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REVIEW

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Depolarizing GABA and Developmental Epilepsies

Roustem Khazipov,[1,2,3] Guzel Valeeva[3] & Ilgam Khalilov[1,2,3]

1 INMED-INSERM U901, Marseille, France
2 Aix-Marseille University, Marseille, France
3 Laboratory of Neurobiology, Kazan Federal University, Kazan, Russia

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Correspondence
R. Khazipov, INMED-INSERM U901, 163, Avenue de Luminy, 13273 Marseille Cedex 09, France.
Tel.: +33 6 85141144;
Fax: +33491828101;
E-mail: Roustem.khazipov@inserm.fr
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SUMMARY

Early in development, GABA, which is the main inhibitory neurotransmitter in adult brain, depolarizes immature neurons and exerts dual—excitatory and shunting/inhibitory—effects in the developing neuronal networks. The present review discusses some general questions, including the properties of excitation at depolarizing GABAergic synapse and shunting inhibition by depolarizing GABA; technical issues in exploration of depolarizing GABA using various techniques and preparations, including the developmental aspects of traumatic injury and what is known (or rather unknown) on the actions of GABA *in vivo*; complex roles of depolarizing GABA in developmental epilepsies, including a contribution of depolarizing GABA to enhanced excitability in the immature networks, caused by repetitive seizures accumulation of intracellular chloride concentration that increases excitatory GABA power and its synchronizing proconvulsive effects, and correction of chloride homeostasis as a potential strategy to treat neonatal seizures.

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Introduction

GABA is the main inhibitory neurotransmitter in adult brain, and GABAergic inhibition is considered to be the main brake in the neuronal networks that prevents generation and spread of paroxysmal activities [1]. Many of the currently used antiepileptic drugs act through enhancing GABAergic functions that includes positive allosteric modulators of GABA(A) receptors, blockers of GABA uptake, and blockers of GABA degradation. However, early in development, GABA exerts depolarizing and excitatory actions on immature neurons [2]. Similar paradoxical effects of GABA have been also found in neurons from the hippocampus of adult epileptic patients [3–5]. This raises a series of questions on the roles of GABA in the developing brain functions and in developmental epilepsies. In the present review, we will discuss some of the most frequently asked questions about depolarizing GABA and its relation to developmental epilepsies based on results obtained using animal models, mainly neonatal rodents.

General Questions

Q1: Why GABA Depolarizes Immature Neurons?

Developmental depolarizing actions of GABA are due to elevated intracellular chloride concentration $[Cl^-]_i$, which is set in the immature neurons primarily as a result of high expression of chloride loader NKCC1 and low expression of chloride extruder KCC2 [6–8] (for reviews, see [9,10]). Such a combination of chloride cotransporters, with a chloride load prevailing over chloride extrusion, sets $[Cl^-]_i$ at elevated levels at resting state. As a result, chloride Nernst potential and the reversal potential of currents through GABA(A) channels (E_{GABA}) are set in the immature neurons at values more positive (typically by 10–30 mV) than the resting membrane potential (E_m). This generates positive driving force acting on chloride currents through GABA(A) receptors (DF_{GABA}), and when GABA(A) channels open, negatively charged chloride ions flow out from the cell generating inwardly directed electrical current which produces depolarizing GABA(A) receptor-mediated postsynaptic potentials (GABA-PSPs). Depolarizing action of GABA is either eliminated or reduced by NKCC1 antagonist bumetanide [7,8,11,12]. However, other mechanisms to accumulate chloride may maintain GABA depolarizing even in the absence of NKCC1 such as anion exchanger AE3, which accumulates chloride in exchange for intracellular bicarbonate [13,14] and sodium-dependent anion exchangers [15]. In addition, chloride gradient maybe also influenced by cytoplasmic impermeant anions and polyanionic extracellular matrix glycoproteins that constrain the local $[Cl^-]$[16]. According to this hypothesis, the developmental reduction in $[Cl^-]_i$ would follow from the increase in cytoplasmic impermeant anions [17] and proteoglycans of the extracellular matrix [18].

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Q2: Is Excitatory Action a Synonym of Depolarizing Action?

In strict terms, excitatory neurotransmitter is the one that is capable of triggering action potentials (APs) in the postsynaptic neuron. This is the case when (i) the reversal potential of the conductance activated by the neurotransmitter is more positive than the AP threshold; characteristic of classical excitatory neurotransmitters glutamate in CNS and acetylcholine at the neuromuscular junction with the reversal potential near 0 mV, but also when (ii) the reversal potential is below the AP threshold, but the depolarization evoked by neurotransmitter activates voltage-gated conductances such as persistent sodium currents or low-threshold calcium currents that further depolarize the neuron to reach the AP threshold. The latter mode of excitation is typical for GABA in the immature neurons where E_{GABA} barely reaches AP threshold and persistent sodium current is required to trigger postsynaptic APs [19–22]. Moreover, not all cells are excited by depolarizing GABA even at the most immature state, and the proportion of cells with excitatory GABA response progressively decreases with age [12,20].

In a broader sense, excitatory actions also include APs facilitation by other otherwise subthreshold inputs [23]. Similar effects have been also reported in some types of adult neurons such as L5 pyramidal cells where GABA evokes subthreshold depolarizing responses [24]. It also involves activation of the voltage-gated calcium channels that evokes calcium transients in the immature neurons [25,26], and attenuation of the voltage-dependent magnesium block of NMDA channels [27,28].

It is important to add that in some adult neurons, hyperpolarizing GABAergic responses are also capable of evoking APs through rebound excitation which involves hyperpolarization-activated *Ih* currents and low-threshold calcium currents that for example occur during spindle oscillations in thalamus [29].

Q3: Are There Differences in the Excitation by GABA and Glutamate?

Excitatory power of GABA is much less than that of glutamate because of a difference in the driving forces: nearly 70–80 mV for glutamate and only 10–30 mV for GABA. As a result, transmission of excitation at depolarizing GABAergic synapses is characterized by relatively low probability and long and variable AP delays. Thus, in the neonatal rat hippocampal slices, the delays of APs evoked by synaptic activation of GABA(A) receptors are long (mean, 65 ms) and variable (within a time window of 10–200 ms) [22]. Depolarizing GABAergic responses are typically subthreshold and their amplification by persistent sodium conductance is required to trigger APs. Recruitment of this intermediate step explains long and variable AP delays. AP delays maybe artificially shortened and their variability reduced with an increase in $[Cl^-]_i$ during whole-cell dialysis so that at symmetric $[Cl^-]_{i/o}$, the transmission of excitation at GABAergic synapse becomes similar to the glutamatergic synapses. Slow and variable transmissions of excitation at depolarizing GABA synapses together with shunting effects of depolarizing GABA (see *Q4*) have major impact at the network level explaining low synchrony and slow propagation of the giant depolarizing potentials (GDPs) in the neonatal hippocampus, an immature network activity pattern driven by depolarizing GABA and glutamatergic synapses [22,27,28,30–32]. GDPs are desynchronized and slowed down with a low concentration of bumetanide, whereas complete blockade of NKCC1 eliminates GDPs [8,22,33,34]. Positive allosteric modulator of GABA(A) receptors diazepam increases GDPs' frequency but also desynchronizes neurons during GDPs and slows down GDPs' propagation [22,35]. An increase in GABAergic excitatory power and synchronizing shift in GABA actions is observed following multiple recurrent seizures as a result of accumulation of $[Cl^-]_i$ [34,36–38].

Q4: What is a Shunting Inhibition by Depolarizing GABA?

