KCC2 as a promising therapeutic target.[88]

Fragile X syndrome is caused by CGG repeats in *FMR1*, encoding a protein involved in mRNA translation of predominantly synaptic proteins.[89] The CGG repeats lead to silencing of the *FMR1* gene.[90] The *Fmr1*$^{-/y}$ KO mouse displayed high levels of NKCC1 expression in cortical neurons, causing a delay in the hyperpolarizing GABA shift.[91] Another study showed that *Fmr1*$^{-/-}$ KO mice had elevated intracellular Cl^- levels, paralleled by an increased excitatory GABAergic transmission.[92] Blocking NKCC1 with bumetanide rectified Cl^- imbalance and restored E_{GABA} in the somatosensory cortex of *Fmr1*$^{-/y}$ KO mice, resulting in a corrected development of excitatory synapses and long-lasting improvement of somatosensory circuit function and behavioral deficits.[93]

Together, these studies indicate a possible contributing role of altered Cl^- homeostasis to the epileptogenesis associated with NDDs.[51] Furthermore, preventing the normal phosphorylation of KCC2 in healthy mice resulted in neurodevelopmental deficits, providing more evidence that KCC2 dysfunction not only leads to increased susceptibility to epilepsy, but can also result in altered neurodevelopment.[94] Depolarizing GABA could be a possible therapeutic target during specific developmental time windows, potentially targeting not only epilepsy, but also neurodevelopmental defects in NDDs. Even when considering the ongoing controversy on the beneficial effects of bumetanide in patients with epilepsy, treatment with bumetanide or other NKCC1 inhibitors has proven beneficial in (pre-)clinical studies for NDDs, including ASDs and tuberous sclerosis syndrome (reviewed by Ben-Ari

et al.).[39,95,96] However, more evidence is needed to directly link neurodevelopmental defects and depolarizing GABA, as it remains unclear through which mechanisms sustained depolarizing GABA results in altered neurodevelopment and cognitive functioning.

6 | DISCUSSION: FROM DEPOLARIZING GABA TO SEIZURE GENERATION AND VICE VERSA

Previous paragraphs describe the implication of depolarizing GABA due to KCC2 dysfunction in epilepsies both with or without a genetic origin, and in NDDs with epilepsy as a comorbidity. However, the specific mechanisms underlying seizurogenesis due to impaired KCC2 function in these patient groups remain unclear. Most examples come from research in animal models or from postmortem tissue, where the spatiotemporal occurrence of depolarizing GABA could be different, and more direct evidence from human patients is necessary. Furthermore, the current paradigm that disrupted KCC2 activity leads to seizures by elevation of intracellular Cl^-, depolarizing $E_{GABA,}$ and therefore causing an E/I imbalance, requires refinement. As explained by Staley,[97] seizure activity represents only 1% of total brain activity in epilepsy patients, and is therefore a rare and often abrupt event. How does continuously decreased KCC2 activity result in these rare and abrupt transitions to epileptiform activity? Moreover, is KCC2 dysfunction a result from ongoing seizure activity, or is epileptogenisis a result from sustained depolarizing GABA due to KCC2 dysfunction? Evidence shows that KCC2 might be one of the contributors to the two principal mechanisms behind epileptogenesis, both inducing aberrant network activity and conferring vulnerability to disinhibition through activity-dependent mechanisms, resulting in a lower seizure threshold (Figure 1).[69,97]

Dysfunction of KCC2 and the resulting depolarized E_{GABA} are clearly associated with epileptogenesis, as proven by mutations in *KCC2* in patients and animal models that result in epilepsy. In addition to the regulation of intracellular Cl^- and the switch of GABA from depolarizing to hyperpolarizing, KCC2 also plays a vital part in functional maturation. KCC2 signaling is of fundamental importance in the migration of inhibitory precursors.[19] Moreover, KCC2 interacts with the dendritic cytoskeleton and the organization of dendritic spines.[98,99] Interestingly, KCC2 also plays a role in delivering α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors to the membrane,[98] and is therefore important for the development of both GABAergic and glutamatergic signaling. In patients, these disrupted developmental processes therefore might predispose to epilepsy and the effect of KCC2 on Cl^- homeostasis in mature neurons, resulting in aberrant network activity.

Acute disruption of KCC2 activity may confer vulnerability to activity-dependent disinhibition, which might also explain the abrupt transition into seizures. Studies show that exposure to excess glutamate in preseizure stages results in Ser940 dephosphorylation and therefore reduced KCC2 activity and membrane expression.[100] This decreased activity results in reduced Cl^- extrusion, which in turn results in disinhibition. In rodents, expression of $Slc12a^{S940A/S940A}$ mutant leads to aberrant Ser940 phosphorylation and increases the lethality of kainate-induced seizures.[54] In addition, patients with mutations in *KCC2* have been identified who show decreased phosphorylation at Ser940.[65] Similarly, brain-derived neurotrophic factor (BDNF) is an important negative regulator of KCC2 expression and function through the TrkB pathway, reviewed in detail by Moore et al.[51] BDNF levels increase within the brain during seizure activity.[101] It is proposed that a glutamate- or BDNF-mediated decrease in KCC2 activity confers susceptibility to acute transitions to seizures[69] and the reduction of KCC2 expression observed in epilepsy patients.[51] Finally, KCC2 downregulation is associated with immediate severe seizures, supporting the hypothesis that altered expression of KCC2 might not be the consequence of epileptic seizures, but rather one of the contributing factors.[102]