Activation of GABA(A) receptors increases membrane conductance. Therefore, according to the Ohm's law, voltage change produced by any other current (e.g., glutamatergic EPSCs) will reduce proportionally to the increase in membrane conductance caused by GABA. This shunting mechanism underlies inhibitory effects of depolarizing GABA at primary afferents as well as at cortical depolarizing GABAergic synapses in adult L5 pyramidal cells and granular cells of dentate gyrus [24,39]. In addition to the direct shunting effect, GABAergic depolarization may activate voltage-gated potassium channels, and cause an inactivation of voltage-gated sodium channels that decreases cell excitability further contributing to the inhibitory actions of depolarizing GABA [40,41]. Shunting GABAergic inhibition prevents paroxysmal activity in the immature brain, and the GABA(A) receptor antagonists evoke interictal-like events in hippocampal slices and tonic–clonic ictal-like discharges in the intact hippocampus preparation and *in vivo* [27,35,42–47].

Thus, GABA depolarizes immature neurons and exerts dual—excitatory and shunting/inhibitory—effects in the developing neuronal networks. Depolarizing GABA is essential for the generation of the network-driven GDPs, whereas slow and variable excitations at depolarizing GABAergic synapses and shunting effects of GABA determine low neuronal synchrony during GDPs and their slow propagation. Blockade of GABA(A) receptors evokes seizure-like activity in the neonatal cortical networks through the elimination of shunting inhibition.

Methodological Aspects of Some Techniques and Preparations

Depolarizing and excitatory actions of GABA on immature neurons have been initially demonstrated in preparations of isolated neurons and brain slices using intracellular sharp-electrode recordings and later on confirmed using gramicidin-perforated patch recordings, cell-attached recordings of single GABA channels and imaging techniques using intracellular calcium indicators and by direct measurements of intracellular chloride using chloride sensors (for review, [2]). There are several important technical issues that should be taken into account when measuring GABAergic signals, however.

Q5: How Cell Dialysis Affects GABA Signals?

Primary rule in measuring GABAergic responses is that the recordings should not modify $[Cl^-]_i$. This is not a trivial task,

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however. Indeed, $[Cl^-]_I$ is easily modified during whole-cell patch clamp or intracellular recordings as a result of cell dialysis by the pipette solution. This change is not uniform within the entire cell, however. Chloride transporters act to maintain natural chloride concentration and the changes in $[Cl^-]_i$ caused by dialysis reduce at a distance from the electrode. For example, imposing elevated chloride during somatic whole-cell recordings evokes depolarizing GABAergic responses at the soma with a reversal potential close to the one predicted by the Nernst equation [48]. However, GABA (A) reversal potential shifts toward more negative values in dendrites proportionally to the distance from the soma, and the somato-dendritic gradient is diminished by KCC2 blockade. Second problem resulting from dialysis is associated with an alteration in the concentration of ions (such as sodium and potassium) that are implicated in the function of chloride cotransporters that may occur during intracellular recordings with molar concentrations of $[K^+]_i$ or during gramicidin-perforated patch-clamp recordings with nonphysiological $[K^+]_i$ or $[Na^+]_i$ or as a result of effect on the activity of chloride cotransporters, like Cs^+ that blocks KCC2.

Q6: How Important is to Know the True Resting Membrane Potential?

DF_{GABA} is defined as a difference between E_{GABA} and resting membrane potential of the cell (E_m). Therefore, correct measurement of GABA actions requires not only maintenance of physiological $[Cl^-]_i$ levels but also intact E_m. Unfortunately, most of the invasive techniques introduce error in E_m measurements which is associated with an introduction of the leak conductance during recordings through a contact between the electrode and cell membrane. This leak conductance is around 500 MOhms in case of intracellular recordings and several GOhms in case of whole-cell or perforated patch recordings. Leak conductance may introduce an important error in the measurement of E_m particularly in small neurons with high membrane resistance where the leak resistance is comparable to the membrane resistance [49]. For example, in the neonatal rat hippocampal neurons which have membrane resistance in the gigaohm range, membrane potential measured during whole-cell or gramicidin-perforated patch recordings was of −44 mV [50]. However, membrane potential deduced from the reversal potential of the currents through single NMDA channels recorded in cell-attached mode was of −77 mV (see also [19,51,52] (Figure 1). Leak-induced error in E_m measurement is also considerable in adult neurons accounting for 10–13 mV depolarized values of E_m [50,53,54]. In addition to an error in the

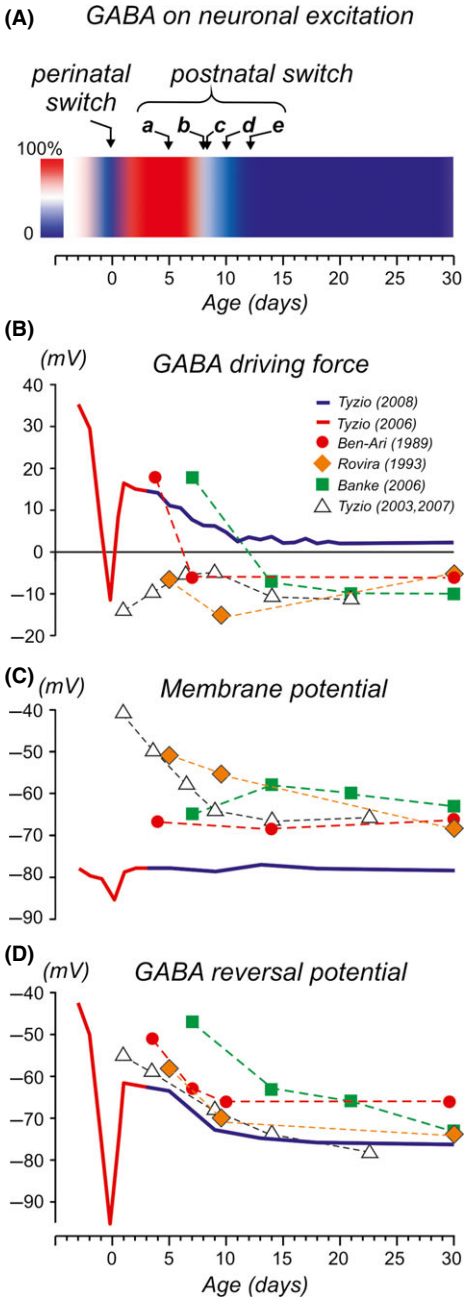

Figure 1 Developmental profile of GABA signaling in hippocampal CA3 pyramidal neurons. (**A**) Excitatory-to-inhibitory (*E–I*) developmental switches in the action of GABA on Wistar rat hippocampal CA3 pyramidal cells were estimated by pooling the results of cell-attached and gramicidin-perforated patch recordings [12,20]; the color code indicates the proportion of cells with excitatory GABA. Note the first transient switch near term and the second permanent switch during the second postnatal week. Arrows (*a* to *e*) indicate the estimates of the second permanent E–I switch obtained using different methodological approaches: (a) at postnatal day (P)5, using sharp electrodes [30,114]; (b) at P8, using extracellular multiple unit activity (MUA) recordings and synaptic activation of GABA(A) receptors [20]; (c) at P8, using gramicidin-perforated patch recordings [20]; (d) at P10, using MUA recordings and brief bath application of the GABA(A) agonist isoguvacine [20] [at P13 in Sprague–Dawley rats [44]]; (e) at P12, using MUA recordings and the GABA(A) antagonist bicuculline [99]. Estimates of the E–I switch obtained using $[Ca^{2+}]_i$ imaging techniques also placed it between P5 and P12 [115,116]. (**B–D**) Developmental changes in the GABA driving force (DF_{GABA}), resting membrane potential (E_m), and GABA(A) reversal potential (E_{GABA}) in CA3 pyramidal cells inferred from cell-attached recordings of single GABA and *N*-methyl-D-aspartate (NMDA) channels [19] and from the results obtained using intracellular [30,117], gramicidin-perforated patch [20,118] and cell-attached recordings of potassium channels [118]. Adapted from [19].

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estimation of the true E_m, neuronal depolarization also likely affects E_{GABA} because $[Cl^-]_i$ is sensitive to E_m. Indeed, prolonged depolarization and hyperpolarization cause transient positive and negative shifts in E_{GABA}, respectively [11,55–57].

Q7: How to Deduce GABA Polarity from Cell-Attached Recordings of GABA Channels?

Cell-attached recordings of single GABA(A) channels measure directly DF_{GABA} affecting neither E_{GABA} nor E_m. In cell-attached recordings, DF_{GABA} is estimated from the current–voltage relationships of the currents through GABA(A) channels and equals—*Vp*, where *Vp* is the potential at the patch pipette. The technique has been used to show depolarizing actions of GABA in various types of immature neurons [12,19,58] including epileptic tissue [34,59] (Figure 1). Limitations of this technique include eventual contamination of non-GABA channels in the recorded patch of membrane that are hard to control because with this technique, recordings from the same patch of membrane without GABA are unavailable (unless GABA is infused into the tip of the pipette [60]). In addition, currents via GABA channels show poor signal/noise ratio near the reversal potential (which is often <10 mV from the resting membrane potential), and thus, the polarity of the GABAergic signals and DF_{GABA} values can be only deduced from approximations of the current–voltage relationships based on values distant from E_{GABA}. Some of these problems can be solved using cell-attached recordings of integral currents through GABA(A) receptors activated by photouncaging of GABA from its caged forms, such as RuBi-GABA [56]. Analysis of cell-attached recordings of GABA channels faces a question of what formula should be used for the I–V approximations? Theoretically, the best fit is provided by Goldman–Hodgkin–Katz (GHK) equation. In practice, these approximations are usually performed using exponential or linear fits of the I–V relationships. While the exponential fit is close to the near-reversal region of GHK relationships, linear fits, in particular those approximating only negative part of the I–V curve, seem to provide erroneous DF_{GABA} values. Clearly, further methodological work is required to find the best formula to fit the I–V relationships of the currents through GABA channels.

Q8: Is Depolarizing GABA a Result of Neuronal Trauma?

A seminal study by Van den Pol and colleagues revealed that neurons may acquire a secondary excitatory GABA phenotype following various types of trauma including neurites transection [61] that has been confirmed in various models of neuronal trauma [62–65]. Moreover, existing evidence for depolarizing and excitatory action of GABA on the immature neurons has been primarily obtained using preparations of brain slices, dissociated neurons, or a few days *in vitro* neuronal cultures. Yet, neurons are severely injured during slice preparation, in particular those of them located close to the slice surface [66–69]. This raised a hypothesis that excitatory action of GABA seen in the immature neurons actually reflects not a physiological phenomenon but rather a developmental aspect of traumatic injury, debated in [70,71]. Through the exploration of developmental GABA actions in the intact hippocampal preparation and at different depths of hippocampal slices, the following findings have been made [68,72]: (i) *in the intact hippocampus in vitro:* bumetanide-sensitive GDPs and depolarizing/excitatory actions of GABA were observed during postnatal days P1-3 and switched to hyperpolarizing and inhibitory actions at around P4-5; (ii) *in the hippocampal slices*: excitatory actions of GABA were revealed at all depths of slices including the core and surface until ≤P5; during the second postnatal week (P7-14), damaged neurons at the slice surface displayed elevated $[Cl^-]_i$ and excitatory GABA phenotype, whereas neurons in the slice core showed low $[Cl^-]_i$ and were inhibited by GABA; in >P14 slices, GABA uniformly inhibited neurons through all depths, whereas the surface neurons did not survive. Thus, depolarizing/excitatory actions of GABA during development involve two age-specific mechanisms: (i) *developmental*, which is present during the first postnatal days and which fades by the end of the first postnatal week both in slices and the intact hippocampus and other intact neonatal preparations including immature retina [73–76], optic tectum of Xenopus laevis tadpoles isolated brain *in vitro* [77], and embryonic and neonatal rat spinal cord *in vitro* [78–80]) and (ii) *related to injury* elevation of $[Cl^-]_i$ associated with an acquisition of a secondary excitatory GABA phenotype, which is primarily expressed at the slice surface during the second postnatal week. Evidently, the trauma-related alterations in $[Cl^-]_i$ and GABA actions at the slice surface should be considered during exploration of GABAergic functions using slice preparations from adolescent animals.

Q9: Does GABA Excite Immature Cortex *in vivo*?

While considerable evidence indicates that GABA exerts depolarizing and excitatory actions in the isolated immature preparations *in vitro*, there is critical lack of knowledge on whether GABA exerts or not depolarizing and excitatory actions on immature cortical neurons *in vivo*. This question is technically difficult to address as an access to cells, pharmacological manipulations, and stimulations are much more difficult to perform than *in vitro* and, as discussed above, because the E_m and E_{GABA} are more severely compromised during recordings from small immature neurons. These problems were well illustrated by Purpura and colleagues [81]. In their study, intracellular recordings from the hippocampal neurons in neonatal kittens revealed large amplitude hyperpolarizing IPSPs in response to fornix stimulation. However, cells were strongly depolarized upon electrode impalement to approximately −20 mV and showed depolarization block of the action potentials. This artificial depolarization of neurons likely results from the leak conductance introduced to the cells during sharp-electrode recordings (see *Q6*). While these results convincingly show early establishment of GABAergic synapses on hippocampal neurons, they preclude from any conclusion on whether GABA depolarizes or hyperpolarizes immature neurons in their intact state. Measurement of DF_{GABA} using cell-attached recordings of GABA channels in the superfused hippocampus preparation during the first postnatal week revealed depolarizing action of GABA in the somatic region of immature hippocampal neurons [19]. So far, all other available arguments on the polarity and excitatory/inhibitory GABA actions in the immature cortex *in vivo* are indirect, and they are mainly based on the effects of the drugs or genetic

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manipulations with GABA(A) receptors or chloride cotransporters on the animal behavior, network-driven activity patterns, neuronal migration and differentiation [82–89], for a review, see [71]. Therefore, physiological actions of GABA on immature cortical neurons *in vivo* and the roles of depolarizing GABA in brain development remain an important question for the future investigations.

Thus, exploration of depolarizing and excitatory actions of GABA requires the techniques and preparations in which neither resting membrane potential nor intracellular chloride concentration is altered. Early depolarizing and excitatory actions of GABA are not related to neuronal trauma during preparation of tissue for recordings; however, injury during slice preparation may cause an acquisition of excitatory GABA phenotype in the neurons located at slice surface in the adolescent animals. Most importantly, the actions of GABA on immature cortical neurons *in vivo* remain unresolved.

Depolarizing GABA and Neonatal Seizures

As discussed above, depolarizing GABA with its dual excitatory and shunting/inhibitory actions critically influences excitability of the developing neuronal networks contributing to generation of the early network-driven activity patterns but also has a strong impact on seizure susceptibility and the effects of the GABA-acting drugs on seizures that will be addressed below. Many aspects of depolarizing GABA in relation to epilepsy in the developing and adult brain have been previously discussed in detail in a number of previous reviews [2,90–97].

Q10: Does Depolarizing GABA Contribute to Seizures in the Immature Brain?