Moreover, studies have shown that intracellular rises in Cl^- can occur dynamically within minutes through a process called Cl^- loading. During epileptic seizures, the firing rate of interneurons increases as a result of general network hyperexcitability. This leads to overloading of GABA on the postsynaptic cell and as a result a rapid increase in intracellular Cl^- and a dynamic shift of GABA from depolarizing to hyperpolarizing.[103] Cl^- loading is typically a short-lasting effect, as the KCC2 rapidly extrudes Cl^- from the cytoplasm. However, during pathological conditions, when KCC2 expression or functioning is aberrant, inadequate extrusion of Cl^- under hyperexcitability aggravates seizure severity.[104] In conclusion, loss of KCC2 function contributes to activity-dependent disinhibition, although the exact molecular mechanisms still need to be further investigated.

Finally, it needs to be stressed that KCC2 dysfunction and a depolarizing shift in GABAergic signaling are highly relevant, but not the only contributors to altered E/I balance in epilepsy and neurodevelopment. Previous reviews on E/I balance, in the fields of psychiatric disorders,[8] epilepsies, and NDDs,[105,106] outlined the various mechanisms at play. When considering GABAergic signaling, in addition to alterations in variants encoding for CCCs, GABA-related pathogenic variants equally contribute to altered E/I balance and could result in a range of clinical

TABLE 1 Overview of the evidence implicating depolarized γ-aminobutyric acid in epilepsies without a genetic origin, epilepsies with a genetic origin, and neurodevelopmental disorders with epilepsy as comorbidity.

Epilepsies without a genetic origin		
Neonatal seizure syndromes	Mouse model	
	Ischemic seizures	Kang et al. (2015)[115] Carter et al. (2018)[116] Kipnis et al. (2020)[133] Sullivan et al. (2021)[43]
	Rat model	
	Bumetanide as treatment	Dzhala et al. (2005, 2008)[27,33] Cleary et al. (2013)[117]
	Patients	
	Bumetanide as treatment	Soul et al. (2021)[35]
TLE	Mouse model	
	Kcc2 ablation (hippocampus)	Kelley et al. (2018)[133]
	Patients	
	Brain tissue	Cohen et al. (2002)[45] Huberfeld et al. (2007)[46] Palma et al. (2006)[47] Muñoz et al. (2007)[118]
	Bumetanide as treatment	Eftekhari et al. (2013)[36,119] Gharaylou et al. (2019)[119]
	Computational model	Buchin et al. (2016)[120]
Induced seizures	Rat model	
	CTZ induced	Chen et al. (2017)[102]
	Pilocarpine induced	Pathak et al. (2007)[57] Li et al. (2008)[56] Bragin et al. (2009)[59] Barmashenko et al. (2011)[29] Yu et al. (2013)[58]
	Mouse model	
	0-Mg^{2+} and 4AP induced	Kelley et al. (2016)[121]
	Pilocarpine induced	Lee et al. (2010)[55]
Brain tumors, gliomas, brain injury, and CD	Mouse model	
	Glioma	Campbell et al. (2015)[122] MacKenzie et al. (2016)[123]
	Brain injury	Bonislawski et al. (2007)[60]
	Ischemic seizures	Jaenisch et al. (2016)[61]
	Patients	
	Tissue from gliomas	Conti et al. (2011)[48] Pallud et al. (2014)[50]
	Tissue from CD	Munakata et al. (2007)[124] Shimizu-Okabe et al. (2011)[125] Talos et al. (2012)[126] Han et al. (2019)[127]

TABLE 1 (Continued)

Epilepsies with a genetic origin		
SLC12A-related epilepsy syndromes	Drosophila model	
	kcc2$^{-/-}$	Hekmat-Scafe (2006)[64]
	Mouse model	
	Slc12a$^{-/+}$	Woo et al. (2002)[63]
	Slc12a phosphorylation-deficient mice	
	Slc12a$^{S940A/S940A}$	Silayeva et al. (2015)[54]
	Slc12a$^{T906A\ /T906A}$	Moore et al. (2018)[128]
	Slc12a$^{T906E/+}$ and *Slc12a*$^{T1007E/+}$	Pisella et al. (2019)[94]
	SLC12A variants in patients	
	IGE patients	Kahle et al. (2014)[65]
		Puskarjov et al. (2014)[66]
	EIMFS patients	Stödberg et al. (2015)[67]
		Saitsu et al. (2016)[68]
		Saito et al. (2017)[129]
DS	Mouse model	
	Scn1b$^{-/-}$	Brackenbury et al. (2013)[72]
	Scn1a$^{+/-}$ and *Scn1b*$^{-/-}$	Yuan et al. (2019)[73]
	DS patients	
	Postmortem tissue	Ruffolo et al. (2018)[74]
	iPSC-derived neurons	Scalise et al. (2022)[75]
	Computational model	Kurbatova et al. (2016)[76]
NDDs with comorbid epilepsy		
Down syndrome	Mouse model	
	Ts65Dn	Deidda et al. (2015)[82]
	Patients	
	Postmortem tissue	Deidda et al. (2015)[82]
Rett syndrome	Mouse model	
	Mecp2$^{-/y}$	Banerjee et al. (2016)[85]
	Mecp2$^{-/y}$	Tang et al. (2019)[88]
	Patients	
	CSF	Duarte et al. (2013)[86]
	Postmortem tissue	Hinz et al. (2019)[87]
	iPSC-derived neurons	Tang et al. (2016)[84]
		Tang et al. (2019)[88]
SYNGAP1	Mouse model	
	Syngap1$^{+/-}$	Verma et al. (2022)[77]
OPHN1	Mouse model	
	Ophn1$^{-/y}$	Maset et al. (2021)[130]
Tuberous sclerosis syndrome	Patients	
	Tissue from TSC patients	Talos et al. (2012)[127]
		Ruffolo et al. (2016)[131]
	Bumetanide as treatment	van Andel et al. (2020)[111]
		Juarez-Martinez et al. (2022)[114]

(Continues)

TABLE 1 (Continued)

Fragile X syndrome	Mouse model	
	Fmr1$^{-/y}$	He et al. (2014)[91]
	Fmr1$^{-/-}$	Tyzio et al. (2014)[92]
	Fmr1$^{-/y}$	He et al. (2019)[93]

Abbreviations: 4AP, 4-aminopyridine; CD, cortical dysplasia; CSF, cerebrospinal fluid; CTZ, cyclothiazide; DS, Dravet syndrome; EIMFS, epilepsy of infancy with migrating focal seizures; IGE, idiopathic generalized epilepsy; iPSC, induced pluripotent stem cell; NDD, neurodevelopmental disorder; TLE, temporal lobe epilepsy; TSC, tuberous sclerosis complex.

phenotypes, including epilepsy and NDDs. For example, pathogenic variants in genes encoding for GABA receptor subunits and GABA transporters, in particular *SLC6A1*,[107] contribute to altered E/I balance and as a result can lead to a clinical phenotype encompassing both epilepsy and altered neurodevelopment. Mutations in the genes *GABRA2* and *GABRA5*, encoding for GABA receptor subunits, have been found in patients with different types of DEE.[108,109] Furthermore, a splice site variant in *Gabra2* is associated with earlier seizure onset in *Scn8a* conditional KO mice, suggesting that gene interactions could play an additional role in seizure susceptibility.[110] This opens the way to further speculations on GABA-related E/I misbalance as a contributor to both seizure generation and altered neurodevelopment.

7 | CONCLUSIONS

Abundant evidence shows that depolarizing GABA might contribute to seizure generation in multiple ways. Moreover, this might trigger epileptogenesis not only in patients with genetic or acquired epilepsies, but also in patients with NDD with epilepsy as a comorbidity, suggesting a common underlying mechanism. The evidence implicating depolarizing GABA in these patient groups is summarized in Table 1. Treatment strategies that enhance intracellular Cl^- concentrations are currently under investigation in both patient groups, either as primary medication or as combinational therapies.[35,111,112,113,114] However, several hurdles need to be overcome. The idea that GABA is depolarizing early in development and hyperpolarizing later in development might be too simple.[113] NKCC1 might actually increase as well, rather than decline, as KCC2 expression increases during development. NKCC1 and KCC2 might not be the only determinants of intracellular Cl^- levels, and the distribution of intra- and extracellular Cl^- might differ between cell type or cell compartment, which might play a critical role in the direction of E_{GABA}. Direct measurements of intracellular Cl^- levels, instead of indirect measurements through quantification of KCC2/NKCC1 ratios, are necessary to fully understand the direction of E_{GABA}. In addition, how transporter dependent or independent functions of KCC2 in neurodevelopment contribute to pathology remains an outstanding question. To fully grasp the therapeutic potential of Cl^- homeostasis in epilepsy, future studies need to disentangle these specific components. Finally, as discussed briefly, other molecular and cellular mechanisms also contribute to altered E/I balance in NDDs and epilepsies. A therapy targeting depolarizing and immature GABA signaling might thus not rescue the complete phenotype, but could be explored as an additional therapeutic target. In the end, seizures are not only a debilitating symptom of disease, but will also contribute to further neurological decline, which will make these investigations worth the effort.

AUTHOR CONTRIBUTIONS

Eline J. H. van Hugte wrote the manuscript. Dirk Schubert and Nael Nadif Kasri edited the manuscript. Dirk Schubert made the figure.

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CONFLICT OF INTEREST STATEMENT

None of the authors has any conflict of interest to disclose.

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