The immature brain is prone to seizures, and considerable evidence indicates that depolarizing GABA is one of the factors contributing to the developmental seizure susceptibility [98]. Firstly, there is a strong developmental correlation between the depolarizing/excitatory effects of GABA and seizure susceptibility. For example, elevation of $[K^+]_o$ evokes ictal-like activity in hippocampal slices during the second postnatal week, and this developmental period of enhanced excitability is within the time window when the GABA(A) receptor agonists enhance neuronal activity (Figure 2; note that in Sprague–Dawley rats, excitatory-to-inhibitory GABA switch occurs later than in Wistar rats shown on Figure 1), and these paroxysmal events are increased in frequency and duration by the GABA(A) receptor agonists [44,99]. Secondly, blockade of GABA(A) receptors under certain conditions suppresses seizures or transforms them to interictal-like activity [37,44,99]; these effects are nonubiquitous, however, and in some other models, GABA(A) receptor antagonists aggravate neonatal seizures (see also Q4). Thirdly, restoration of low $[Cl^-]_i$ with

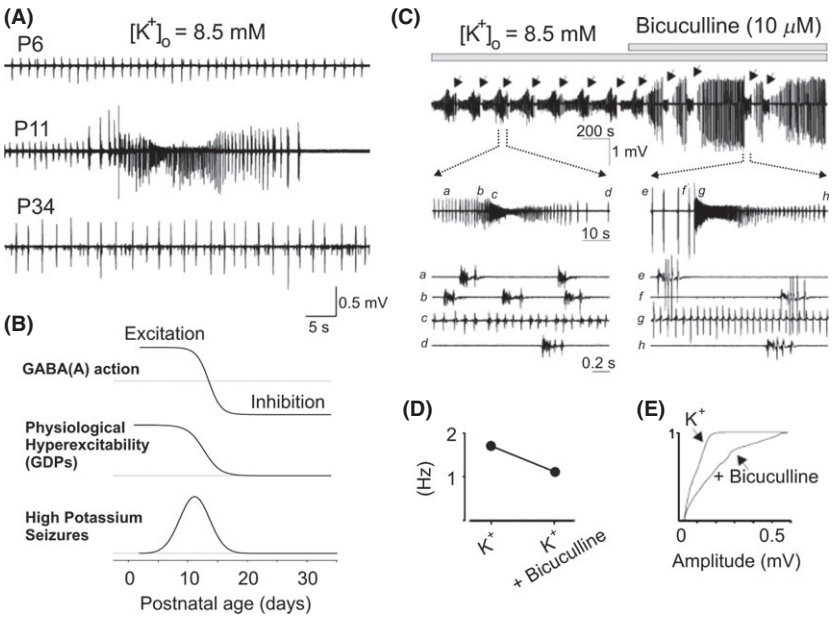

Figure 2 Age dependence of the occurrence of seizure-like activity evoked by high potassium and the effects of the GABA(A) receptor blockade. **(A)** Extracellular field potential recordings from the hippocampal slices in the presence of 8.5 mM $[K^+]_o$. In slices from P6 to P34 rats, only interictal-like activity is observed, whereas at P11, a seizure-like event (SLE) is generated. (**B**) Developmental profile of the effects of the GABA(A) agonist isoguvacine on MUA, occurrence of GDPs and occurrence of SLRs in response to of 8.5 mm $[K^+]_o$ (based on 118 hippocampal slices of P2–37 Sprague–Dawley rats). (**C**) Effect of the blockade of GABA(A) receptors on the high-potassium-induced epileptiform activity in a P9 hippocampal slice. Note that SLEs still occurred after addition of bicuculline, yet at lower frequency. Below, two of the SLEs before and after addition of bicuculline are shown on an expanded timescale. (**D**) Average frequency of the population spikes; (**E**) cumulative histogram of the population spike amplitude. D and E were obtained from analysis of 1400-s-long epochs before and after addition of bicuculline. Note that blockade of GABA(A) receptors caused a reduction in the average frequency but an increase in the amplitude of the population spikes. Adapted from [44].

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bumetanide, at least in some models, alleviates or even suppresses seizures in the immature preparations, whereas positive allosteric modulators of GABA(A) receptors aggravate seizures.

An important phenomenon that should be taken into account is an accumulation of $[Cl^-]_i$ developing as a result of impairment in KCC2-mediated chloride extrusion or upregulation of NKCC1-mediated chloride load, and associated with profound transformations in the network function and an emergence of spontaneous epileptiform events. An increase in $[Cl^-]_i$ and associated depolarizing shift in E_{GABA} develops during recurrent seizures in the "mirror-focus" model of epileptogenesis using interconnected hippocampi *in vitro* [34,36,37]. Low-magnesium model of ictogenesis in the intact hippocampus also showed a remarkable dependence of $[Cl^-]_i$ on the number of ictal-like episodes [38]. In addition, $[Cl^-]_i$ dynamically accumulates during seizures to promote excitatory GABA actions both in the immature and adult brain [16,100,101]. These depolarizing shifts in $[Cl^-]_I$ are clearly proconvulsive, and they likely underlie different efficacy and even different polarity in the actions of the GABA-acting drugs, including bumetanide and barbiturates/benzodiazepines on seizures at different time points of the epileptogenic process.

Q11: Will Suppression of Depolarizing GABA Alleviate Neonatal Seizures?

Reduction of $[Cl^-]_I$ with the NKCC1 antagonist bumetanide has been shown to efficiently reduce or even suppress epileptiform activity in the immature preparations in various models including high-potassium in hippocampal slices and kainate *in vivo* [8], spontaneous seizures in the "mirror-focus" [34], low-magnesium in the intact hippocampus *in vitro* [38,102], 4-AP in CA3 hippocampus (but not entorhinal cortex) in entorhino-hippocampal slices [103] and somatosensory cortex [104]. On the other hand, bumetanide showed barely consistent efficacy in neonatal mice CA3 hippocampal slices in 4-AP, low-magnesium, kainate, high-potassium models [105], or in flurothyl model *in vivo* [106].

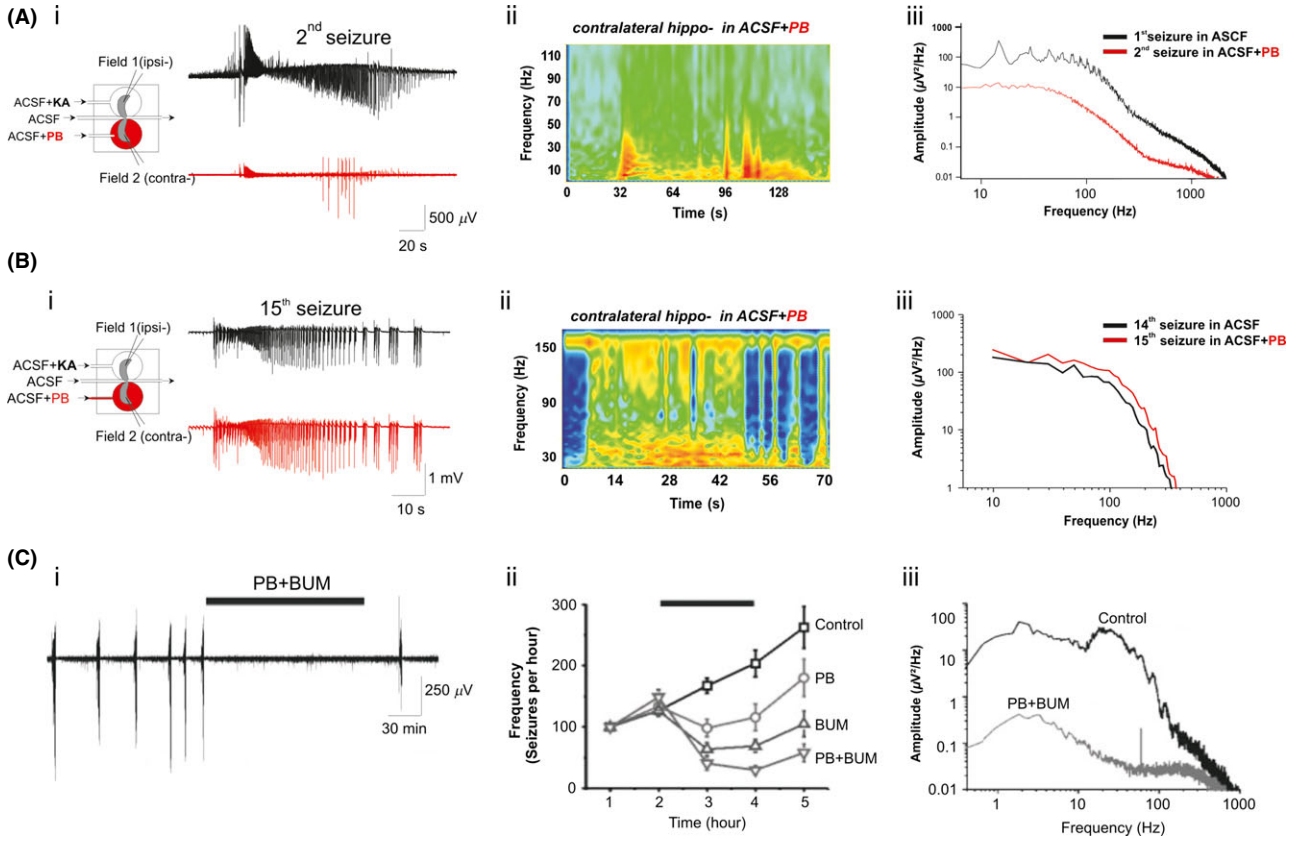

Figure 3 Effects of phenobarbital and bumetanide on neonatal seizures. (**A**) The scheme depicts the triple chamber preparation with the two intact hippocampi and their connecting interhemispheric commissure in independent chambers (left). Field recordings were made in the two hippocampi. (**A**) Kainate (KA) applied to one hippocampus (ipsilateral = ipsi-) generated ictal-like events which contralateral propagation (contra-, red) was suppressed by application of phenobarbital (PB). (**B**) Anticonvulsive efficacy of phenobarbital was lost after 15 seizures. Fourteen seizures were evoked by KA applied repeatedly (every 20 min) to ipsi-hippocampus and propagating to the contralateral hippocampus superfused with artificial cerebrospinal fluid (ACSF) (not shown). Phenobarbital applied to the contra-hippocampus prior to the 15th KA application failed to block propagating ictal-like events (red). (**C**) Application of low-Mg^{2+} ACSF induced recurrent seizures in the CA3 region of intact hippocampus of a postnatal day 5 (P5) rat. Both application of phenobarbital (PB) in combination with the NKCC1 blocker bumetanide (BUM) abolished recurrent seizures. Power spectra of extracellular field potential activity before and during application of drugs are shown (Ciii) and mean frequency of recurrent seizures in 60-min windows in control recordings, and before, during, and after drug applications; black bar indicates the time window of drug application (Cii). Adapted from (**A**–**B**) [57] and (**C**) [102].

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Limited efficacy of bumetanide *in vivo* may be also due to its heavy bounding to plasma proteins and poor permeability through the blood–brain barrier [91,107] that can be overcome by development of prodrugs of bumetanide that penetrate the blood–brain barrier more easily [108].

Pharmacokinetics, efficacy, and side effects of bumetanide treatment in animal models and epileptic patients have been reviewed [94,97,109–113] (see also Dzhala and Staley, present issue). There are two clinical studies currently investigating bumetanide as add-on therapy to treat refractory seizures in neonates (Pilot Study of Bumetanide for Newborn Seizures, Massachusetts, USA (http://www.clinicaltrials.gov/ct2/show/NCT00830531) and NEMO1, an open label exploratory dose finding and pharmacokinetic clinical trial of bumetanide for the treatment of NEonatal Seizure using Medication Off-patent, EU (http://www.clinicaltrials.gov/ct2/show/NCT01434225)).

Q12: How GABA(A) Receptor-Acting Drugs Affect Neonatal Seizures?

Drugs enhancing GABAergic conductance, such as positive allosteric GABA(A) receptor modulators barbiturates and benzodiazepines, exert desynchronizing and anticonvulsive actions at low or moderately elevated physiological levels of $[Cl^-]_i$; however, these are reduced—lost—or even inverted to synchronizing and proconvulsive actions during the states when $[Cl^-]_i$ is increased. For example, phenobarbital efficiently suppresses first seizures evoked by low-magnesium and propagating seizures in the interconnected hippocampi preparation, but it loses its anticonvulsive actions or even aggravates seizures after multiple seizures along with an accumulation of $[Cl^-]_i$ and progressive depolarizing shift in E_{GABA} in these two models [38,57] (Figure 3). Therefore, the best anticonvulsive effect is achieved when a treatment using phenobarbital to enhance GABA(A) receptor-mediated function is combined with bumetanide to restore low $[Cl^-]_i$ [34,57,102] (Figure 3).

Thus, the available wealth of information indicates that (i) depolarizing GABA contributes to enhanced excitability in the immature networks, (ii) accumulation of $[Cl^-]_i$ caused by repetitive seizures increases excitatory GABA drive and its synchronizing effects on the network and shifts its role toward proconvulsing, and (iii) reduction of $[Cl^-]_i$ may be a valid strategy to treat neonatal seizures.

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Conflict of Interests

The authors declare no conflict of interest.

References

1. Trevelyan AJ, Schevon CA. How inhibition influences seizure propagation. *Neuropharmacology* 2013;**69**:45–54.
2. Ben Ari Y, Gaiarsa JL, Tyzio R, Khazipov R. GABA: A pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol Rev* 2007;**87**:1215–1284.
3. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science* 2002;**298**:1418–1421.
4. Huberfeld G, Wittner L, Clemenceau S, et al. Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. *J Neurosci* 2007;**27**:9866–9873.
5. Palma E, Amici M, Sobrero F, et al. Anomalous levels of Cl- transporters in the hippocampal subiculum from temporal lobe epilepsy patients make GABA excitatory. *Proc Natl Acad Sci U S A* 2006;**103**:8465–8468.
6. Rivera C, Voipio J, Payne JA, et al. The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 1999;**397**:251–255.
7. Yamada J, Okabe A, Toyoda H, Kilb W, Luhmann HJ, Fukuda A. Cl- uptake promoting depolarizing GABA actions in immature rat neocortical neurones is mediated by NKCC1. *J Physiol Online* 2004;**557**:829–841.
8. Dzhala VI, Talos DM, Sdrulla DA, et al. NKCC1 transporter facilitates seizures in the developing brain. *Nat Med* 2005;**11**:1205–1213.
9. Payne JA, Rivera C, Voipio J, Kaila K. Cation-chloride co-transporters in neuronal communication, development and trauma. *Trends Neurosci* 2003;**26**:199–206.
10. Blaesse P, Airaksinen MS, Rivera C, Kaila K. Cation-chloride cotransporters and neuronal function. *Neuron* 2009;**61**:820–838.
11. Achilles K, Okabe A, Ikeda M, et al. Kinetic properties of Cl- uptake mediated by Na+-dependent K+-2Cl(−) cotransport in immature rat neocortical neurons. *J Neurosci* 2007;**27**:8616–8627.
12. Tyzio R, Cossart R, Khalilov I, et al. Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. *Science* 2006;**314**:1788–1792.
13. Pfeffer CK, Stein V, Keating DJ, et al. NKCC1-dependent GABAergic excitation drives synaptic network maturation during early hippocampal development. *J Neurosci* 2009;**29**:3419–3430.
14. Gonzalez-Islas C, Chub N, Wenner P. NKCC1 and AE3 appear to accumulate chloride in embryonic motoneurons. *J Neurophysiol* 2009;**101**:507–518.
15. Hubner CA, Holthoff K. Anion transport and GABA signaling. *Front Cell Neurosci* 2013;**7**:177.
16. Glykys J, Dzhala V, Egawa K, et al. Local impermeant anions establish the neuronal chloride concentration. *Science* 2014;**343**:670–675.
17. McClatchy DB, Liao L, Park SK, Venable JD, Yates JR. Quantification of the synaptosomal proteome of the rat cerebellum during post-natal development. *Genome Res* 2007;**17**:1378–1388.
18. Frischknecht R, Heine M, Perrais D, Seidenbecher CI, Choquet D, Gundelfinger ED. Brain extracellular matrix affects AMPA receptor lateral mobility and short-term synaptic plasticity. *Nat Neurosci* 2009;**12**:897–904.
19. Tyzio R, Minlebaev M, Rheims S, et al. Postnatal changes in somatic gamma-aminobutyric acid signalling in the rat hippocampus. *Eur J Neurosci* 2008;**27**:2515–2528.
20. Tyzio R, Holmes GL, Ben-Ari Y, Khazipov R. Timing of the developmental switch in GABA(A) mediated signalling from excitation to inhibition in CA3 rat hippocampus using gramicidin perforated patch and extracellular recordings. *Epilepsia* 2007;**48**:96–105.
21. Rheims S, Minlebaev M, Ivanov A, et al. Excitatory GABA in rodent developing neocortex in vitro. *J Neurophysiol* 2008;**100**:609–619.
22. Valeeva G, Abdullin A, Tyzio R, et al. Temporal coding at the immature depolarizing GABAergic synapse. *Front Cell Neurosci* 2010;**4**:17.
23. Chen G, Trombley PQ, van den Pol AN. Excitatory actions of GABA in developing rat hypothalamic neurones. *J Physiol* 1996;**494**(Pt 2):451–464.
24. Gulledge AT, Stuart GJ. Excitatory actions of GABA in the cortex. *Neuron* 2003;**37**:299–309.
25. Garaschuk O, Linn J, Eilers J, Konnerth A. Large-scale oscillatory calcium waves in the immature cortex. *Nat Neurosci* 2000;**3**:452–459.
26. Bonifazi P, Goldin M, Picardo MA, et al. GABAergic hub neurons orchestrate synchrony in developing hippocampal networks. *Science* 2009;**326**:1419–1424.
27. Khazipov R, Leinekugel X, Khalilov I, Gaïarsa J-L, Ben-Ari Y. Synchronization of GABAergic interneuronal network in CA3 subfield of neonatal rat hippocampal slices. *J Physiol (Lond)* 1997;**498**:763–772.
28. Leinekugel X, Medina I, Khalilov I, Ben-Ari Y, Khazipov R. Ca^{2+} oscillations mediated by the synergistic excitatory actions of $GABA_A$ and NMDA receptors in the neonatal hippocampus. *Neuron* 1997;**18**:243–255.
29. Steriade M, McCormick DA, Sejnowski TJ. Thalamocortical oscillations in the sleeping and aroused brain. *Science* 1993;**262**:679–685.
30. Ben-Ari Y, Cherubini E, Corradetti R, Gaïarsa J-L. Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J Physiol (Lond)* 1989;**416**:303–325.
31. Leinekugel X, Khalilov I, Ben-Ari Y, Khazipov R. Giant depolarizing potentials: The septal pole of the hippocampus paces the activity of the developing intact

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septohippocampal complex in vitro. *J Neurosci* 1998;**18**:6349–6357.
32. Menendez d, Bolea S, Sanchez-Andres JV. Origin of the synchronized network activity in the rabbit developing hippocampus. *Eur J Neurosci* 1998;**10**:899–906.
33. Sipila ST, Schuchmann S, Voipio J, Yamada J, Kaila K. The cation-chloride cotransporter NKCC1 promotes sharp waves in the neonatal rat hippocampus. *J Physiol* 2006;**573**:765–773.
34. Nardou R, Ben-Ari Y, Khalilov I. Bumetanide, an NKCC1 antagonist, does not prevent formation of epileptogenic focus but blocks epileptic focus seizures in immature rat hippocampus. *J Neurophysiol* 2009;**101**:2878–2888.
35. Khalilov I, Dzhala V, Ben-Ari Y, Khazipov R. Dual role of GABA in the neonatal rat hippocampus. *Dev Neurosci* 1999;**21**:310–319.
36. Khalilov I, Le Van QM, Gozlan H, Ben Ari Y. Epileptogenic actions of GABA and fast oscillations in the developing hippocampus. *Neuron* 2005;**48**:787–796.
37. Khalilov I, Holmes GL, Ben Ari Y. In vitro formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures. *Nat Neurosci* 2003;**6**:1079–1085.
38. Dzhala VI, Kuchibhotla KV, Glykys JC, et al. Progressive NKCC1-dependent neuronal chloride accumulation during neonatal seizures. *J Neurosci* 2010;**30**:11745–11761.
39. Staley KJ, Mody I. Shunting of excitatory input to dentate gyrus granule cells by a depolarizing GABAA receptor-mediated postsynaptic conductance. *J Neurophysiol* 1992;**68**:197–212.
40. Lu T, Trussell LO. Mixed excitatory and inhibitory GABA-mediated transmission in chick cochlear nucleus. *J Physiol* 2001;**535**:125–131.
41. Monsivais P, Rubel EW. Accommodation enhances depolarizing inhibition in central neurons. *J Neurosci* 2001;**21**:7823–7830.
42. Khalilov I, Khazipov R, Esclapez M, Ben-Ari Y. Bicuculline induces ictal seizures in the intact hippocampus recorded in vitro. *Eur J Pharmacol* 1997;**319**:R5–R6.
43. Khazipov R, Esclapez M, Caillard O, et al. Early development of neuronal activity in the primate hippocampus in utero. *J Neurosci* 2001;**21**:9770–9781.
44. Khazipov R, Khalilov I, Tyzio R, Morozova E, Ben Ari Y, Holmes GL. Developmental changes in GABAergic actions and seizure susceptibility in the rat hippocampus. *Eur J Neurosci* 2004;**19**:590–600.
45. Baram TZ, Snead OC. Bicuculline induced seizures in infant rats: Ontogeny of behavioral and electrocortical phenomena. *Dev Brain Res* 1990;**57**:291–295.
46. Kolbaev SN, Sharopov S, Dierkes PW, Luhmann HJ, Kilb W. Phasic GABAA -receptor activation is required to suppress epileptiform activity in the CA3 region of the immature rat hippocampus. *Epilepsia* 2012;**53**:888–896.
47. Isaev D, Isaeva E, Khazipov R, Holmes GL. Anticonvulsant action of GABA in the high potassium-low magnesium model of ictogenesis in the neonatal rat hippocampus in vivo and in vitro. *J Neurophysiol* 2005;**94**:2987–2992.
48. Khirug S, Huttu K, Ludwig A, et al. Distinct properties of functional KCC2 expression in immature mouse hippocampal neurons in culture and in acute slices. *Eur J Neurosci* 2005;**21**:899–904.
49. Barry PH, Lynch JW. Liquid junction potentials and small cell effects in patch-clamp analysis. *J Membr Biol* 1991;**121**:101–117.
50. Tyzio R, Ivanov A, Bernard C, Holmes GL, Ben Ari Y, Khazipov R. Membrane potential of CA3 hippocampal pyramidal cells during postnatal development. *J Neurophysiol* 2003;**90**:2964–2972.
51. Maric D, Maric I, Smith SV, Serafini R, Hu Q, Barker JL. Potentiometric study of resting potential, contributing K+ channels and the onset of Na+ channel excitability in embryonic rat cortical cells. *Eur J Neurosci* 1998;**10**:2532–2546.
52. Verheugen JA, Vijverberg HP, Oortgiesen M, Cahalan MD. Voltage-gated and Ca(2 +)-activated K+ channels in intact human T lymphocytes. Noninvasive measurements of membrane currents, membrane potential, and intracellular calcium. *J Gen Physiol* 1995;**105**:765–794.
53. Fricker D, Verheugen JA, Miles R. Cell-attached measurements of the firing threshold of rat hippocampal neurones. *J Physiol* 1999;**517**:791–804.
54. Verheugen JA, Fricker D, Miles R. Noninvasive measurements of the membrane potential and GABAergic action in hippocampal interneurons. *J Neurosci* 1999;**19**:2546–2555.
55. Ehrlich I, Lohrke S, Friauf E. Shift from depolarizing to hyperpolarizing glycine action in rat auditory neurones is due to age-dependent Cl- regulation. *J Physiol (Lond)* 1999;**520**:121–137.
56. Minlebaev M, Valeeva G, Tcheremiskine V, Coustillier G, Khazipov R. Cell-attached recordings of responses evoked by photorelease of GABA in the immature cortical neurons. *Front Cell Neurosci* 2013;**7**:83.
57. Nardou R, Yamamoto S, Chazal G, et al. Neuronal chloride accumulation and excitatory GABA underlie aggravation of neonatal epileptiform activities by phenobarbital. *Brain* 2011;**134**:987–1002.
58. Serafini R, Valeyev AY, Barker JL, Poulter MO. Depolarizing GABA-activated Cl^- channels in embryonic rat spinal and olfactory bulb cells. *J Physiol (Lond)* 1995;**488**:371–386.
59. Tyzio R, Khalilov I, Represa A, et al. Inhibitory actions of the gamma-aminobutyric acid in pediatric Sturge-Weber syndrome. *Ann Neurol* 2009;**66**:209–218.
60. Curmi JP, Premkumar LS, Birnir B, Gage PW. The influence of membrane potential on chloride channels activated by GABA in rat cultured hippocampal neurons. *J Membr Biol* 1993;**136**:273–280.
61. van den Pol AN, Obrietan K, Chen G. Excitatory actions of GABA after neuronal trauma. *J Neurosci* 1996;**16**:4283–4292.
62. Nabekura J, Ueno T, Okabe A, et al. Reduction of KCC2 expression and GABAA receptor-mediated excitation after in vivo axonal injury. *J Neurosci* 2002;**22**:4412–4417.
63. De Koninck Y. Altered chloride homeostasis in neurological disorders: A new target. *Curr Opin Pharmacol* 2007;**7**:93–99.
64. Price TJ, Cervero F, Gold MS, Hammond DL, Prescott SA. Chloride regulation in the pain pathway. *Brain Res Rev* 2009;**60**:149–170.
65. Bos R, Sadlaoud K, Boulenguez P, et al. Activation of 5-HT2A receptors upregulates the function of the neuronal K-Cl cotransporter KCC2. *Proc Natl Acad Sci U S A* 2013;**110**:348–353.
66. Bak IJ, Misgeld U, Weiler M, Morgan E. The preservation of nerve cells in rat neostriatal slices maintained in vitro: A morphological study. *Brain Res* 1980;**197**:341–353.
67. Khalilov I, Esclapez M, Medina I, et al. A novel in vitro preparation: The intact hippocampal formation. *Neuron* 1997;**19**:743–749.
68. Dzhala V, Valeeva G, Glykys J, Khazipov R, Staley K. Traumatic alterations in GABA signaling disrupt hippocampal network activity in the developing brain. *J Neurosci* 2012;**32**:4017–4031.
69. Moyer JR Jr, McNay EC, Brown TH. Three classes of pyramidal neurons in layer V of rat perirhinal cortex. *Hippocampus* 2002;**12**:218–234.
70. Bregestovski P, Bernard C. Excitatory GABA: how a correct observation may turn out to be an experimental artifact. *Front Pharmacol* 2012;**3**:65.
71. Ben-Ari Y, Woodin MA, Sernagor E, et al. Refuting the challenges of the developmental shift of polarity of GABA actions: GABA more exciting than ever!. *Front Cell Neurosci* 2012;**6**:35.
72. Valeeva G, Valiullina F, Khazipov R. Excitatory actions of GABA in the intact neonatal rodent hippocampus in vitro. *Front Cell Neurosci* 2013;**7**:20.
73. Yamashita M, Fukuda Y. Calcium channels and GABA receptors in the early embryonic chick retina. *J Neurobiol* 1993;**24**:1600–1614.
74. Fischer KF, Lukasiewicz PD, Wong ROL. Age-dependent and cell class-specific modulation of retinal ganglion cell bursting activity by GABA. *J Neurosci* 1998;**18**:3767–3778.
75. Sernagor E, Young C, Eglen SJ. Developmental modulation of retinal wave dynamics: Shedding light on the GABA saga. *J Neurosci* 2003;**23**:7621–7629.
76. Zheng JJ, Lee S, Zhou ZJ. A developmental switch in the excitability and function of the starburst network in the mammalian retina. *Neuron* 2004;**44**:851–864.
77. Khakhalin AS, Aizenman CD. GABAergic transmission and chloride equilibrium potential are not modulated by pyruvate in the developing optic tectum of Xenopus laevis tadpoles. *PLoS ONE* 2012;**7**:e34446.
78. Nishimaru H, Iizuka M, Ozaki S, Kudo N. Spontaneous motoneuronal activity mediated by glycine and GABA in the spinal cord of rat fetuses in vitro. *J Physiol (Lond)* 1996;**497**:131–143.
79. Fellippa-Marques S, Vinay L, Clarac F. Spontaneous and locomotor-related GABAergic input onto primary afferents in the neonatal rat. *Eur J Neurosci* 2000;**12**:155–164.
80. Bos R, Brocard F, Vinay L. Primary afferent terminals acting as excitatory interneurons contribute to spontaneous motor activities in the immature spinal cord. *J Neurosci* 2011;**31**:10184–10188.
81. Purpura DP, Prelevic S, Santini M. Postsynaptic potentials and spike variations in the feline hippocampus during postnatal ontogenesis. *Exp Neurol* 1968;**22**:408–422.
82. Inada H, Watanabe M, Uchida T, et al. GABA regulates the multidirectional tangential migration of GABAergic interneurons in living neonatal mice. *PLoS ONE* 2011;**6**:e27048.
83. Wang DD, Kriegstein AR. Blocking early GABA depolarization with bumetanide results in permanent alterations in cortical circuits and sensorimotor gating deficits. *Cereb Cortex* 2011;**21**:574–587.
84. Wilhelm JC, Rich MM, Wenner P. Compensatory changes in cellular excitability, not synaptic scaling, contribute to homeostatic recovery of embryonic network activity. *Proc Natl Acad Sci U S A* 2009;**106**:6760–6765.
85. Heck N, Kilb W, Reiprich P, et al. GABA-A receptors regulate neocortical neuronal migration in vitro and in vivo. *Cereb Cortex* 2007;**17**:138–148.
86. Minlebaev M, Ben-Ari Y, Khazipov R. Network mechanisms of spindle-burst oscillations in the neonatal rat barrel cortex in vivo. *J Neurophysiol* 2007;**97**:692–700.
87. Koyama Y, Andoh T, Kamiya Y, et al. Bumetanide, an inhibitor of cation-chloride cotransporter isoform 1, inhibits gamma-aminobutyric acidergic excitatory actions and enhances sedative actions of midazolam in neonatal rats. *Anesthesiology* 2013;**119**:1096–1108.
88. Sipila ST, Schuchmann S, Voipio J, Yamada J, Kaila K. The Na-K-Cl cotransporter (NKCC1) promotes sharp waves in the neonatal rat hippocampus. *J Physiol* 2006;**573**:765–773.
89. Minlebaev M, Colonnese M, Tsintsadze T, Sirota A, Khazipov R. Early gamma oscillations synchronize developing thalamus and cortex. *Science* 2011;**334**:226–229.
90. Pavlov I, Kaila K, Kullmann DM, Miles R. Cortical inhibition, pH and cell excitability in epilepsy: What are optimal targets for antiepileptic interventions? *J Physiol* 2013;**591**:765–774.

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91. Loscher W, Puskarjov M, Kaila K. Cation-chloride cotransporters NKCC1 and KCC2 as potential targets for novel antiepileptic and antiepileptogenic treatments. *Neuropharmacology* 2013;**69**:62–74.
92. Cossart R, Bernard C, Ben Ari Y. Multiple facets of GABAergic neurons and synapses: Multiple fates of GABA signalling in epilepsies. *Trends Neurosci* 2005;**28**:108–115.
93. Huberfeld G, Le DC, Le Van QM, Navarro V, Baulac M, Miles R. The paradox of the paroxysm: Can seizure precipitants help explain human ictogenesis? *Neuroscientist* 2013;**19**:523–540.
94. Kaila K, Ruusuvuori E, Seja P, Voipio J, Puskarjov M. GABA actions and ionic plasticity in epilepsy. *Curr Opin Neurobiol* 2014;**26**:34–41.
95. Kahle KT, Staley KJ. The bumetanide-sensitive Na-K-2Cl cotransporter NKCC1 as a potential target of a novel mechanism-based treatment strategy for neonatal seizures. *Neurosurg Focus* 2008;**25**:E22.
96. Staley KJ. Does plasticity of the GABA(A) reversal potential contribute to epileptogenesis? *Epilepsy Curr* 2008;**8**:107–110.
97. Kirmse K, Witte OW, Holthoff K. GABAergic depolarization during early cortical development and implications for anticonvulsive therapy in neonates. *Epilepsia* 2011;**52**:1532–1543.
98. Holmes GL, Khazipov R, Ben Ari Y. New concepts in neonatal seizures. *NeuroReport* 2002;**13**:A3–A8.
99. Dzhala VI, Staley KJ. Excitatory actions of endogenously released GABA contribute to initiation of ictal epileptiform activity in the developing hippocampus. *J Neurosci* 2003;**23**:1840–1846.
100. Fujiwara-Tsukamoto Y, Isomura Y, Imanishi M, et al. Prototypic seizure activity driven by mature hippocampal fast-spiking interneurons. *J Neurosci* 2010;**30**:13679–13689.
101. Fujiwara-Tsukamoto Y, Isomura Y, Nambu A, Takada M. Excitatory GABA input directly drives seizure-like rhythmic synchronization in mature hippocampal CA1 pyramidal cells. *Neuroscience* 2003;**119**:265–275.
102. Dzhala VI, Brumback AC, Staley KJ. Bumetanide enhances phenobarbital efficacy in a neonatal seizure model. *Ann Neurol* 2008;**63**:222–235.
103. Wahab A, Albus K, Heinemann U. Age- and region-specific effects of anticonvulsants and bumetanide on 4-aminopyridine-induced seizure-like events in immature rat hippocampal-entorhinal cortex slices. *Epilepsia* 2011;**52**:94–103.
104. Rheims S, Represa A, Ben-Ari Y, Zilberter Y. Layer-specific generation and propagation of seizures in slices of developing neocortex: Role of excitatory GABAergic synapses. *J Neurophysiol* 2008;**100**:620–628.
105. Kilb W, Sinning A, Luhmann HJ. Model-specific effects of bumetanide on epileptiform activity in the in-vitro intact hippocampus of the newborn mouse. *Neuropharmacology* 2007;**53**:524–533.
106. Minlebaev M, Khazipov R. Antiepileptic effects of endogenous beta-hydroxybutyrate in suckling infant rats. *Epilepsy Res* 2011;**95**:100–109.
107. Javaheri S, Davis C, Rogers DH. Ionic composition of cisternal CSF in acute respiratory acidosis: Lack of effect of large dose bumetanide. *J Neurochem* 1993;**61**:1525–1529.
108. Tollner K, Brandt C, Topfer M, et al. A novel prodrug-based strategy to increase effects of bumetanide in epilepsy. *Ann Neurol* 2014;**75**:550–562.
109. Khanna A, Walcott BP, Kahle KT. Limitations of current GABA agonists in neonatal seizures: Toward GABA modulation via the targeting of neuronal Cl(−) transport. *Front Neurol* 2013;**4**:78.
110. Maa EH, Kahle KT, Walcott BP, Spitz MC, Staley KJ. Diuretics and epilepsy: Will the past and present meet? *Epilepsia* 2011;**52**:1559–1569.
111. Puskarjov M, Kahle KT, Ruusuvuori E, Kaila K. Pharmacotherapeutic targeting of cation-chloride cotransporters in neonatal seizures. *Epilepsia* 2014;**55**:806–818.
112. Pressler R, Auvin S. Comparison of brain maturation among species: An example in translational research suggesting the possible use of bumetanide in newborn. *Front Neurol* 2013;**4**:36.
113. Miles R, Blaesse P, Huberfeld G, Wittner L, Kaila K. Chloride homeostasis and GABA signaling in temporal lobe epilepsy. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, editors. *Jasper's Basic Mechanisms of the Epilepsies* [Internet]. 4th edition. Bethesda, MD: National Center for Biotechnology Information, 2012.
114. Swann JW, Brady RJ, Martin DL. Postnatal development of GABA-mediated synaptic inhibition in rat hippocampus. *Neuroscience* 1989;**28**:551–561.
115. Leinekugel X, Tseeb V, Ben-Ari Y, Bregestovski P. Synaptic $GABA_A$ activation induces Ca^{2+} rise in pyramidal cells and interneurons from rat neonatal hippocampal slices. *J Physiol (Lond)* 1995;**487**:319–329.
116. Garaschuk O, Hanse E, Konnerth A. Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampus. *J Physiol (Lond)* 1998;**507**:219–236.
117. Rovira C, Ben-Ari Y. Developmental study of Benzodiazepine effects on monosynaptic GABAA-mediated IPSP of rat hippocampal neurons. *J Neurophysiol* 1993;**70**:1076–1085.
118. Banke TG, McBain CJ. GABAergic input onto CA3 hippocampal interneurons remains shunting throughout development. *J Neurosci* 2006;**26**:11720–11725.

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